ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
19 (1999) 47–53

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Amperometric biosensors for clinical and therapeutic drug monitoring: a review

Joseph Wang *

Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, NM 88003, USA

Received 8 January 1998; received in revised form 26 March 1998; accepted 26 March 1998

Abstract

The coupling of enzymes and electrode transducers permits the rapid and simple determination of endogeneous compounds and therapeutic drugs in clinical samples. New developments in the operation, miniaturization and microfabrication of electrochemical biosensors offer exciting possibilities for biomedical and pharmaceutical analysis. This review focuses on the current state of amperometric enzyme electrodes for biomedicine, with emphasis on recent advances, challenges and trends. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Biosensors; Enzyme electrodes; Electroanalysis; Glucose

1. Introduction

Over the past two decades we have witnessed a tremendous amount of activity in the area of biosensors. Biosensors are small devices employing biochemical molecular recognition properties as the basis for a selective analysis. The major processes involved in any biosensor system are analyte recognition, signal transduction and readout. Such devices hold great promise for the health care and pharmaceutical industries. In particular, due to their specificity, portability, speed, and low cost, biosensors offer exciting opportunities for numerous decentralized clinical applications, ranging from emergency-room screening, bedside monitoring, home self testing and 'alternative-site' testing (e.g. the physician's office).

Electrochemical devices have traditionally received the major share of the attention in biosensor development. The name electrochemical biosensor is applied to a molecular sensing device which intimately couples a biological recognition element to an electrode transducer. The purpose of the transducer is to convert the biological recognition event into a useful electrical signal. Amperometric and potentiometric transducers are most commonly used in conjunction with electrochemical biosensors. In potentiometric devices the analytical information is obtained by converting the biorecognition process into a potential signal, whereas the amperometric types are based on monitoring the current associated with oxidation or reduction of an electroactive species involved in the recognition process. An amperometric biosensor may be more attractive because of its

* Tel.: +1 505 6462505; fax: +1 505 6462549; e-mail: joewang@nmsu.edu

PII S0731-7085(98)00056-9

high sensitivity and wide linear range. Elegant research on new sensing concepts, coupled with numerous technological innovations, have opened the door to a widespread biomedical use of amperometric devices.

In this review article I will focus on the amperometric approach, and more specifically, on amperometric enzyme electrodes. Particular attention will be given to recent advances, trends and biomedical or pharmaceutical applications of these biosensing devices. The reader is referred to several books for comprehensive information on electrochemical biosensors [1–3].

2. Enzyme electrodes

Amperometric enzyme electrodes hold a leading position among biosensor systems presently available, and have already found a large commercial market. Such devices combine the specificity of the enzyme for recognizing a given target analyte, with the direct transduction of the rate of the biocatalytic reaction into a current signal. Such coupling of enzymes and amperometric electrodes permits the rapid, simple and direct determination of various metabolites and therapeutic drugs in biological fluids in connection with single-use, intermittent-use or continuous-monitoring applications.

2.1. Principles

An enzyme electrode consists of a thin layer of an enzyme immobilized on the electrode surface. The enzyme is chosen to catalyze a reaction which generates a product or consumes a reactant which can be monitored amperometrically. Accordingly, amperometric probes are highly suitable in connection with oxidase or dehydrogenase enzymes that generate easily oxidizable hydrogen peroxide or reduced nicotinamide adenine dinucleotide (NADH), respectively:

$$\text{Substrate} + O_2 \overset{\text{oxidase}}{\rightarrow} \text{Product} + H_2O_2 \quad (1)$$

$$\text{Substrate} + NAD^+ \overset{\text{dehydrogenase}}{\rightarrow} \text{Product} + NADH \quad (2)$$

The liberated peroxide or NADH species can be detected by poising the working-electrode transducer at relatively modest potentials ($+0.5$–$+0.8$ V, against a Ag/AgCl reference electrode):

$$H_2O_2 \Rightarrow O_2 + 2H^+ + 2e^- \quad (3)$$

$$NADH \Rightarrow NAD^+ + H^+ + 2e^- \quad (4)$$

to yield a current signal that rises with the substrate concentration. The exact operating potential depends on the working electrode material. The selection of the working electrode transducer is thus strongly dependent upon its reactivity towards the peroxide and NADH products, as well as upon other considerations, including the background current, interfering reactions, surface reproducibility, mechanical properties, and cost. The most popular ones are those involving platinum, gold, carbon and metalized carbon.

The performance of the enzyme electrode depends in part on the immobilization of the enzyme layer. Several methods can be used for immobilizing the enzyme onto the electrode, including entrapment behind a dialysis membrane or within a polymeric film (e.g. Nafion, polypyrrole, polyphenylenediamine), covalent coupling through a cross-linking agent, avidin–biotin binding, or incorporation within the bulk of a carbon composite matrix. Accordingly, the overall sequence of events involves several steps, including diffusion of the substrate molecule towards the surface and its reaction with the immobilized enzyme, which in turn is regenerated into its native form by reaction with the (oxygen or NAD^+) cofactor. The actual response is determined by the rate limiting step in this overall reaction scheme, and may thus reflect mass-transport or kinetic limitations.

Various biomedical applications of oxidase electrodes based on measurements of the liberated peroxide species may be affected by fluctuations in the oxygen level or by interfering endogeneous electroactive compounds (e.g. ascorbic or uric acids) or oxogeneous drugs (e.g. acetaminophen) that add to the signal. Such problems have been alleviated using proper membrane coverage (that excludes potential interferences and restricts the flux of the substrate and hence improves the

relative surface availability of oxygen), as well as in the connection to electrocatalytic transducers (that preferentially catalyze the oxidation of hydrogen peroxide but not of the common interferences). Oxygen-rich electrode materials have also been documented to satisfy the oxygen demand [4]. Dehydrogenase-based biosensors (recently reviewed in [5]) also face the problem of gradual passivation (fouling) of the surface due to accumulation of reaction products. Such stability problems have been minimized through the use of redox mediators (e.g. phenoxazine or phenothiazine compounds) that shuttle electrons between NADH and the surface.

2.2. Electrical communication between redox enzymes and the electrode surface

Ideally, the above problems of oxidase sensors can be eliminated through a direct electron transfer between the enzyme redox center and the electrode surface. In practice, the thick protein shell (surrounding the active center) introduces a kinetic barrier for electron transfer. Diffusional electron mediators, such as ferrocene derivatives, ferrocyanide, conducting organic salts or quinone compounds have thus been widely used to electrically contact redox enzymes [6] (Fig. 1A). As a result of using artificial electron acceptors, measurements become insensitive to oxygen fluctuations and can be performed at lower potentials that do not provoke interfering reactions. In order to function effectively, the mediator should react rapidly with the reduced enzyme, must be nontoxic and chemically stable (in both reduced and oxidized forms), and must have a low redox potential. Commercial blood glucose self-testing meters commonly rely on the use of ferrocene or ferrocyanide mediators.

Ohara et al. [7] developed an elegant nondiffusional route for establishing electrical communication between redox enzymes and electrodes based on 'wiring' the enzyme to the surface with a long flexible polymer having a dense array of osmium-complex electron relays (Fig. 1B). The resulting three-dimensional redox–polymer/enzyme networks offer high current outputs and stabilize the mediator to the electrode surfaces. Willner et al. [8] reported on a novel approach for modifying glucose oxidase for facilitating the electron transfer between its redox center and the electrode surface (Fig. 1C). For this purpose, the FAD active center of the enzyme was removed to allow positioning of an electron-mediating ferrocene unit prior to the reconstitution of the enzyme. Such enzyme reconstitution strategy has been ex-

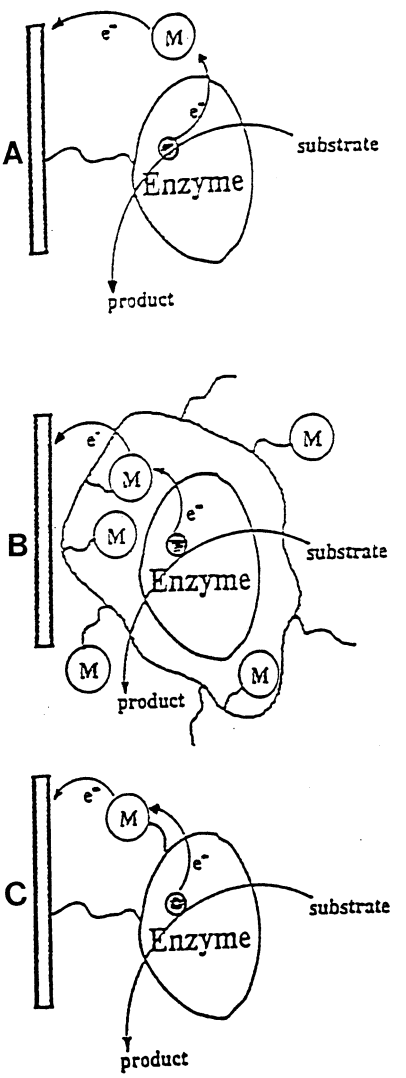

Fig. 1. Electrical contact of a redox enzyme with electrode surfaces: (A) by a diffusional mediator; (B) by immobilization of the enzyme in a redox polymer; (C) by tethering redox relay units to the protein (reproduced with permission from Willner et al. [9]).

panded recently to NAD^+-dependent dehydrogenase enzymes [9].

2.3. Biosensors for in vivo monitoring

Continuous monitoring of endogenous compounds or drugs by implantable sensors enables a closer surveillance of patients via a rapid return of clinical information [10,11]. The continuous signal provided by an amperometric enzyme electrode can be exploited for continuous in vivo monitoring of unstable patients (in which rapid biochemical changes can be missed by discrete measurements). Such real-time measurements are thus highly desired in intensive care units, during surgery, or for the management of diabetes, as they offer the option of early warning of an unexpected deterioration, and rapid corrective action (eventually via an artificial biofeedback system). The ideal in vivo biosensor should thus respond continuously and specifically to the target analyte over a wide concentration range. It can monitor the analyte intravascularly (i.e. in the blood stream) or transcutaneously (i.e. across the skin surface). Such monitoring requires special adaptation of biosensors in term of miniaturization, biocompatability (involving both the tissue and blood response), enzyme stability, cofactor dependency (oxygen deficit), drift, in vivo calibration, safety, or convenience. In spite of these major challenges, significant progress has been made towards the continuous monitoring of physiologically important molecules, such as glucose, lactate or glutamate.

Most of the attention has been given to the probing of blood glucose levels as an aid to diabetes therapy [12,13]. In particular, subcutaneously implantable needle-type amperometric glucose sensors, being developed in various laboratories (e.g. Fig. 2 [14]), should offer hypo- or hyperglycemia alarm capabilities to enable swift and appropriate corrective action (through a closed-loop insulin delivery system, i.e. an artificial pancreas). While success in this research has reached the level of short-term human implantation, a continuously functioning implantable glucose sensor possessing long-term stability has not been realized. Besides the obvious biocompatability (biofouling) challenge, such implantable glucose sensors may be prone to errors due to low oxygen tension or electroactive interferences. Non-invasive approaches for continuous glucose monitoring, such as the amperometric 'GlucoWatch' (being developed by Cygnus and relying on transdermal extraction), represent a promising route for addressing the challenges of implantable glucose devices. Analogous developments of implantable lactate biosensors would benefit the monitoring of heart attack patients.

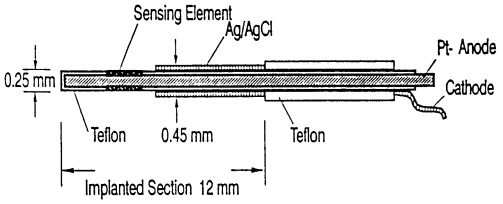

Fig. 2. Schematic of an implantable needle-type glucose sensor (reproduced from Zhang and Wilson [14]).

2.4. Practical examples

Several important metabolites and drugs (in addition to glucose) can be readily detected in connection with amperometric biosensors based on the judicious selection of the immobilized enzyme. Because of the involvement of lactate in several severe diseases there are strong demands for the development of reliable amperometric sensors for decentralized lactate monitoring. The majority of these devices rely on the enzyme lactate oxidase, although lactate–dehydrogenase sensors have also been described. Kyrolainen et al. [15] developed an on-line biosensor system for monitoring blood lactate during open heart surgery, Meyerhoff et al. [16] reported on a catheter-based portable device for continuous monitoring of blood lactate, and Baker and Gough [17] introduced an implantable lactate sensor.

The determination of creatinine in body fluids is of significant value for diagnosis of renal function. Multienzyme electrode systems have thus been developed for amperometric monitoring of creatinine [18,19]. Continuous concerns about cholesterol levels in body fluids have led to the

coupling of cholesterol esterase and cholesterol oxidase to electrode surfaces for monitoring total cholesterol (free cholesterol + cholesterol ester) in serum [20] and whole blood [21]. Useful amperometric enzyme electrodes have been developed for the determination of bilirubin [22], galactose [23], amino acids [24] or peptides [25], in connection to immobilized bilirubin oxidase, galactose oxidase, amino acid oxidase, and tyrosinase, respectively.

Amperometric biosensors for monitoring drugs have also received considerable attention. These include the use of the enzyme aryl acylamidase for assays of acetaminophen (paracetamol) in connection with the detection of the liberated aminophenol [26], the use of theophylline oxidase and a ferrocyanide mediator for the biosensing of theophylline [27], an indirect enzymatic amplification strategy for chlorpromazine [28], or the immobilization of salicylate hydroxylase for the detection of salicylate in the presence of NADH and dissolved oxygen [29]. The latter was applied to rapid flow injection measurements of salicylate in pharmaceuticals [30]. Organic-phase tyrosinase and peroxidase electrodes have been used for the monitoring of phenolic and peroxide antiseptics in anti-infective pharmaceutical formulations [31]. Such organic-phase operation obviates the need for extensive sample manipulations and facilitates rapid assays of pharmaceutical products. Amperometric biosensors are also attractive for monitoring the levels of clinically-important enzymes. For example, the detection of α-amylase, which is important in the diagnosis of pancreatitis, can be accomplished in the presence of its oligosaccharide substrate and another enzyme (α-glucosidase) to yield a detectable aminophenol product [29].

2.5. Manufacturing considerations

In order to address the needs of decentralized clinical testing, it is necessary to move away from the use of conventional electrochemical protocols and cells (common in research laboratories). The exploitation of advanced microfabrication techniques allows the replacement of traditional ('beaker-type') electrochemical cells and bulky electrodes with easy-to-use sensor strips. These strips can be considered as disposable electrochemical cells onto which the sample droplet is placed. Appropriate fabrication technology is essential not only for simplifying clinical assays, but also for meeting the demand of large-scale production of reproducible and low cost biosensing devices.

One of the most promising routes for mass production of enzyme electrodes is to employ screen-printing (thick-film) technology. Such technology relies on printing patterns of the conductor or insulator onto planar substrates via placement of a proper ink on a patterned screen, followed by forcing it through the openings with the aid of a squeegee. Various commercial strips for self-monitoring of blood glucose rely on single-use printed carbon electrodes in connection with immobilized

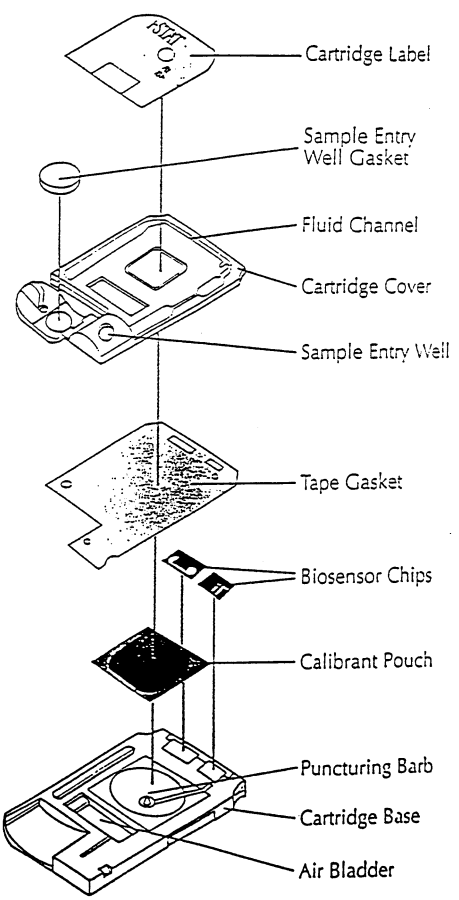

Fig. 3. Design of the disposable sensor-array cartridge used in the i-STAT portable clinical analyzer (courtesy of i-STAT).

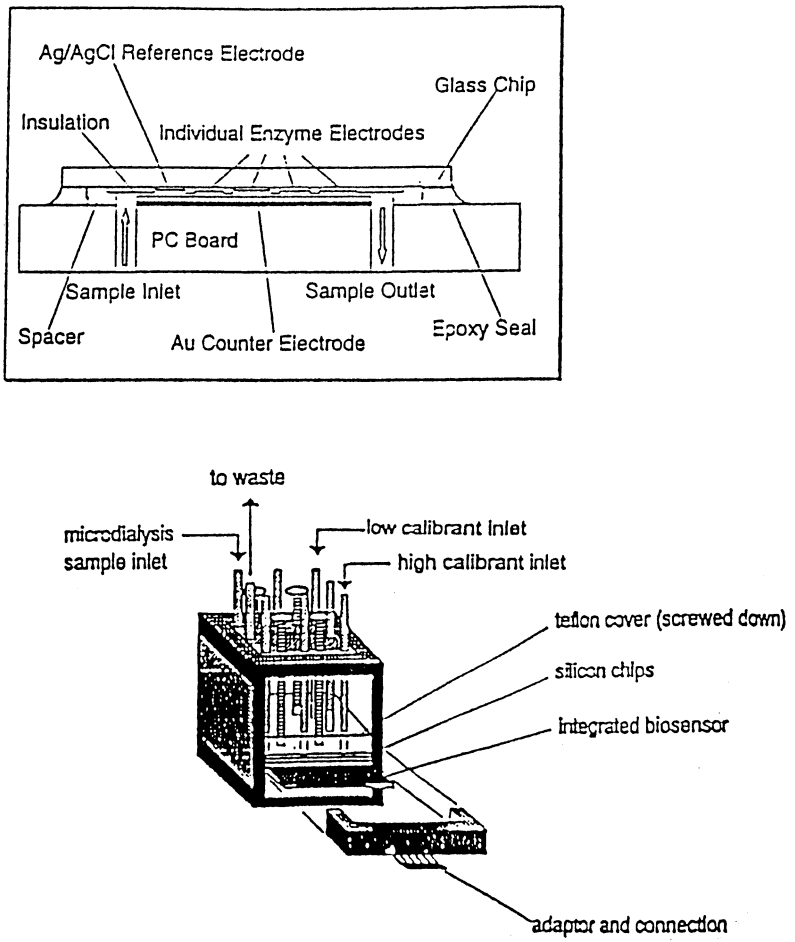

Fig. 4. Schematics of a microfabricated biosensor flow cell (top) and the integrated sampling-sensing micromachined system (bottom) for on-line monitoring of glucose and lactate (reproduced with permission from Dempsey et al. [35]).

glucose oxidase and a (ferrocene or ferrocyanide) mediator [32]. Similar strip electrodes have been reported for lactate [33] or creatinine [18], or salicylate [29], but have not reached the commercial stage.

Thin-film silicon technology represents another attractive route for the microfabrication of biosensors. Such fabrication technology couples various processes, used in the manufacture of electronic circuits, including film deposition, photolithographic patterning, and etching. It offers a greater resolution (down to submicrometer structures) at higher capital and manufacturing costs, and is thus particularly suitable for the production of sensor arrays. One successful example is the i-STAT portable (hand-held) clinical analyzer that simultaneously performs eight common clinical tests on a 60 ml patient blood sample in about 90 s ([34], Fig. 3). Upon completion of the assay, the results are displayed on a liquid-crystal screen, along with the patient identification number and time.

Thin-film biosensors can be readily integrated with miniaturized total analytical systems (μTAS), produced by silicon micromachining technology. Miniaturized analytical systems based on fluid-

handling silicon microstructures, offer an improved efficiency with respect to sample size, response time and reagent consumption. A recent example is the development of a μTAS system for on-line monitoring of lactate and glucose in biological systems, based on integrated microdialysis sampling and photolithographically-prepared lactate and glucose electrodes ([35], Fig. 4). Various approaches (e.g. ink-jet dispersion) have been developed for spatially controlling and automating the deposition of enzymes and membranes onto microfabricated strip electrodes, and hence for providing reproducible immobilization schemes.

3. Conclusions

Biosensors technology is a field in the quest for innovative approaches to analysis. While the concept of biosensors is simple, its commercial realization is far from simple. As a result, only a few of the innovative ideas described in this review have reached the marketplace. The majority of these deal with the self-testing of glucose. This market is unique in that it is large enough to encourage stand-alone products. The success of pocket-sized blood glucose monitors has stimulated tremendous interest in new devices (e.g. the i-STAT analyzer) offering a panel of blood tests at the patient's side or valuable real-time information on key metabolites or drugs. On-going fundamental studies on mediated and direct electron-transfer electrochemistry, on new sensing principles, and on enzyme stabilization, coupled to extensive commercial efforts, should have a tremendous impact on point-of-care clinical testing, and upon biomedicine, in general.

References

[1] A.P. Turner, I. Karube, G.S. Wilson (Eds.), Biosensors: Fundamentals and Applications, Oxford University Press, Oxford, 1987.
[2] R.F. Taylor, J.S. Schultz (Eds.), Handbook of Chemical and Biological Sensors, Institute of Physics Publishing, Bristol, 1996.
[3] J. Wang, Analytical Electrochemistry, VCH, New York, 1994.
[4] J. Wang, F. Lu, J. Am. Chem. Soc. 120 (1998) 1048.
[5] M. Lobo, A. Miranda, P. Tunon, Electroanalysis 9 (1997) 191.
[6] J. Frew, H.A. Hill, Anal. Chem. 59 (1987) 933A.
[7] T. Ohara, R. Rajogopalan, A. Heller, Anal. Chem. 66 (1994) 2451.
[8] I. Willner, V. Heleg-Shabtai, R. Blonder, E. Katz, G. Tao, J. Am. Chem. Soc. 118 (1996) 10321.
[9] I. Willner, E. Katz, B. Willner, Electroanalysis 9 (1997) 965.
[10] M. Collison, M.E. Meyerhoff, Anal. Chem. 62 (1990) 425A.
[11] P. Vadgama, M. Desai, P. Crump, Electroanalysis 3 (1991) 597.
[12] G. Reach, G.S. Wilson, Anal. Chem. 64 (1992) 381A.
[13] S. Jaffari, A.P. Turner, Physiol. Meas. 16 (1995) 1.
[14] Y. Zhang, G.S. Wilson, Anal. Chim. Acta 281 (1993) 513.
[15] M. Kyrolainen, H. Hakanson, R. Ekroth, B. Mattiasson, Anal. Chim. Acta 279 (1993) 149.
[16] C. Meyerhoff, F. Bischof, F. Mennel, F. Sternberg, J. Bican, E. Pfeiffer, Biosens. Bioelectron. 8 (1993) 409.
[17] D. Baker, D. Gough, Anal. Chem. 67 (1995) 1536.
[18] M. Madaras, I. Popesco, S. Ufer, R. Buck, Anal. Chim. Acta 319 (1996) 335.
[19] G. Khan, W. Wernet, Anal. Chim. Acta 351 (1997) 151.
[20] Y. Hahn, C.L. Olson, Anal. Chem. 51 (1979) 444.
[21] A. Crumbliss, J. Stonehuerner, R. Henkens, J. Zhao, J. O'Daly, Biosens. Bioelectron. 8 (1993) 331.
[22] J. Wang, M. Ozsoz, Electroanalysis 2 (1990) 647.
[23] P. Taylor, E. Kmetec, J. Johnson, Anal. Chem. 49 (1977) 789.
[24] V. Kacanickle, K. Johansson, G. MarkoVarga, L. Gorton, G. Jonsson-Petterson, E. Csoregi, Electroanalysis 6 (1994) 381.
[25] H. Bramwell, A. Cass, P. Gibbs, M. Green, Analyst 115 (1990) 185.
[26] A. Eremenko, A. Makower, C. Bauer, I. Kurochkin, F. Scheller, Electroanalysis 9 (1997) 288.
[27] J. Wang, E. Dempsey, M. Ozsos, M. Smyth, Analyst 116 (1991) 997.
[28] T. Moore, G. Nam, L. Pipes, L. Coury, Anal. Chem. 66 (1994) 3158.
[29] J. Frew, M. Green, Anal. Proc. 26 (1989) 334.
[30] M. Neumayr, G. Sontag, F. Pittner, Anal. Chim. Acta 305 (1995) 26.
[31] J. Wang, Y. Lin, L. Chen, Analyst 118 (1993) 277.
[32] B. Lewis, Clin. Chem. 38 (1992) 2093.
[33] S. Sprules, J. Hart, S. Wring, R. Pittson, Anal. Chim. Acta 304 (1995) 17.
[34] E. Jacobs, E. Vadasdi, L. Sarkozi, N. Colman, Clin. Chem. 39 (1993) 1069.
[35] E. Dempsey, D. Diamond, M. Smyth, et al., Anal. Chim. Acta 346 (1997) 341.