Pure & Appl. Chem., Vol. 63, No. 8, pp. 1147–1152, 1991.
Printed in Great Britain.

ADONIS 206922209100107C

Chemical sensors and biosensors: nearer the patient

Pankaj Vadgama, Mohamed A. Desai, Ian Christie and Zahra Koochaki

Department of Medicine (Clinical Biochemistry), The University of Manchester, Hope Hospital, Salford, M6 8HD, England

Abstract - Rapid biochemical testing can expedite therapy in key patient categories, particularly intensive care. Dry reagent chemistries have gone some way to satisfying some of these requirements. However, chemical and biosensors offer the prospects for more robust and operationally simpler near-patient analysers which, furthermore, are well suited to the assay of optically opaque biofluids, as well as the development of cheaper instrumentation. The wide range of transduction principles on offer with sensing technology not only enhances prospects for success in this area, but permits more precise tailoring of sensor performance to clinical requirements. Background considerations for extra-laboratory testing are reviewed here, together with discussion of key sensor properties and fabrication needs that are of relevance to measurement in these less controlled environments. An outline will then be given of the main transducer types, including ion-selective and amperometric electrodes, thermistors, piezoelectric crystals and optical wave guides. Use of biomolecules will centre on a description of enzyme and antibody incorporation into practical systems.

INTRODUCTION

The hospital biochemistry laboratories have grown from being an offshoot of pathology departments early this century, to being a self-contained, diagnostic resource in its own right. The practice of modern-day medicine requires a rapid return of high quality biochemical data, no longer merely as a luxury contributing to diagnostic precision, but as a virtually obligatory extension of the physical examination. There is added significance to biochemical data, since they provide a quantitative indication of patient status, especially when therapy requires careful titration, as is the case in critical care medicine. Though administratively efficient and organisationally cohesive, location of an entire analytical capability of a hospital on a single site can no longer be accepted as ideal with respect to efficient patient care (ref. 1). A key failing of centralisation is the relatively long turn around times for specimens requiring stat analysis, and which by their very nature are presented to the laboratory with a frequency and timing likely to disturb the untrammelled throughput of the "batch" specimens (ref. 2).

A logical response to current needs is a greater flexibility in specimen analysis, with the setting up of satellite laboratories, frequently dedicated to specific specialist units, e.g. neonatal and adult intensive care, renal dialysis, cardiothoracic surgery. Such laboratories have been characterised by smaller test repertoires serviced by small-scale analytical systems, sufficiently user-friendly to be operated, though not necessarily serviced by staff with rudimentary analytical training. Here, the development of automated blood gas analysers has been quickly followed by multi-ion (Ca^{2+}, Na^{+}, K^{+}, Li^{+}) and dry reagent analysers. Additional attractive features of many of these systems have been automatic trouble-shooting and computational facilities (ref. 3). Many of the operating principles here are a departure from previous 'wet chemistry' systems, and minimise the need for reagent addition, sample preparation and repeat calibration. It is these key attributes which can be more readily realised in chemical and biosensors, and which makes development of such devices for decentralised use particularly attractive. From the early days of the Clark oxygen electrode (ref. 4), experience of the special needs of clinical sensors is accumulating, and provides a pointer to both the prospects and pit-falls of future sensor use. Additional lessons are provided by the parallel and highly successful use of reflectance-based measurement, which has many of the functional attributes of sensors. Thus, successful commercial systems such as the Kodak Ektachem Analyser (Eastman Kodak, Rochester) and the Reflotron (Boehringer, Mannheim), illustrate the way in which reagent membrane layers, including those bearing metastable reagents, can be laminated, manufactured on a large scale, stored and then rapidly reconstituted during use to both form a highly reproducible assay matrix and an optical path for reflectance measurement. This does not, of course, preclude problems of operator error associated with specimen volume measurement, and the timing of a reaction. This possibility is supported by the finding of a generally unacceptable precision and accuracy during evaluation of glucose reflectance meters away from controlled laboratory conditions (ref. 5).

The sensor option

Chemical and biosensors have the potential to exploit a diversity of transduction principles for the type of reagent layer components used in reflectance analysers. This should pave the way towards greater analytical flexibility and targeting to precise clinical applications. A further attraction is that it is now increasingly feasible to adapt the fabrication and signal processing capabilities of the microelectronic and telecommunication industries to sensors whereby miniaturisation, manufacture, multiplexing and signal modification are now much more easily realised.

As constituted at present, analytical advantages of sensors reside in their direct transduction of a given biochemical parameter, often with an electrical output that helps to simplify the attendant instrumentation. Also, in contrast to standard spectrophotometric methods, the analysis of optically opaque samples, notably whole blood and tissue, comes within reach, clearly an advantage for near-patient monitoring. With many devices, the surface chemical or bio-reagent is used principally as a recognition molecule; not only can this permit highly economical reagent reuse, but a non-destructive assay is possible. Such aspects have contributed, for example, to the growing acceptance in clinical laboratories of ion-selective electrodes, the archetype non-destructive sensor. Implicit in this concept of molecular recognition is a reversible binding process which in turn enables response to be reversible. In practice, this may be difficult to achieve, especially when the kinetics of dissociation are too slow (e.g. with immunosensors). However, for many devices, response can be made readily reversible, usually showing no hysteresis. Such systems confer the unique property of enabling continuous monitoring of an analyte, permitting better management of the critically ill patient, and can take the form of transducer elements in artificial biofeedback systems (vide infra). For most clinical requirements, discrete analysis sensors, located in varying proximity to the patient, should prove satisfactory. Feeding into the overall equation in determining whether sensors will be used for home monitoring, at the bed-side, operating theatre, side ward or satellite laboratory, will be factors such as sensor robustness, ease of operation, availability, cost, prevailing medical ethics, and actual clinical value. A clear resolution of benefits is often difficult (ref. 6), at least until any given system has been in place for some time, leading merely to a retrospective assessment.

With growing efforts in industry, directed over the last decade to providing sensors for medical diagnostics, there has been increased understanding of special functional requirements and fabrication needs of near-patient sensor systems. Some of the basic ground-rules are suggested in Table 1. Manufacturer/purchaser requirements are indicated together here as they really form part of a single continuum of need that is compartmentalised at the risk of jeopardising both entities, and the ultimate successful deployment of sensors. A useful segregation, however, is to consider ex vivo devices, used for intensive monitoring, reusable sensors for bench-top analysers, and "dip-sticks" for hand-held meters separately. Table 1 shows some generalisations relating to each of these types and which may help in the choice of measurement principles, though it is necessary to recall that the analytical route will also be dictated by the measurand. It is this latter aspect that dominates current thinking in sensor research, and which will be highlighted in the succeeding section.

SENSOR SYSTEMS FOR SPECIFIC ANALYTES

Blood gases

The Clark electrode with its O_2-permeable membrane compartmentalising and stabilising the electrolytic environment of its integral electrochemical cell, has proved a virtually immutable sensing principle for oxygen (ref. 7). Modernisation has taken the course of more modern materials with, for example, exploitation of semi-conductor fabrication techniques rather than transformation of underlying chemistry and miniature, disposable sensors, economical of electrode components have resulted (ref. 8). The principle of membrane compartmentalised bicarbonate solution has provided the basis of a selective (Severinghaus) CO_2 electrode, ΔpCO_2 in blood here registering selectively as a ΔpH in the internal electrolyte (ref. 9). Miniaturisation of the glass pH membrane has been difficult, but with the advent of the ion-selective field effect transistor (ISFET) (ref. 10), an inherent solid state construction combining with the H^+ responsiveness of the insulator layers (SiO_2, Si_3N_4), miniature, robust devices are possible. In a new design in the Mallinckrodt GEM analyser (ref. 11), an outer CO_2-permeable membrane also combining the function of H^+ response is used to respond to the inner bicarbonate solution, external sample pH variation here being compensated for by a matching electrode containing well-buffered internal electrolyte.

For both O_2 and CO_2, the ideal of non-invasive continuous monitoring has been achieved with heated skin electrodes (ref. 12). The principle exploiter here is the enhanced transcutaneous flux of gases resulting from skin heating (~43°C). Although increased tissue metabolic rate can lead to underestimation of arterial pO_2, and an overestimate of pCO_2, valuable information on pulmonary gas exchange is, nevertheless, obtained on a real-time basis. Usage is largely restricted to critically ill neonates, where not only does the

TABLE 1. Manufacturer/purchaser requirements for clinical sensors

Ex vivo* monitoring	Multiple use electrode	Single use 'dip-stick' sensor
Low volume manufacture	Large scale manufacture needed	
Limited specialist market	Large clinical + over the counter market	
Built-in safety requirements needed	No direct patient hazard presented	
Unique complex chemistry/ technology acceptable	Generic preferably simple chemistry/ technology required	
Compromise on accuracy permitted	High level of accuracy and precision needed	
Reversible response required for continuous operation		Irreversible response acceptable
Pre-calibration permitted		Calibration-free operation required
Less stringent storage stability demanded		High storage stability needed for response stability
High operational stability preferred		Operational stability not relevant
Expensive components acceptable		Cheap fabrication demanded
Miniaturisation an advantage		Miniaturisation of disposable component vital
Fragile components acceptable		Robust solid state construction necessary
Slow reconstitution on hydration acceptable		Immediate reconstitution required
Flow dependance not critical		Stir-independent response required
Thermostatting possible		Low temperature dependence demanded
Sample dilution possible		Undiluted specimen presented
High biocompatibility requirement	Biocompatibility an advantage	Minimal biocompatibility requirement

*In vivo catheter devices are not considered here

relatively thin skin allow greater gas permeability for more accurate monitoring, but precipitous changes in blood gases warrant closer monitoring (ref. 13). This non-invasive technique carries the possibility of heat damage to skin, and, therefore, regular change to the monitoring site is necessitated (2-4 hourly for neonates, 4-8 hourly in adults).

Optical sensing principles have been exploited for ex vivo monitoring of blood gases. Here, an immobilised indicator dye, reacting reversibly with the measured parameter, is placed in an optical path configured with appropriate optical fibers. A fluorescent pH indicator dye has been used to follow internal electrolyte pH change in one type of commercial optical pCO_2 sensor (CDI 300, Cardiovascular Devices Inc., Irvine CA) (ref. 14); in an alternative approach, an absorbance dye indicator has been employed (Cardiomet 4000, Shiley Inc., Irvine CA) (ref. 15). For pO_2, concentration dependent oxygen quenching of dye fluorescence has proved to be applicable (ref. 14), though dye photobleaching remains a problem.

Ions

Arterial pH has been a counterpart of respiratory gas measurement from the early days of monitoring. Most advances here have been those of sample presentation and flow-cell design, with associated modifications to reference/glass electrode geometry (ref. 16), directed to minimising maintenance requirements. The pH ISFET has been one important advance, furnishing a low impedance signal from a high impedance input (ref. 10); alternatives to the glass pH membrane have included liquid membranes incorporating protein ionophore (ref. 17).

Ion measurement using potentiometric electrochemical cells has seen wide application in the central laboratory, now extended to small bench-top analysers and through to bedside systems. Selectivity and sensitivity limitations of current ion-selective membranes has meant a restriction in the measurement range of Na^+, K^+, Ca^{2+}, Cl^- and Li^+. The validity of correlations with spectrophotometric methods which measure ion concentration rather than activity have led to much debate and confusion in the past. However, recent recommendation by one authority simply to standardise electrodes (Na^+, K^+) against flame photometry (ref. 18) is a constructive response to practical needs. Technological advances in electrode design are well demonstrated by the flat film format adapted in the Kodak and Chem Pro (Johnson and Johnson, Roseville, MN) disposable single use electrodes.

Metabolites

Enzymes have proved invaluable as selective substrate-degrading reagents for metabolite assay in Clinical Chemistry. Their incorporation as active layers in transducer elements has been both conceptually attractive and now practically realisable. The amperometric detection of hydrogen peroxide generated from oxidase enzymes, typically using a noble metal polarised at +0.6V (vs Ag/AgCl) has permitted assay of many metabolites of clinical interest, in particular, blood glucose and lactate. The Yellow Springs glucose electrode (YSI Inc., Ohio) has become a model for many other commercial instruments. Membrane technology was exploited here, whereby an external microporous membrane prevented contamination of the immobilised enzyme layer by sample colloids, with a subjacent permselective barrier reducing interference from diffusible electrochemically active species in blood. Need for sample dilution restricted use of this analyser to the laboratory environment. Extension of the linear operating range of such an enzyme electrode using external substrate limiting barrier membranes (refs. 19, 20) has made it possible to analyse undiluted whole blood samples; this principle has been used in the NOVA Stat-Profile glucose electrode (ref. 21). A single use electrode, now marketed for home glucose monitoring, employs an electrochemically recyclable electron mediator to substitute for O_2 in the glucose oxidase reaction (Exact Tech, Medi Sense, Cambridge MA); this also permits assay of undiluted blood.

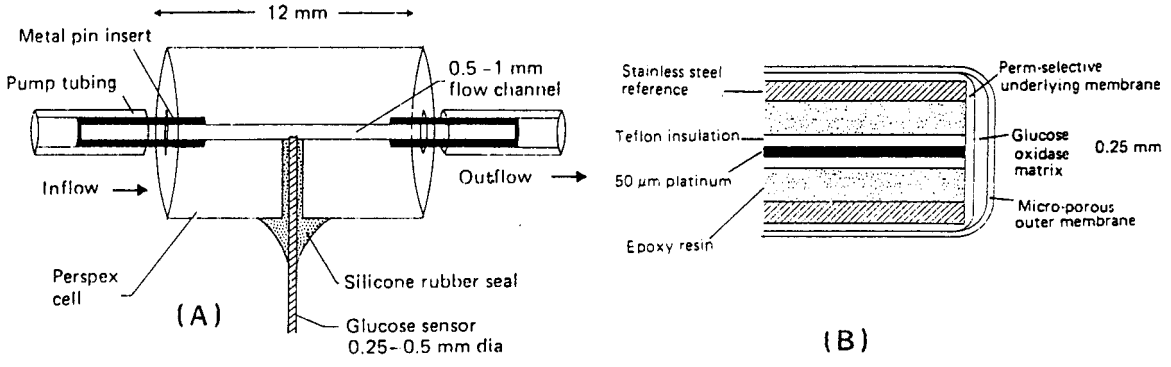

Fig. 1. (A) Needle electrode in flow cell. (B) Basic needle electrode construction for enzyme electrode. (From ref. 23 with permission).

Continuous, extracorporeal monitoring of glucose has been possible with the Biostator (Miles Lab., Elkhart) which houses a Yellow Springs-type membrane electrode in a low volume flow cell designed to take diluted blood (ref. 22). More recently, such an electrode has been used in a system for monitoring haemofiltered blood (Betalike, Esacontrol SpA, Genova). Both monitors sample venous blood via a double lumen catheter designed to achieve localised rather than systemic anticoagulation. Their additional clinical benefit resides in there being closed-loop systems for the computer controlled administration of insulin. Although needle enzyme electrodes are designed for invasive monitoring (ref. 23), they are usable in miniature flow cells (Fig. 1) and could help to reduce the bulk size of such extracorporeal systems. Non-invasive glucose monitoring was reported by Ito et al (ref. 24) who sampled effusion fluid from skin. After prior removal of the impermeable stratum corneum; negative pressure was employed to promote fluid transfer. The electrode used was an oxidase coated ISFET responding to local pH change resulting from the enzymic reaction. The drawback of such enzyme field effect transistors (ENFETs) is their strong dependence on sample buffer capacity and pH. Nevertheless, the integration of a biolayer with microelectronics is attractive, and pH sensing, furthermore, constitutes a near-universal detection for an enzymic reaction. Olthuis et al (ref. 25) have accordingly combined an activator system with the pH ISFET (Fig. 2). This is designed to maintain local pH constant, by coulometric generation of OH^- (or H^+), the magnitude of the coulometric current for neutralisation providing a measure of enzymic H^+ generation and, therefore, substrate level, without reference to buffer capacity. Another near-universal transducer element is the thermistor; its use in conjunction with immobilised enzyme reactor columns has enabled reagentless, though distinctive, substrate measurement by means of the detection of the heat of reaction (ref. 26). While only small temperature changes result, this approach holds promise for more robust, low-drift bedside monitors.

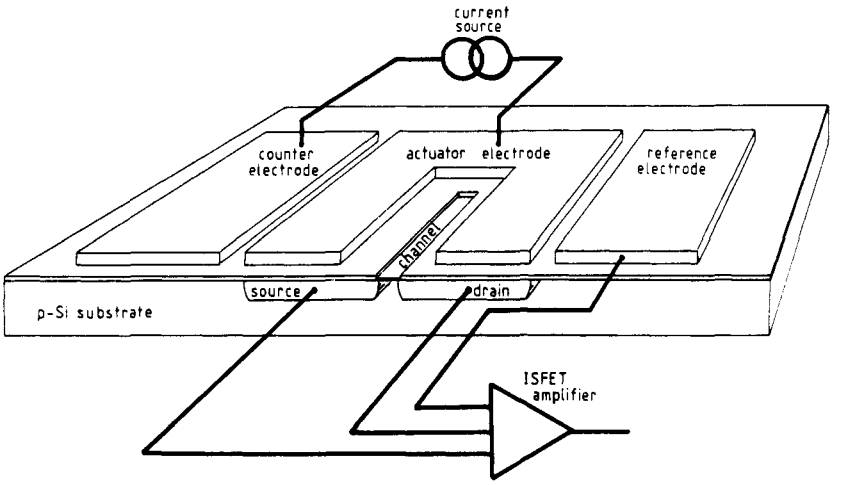

Fig. 2. ISFET based sensor-actuator OH^- generated at the actuator electrode neutralises H^+ from an enzymic reaction (not shown); feedback provided by the ISFET. (From ref. 24 with permission).

The spectral selectivity of optical sensors will undoubtedly be exploited in future bedside biochemical analysers. This area of development is at an earlier stage (ref. 27), but recent reports are promising. Lactate dehydrogenase immobilised at the tip of a bifurcated optical fiber, for example, has been used for lactate/pyruvate based on the measured fluorescence intensity of NADH (ref. 28). Luminescence has also been exploited, where immobilised firefly luciferase has enabled ATP detection and an NADH:FMN oxidoreductase and luciferase combination has permitted NADH to be measured (ref. 29). Such biosensors (Fig. 3) could form the basic detector elements for a family of optical sensors employed for the assay of kinase and dehydrogenase substrates.

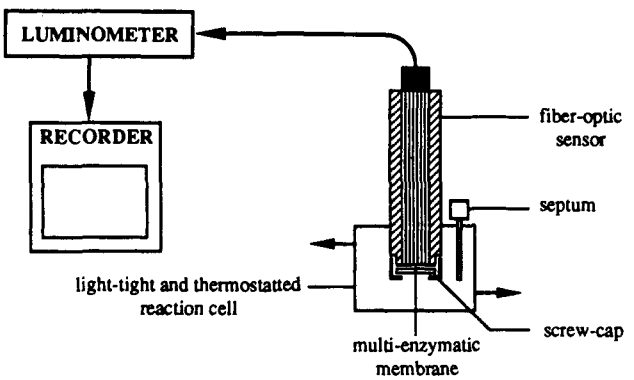

Fig. 3.
Bioluminescence fiberoptic biosensor; sample injection through septum.
(From ref. 29 with permission).

Antigenic species

An antigen at low concentration can be recognised by a sufficiently specific, high affinity antibody. Immunosensors have been designed to utilise this recognition property of antibodies for the assay of drugs and protein molecules. In one general type of device, the direct immunosensor, the binding event itself is monitored. Thus, with an antibody immobilised to the surface of a potentiometric sensor, interfacial charge effects lead to electrode potential changes (ref. 30); over a piezoelectric crystal a change in vibration frequency of the crystal due to weight change from the antigen mass bound may be monitored (ref. 31). While operationally highly convenient, the susceptibility of these probes to simple background solution variables, and the inadequate sensitivity of the underlying transducer, still consigns them to the experimental bench. This situation may change with improvements in signal accessing and the multiplexing of devices possessing a spectrum of response characteristics. More realistic has been the direct antibody coating of optical wave guides; here the evanescent wave, the electric vector of the light which extends into the surface antibody phase, can be used to interrogate any surface-bound antigen (analyte) provided it contains a suitable chromophore (ref. 32). In the second general type of transducer, the indirect immunosensor, the transducer element is used to detect a change in the property of the labelled antigen following the immunoreaction, and if required, after separation of free from bound antigen. Assay here is along the lines of more conventional immunoassay and is more properly regarded as being in the genre of non-isotopic immunoassay. Aizawa's group (ref. 33) measured α-fetoprotein (AFP) by immobilisation of the appropriate antibody on an O_2 electrode. Competition between catalase labelled and unlabelled AFP led to variable catalase loading of the electrode,

which was then determined from the electrode response to H_2O_2; this typifies one popular approach with electrochemical detection. Antibodies immobilised on magnetic particles (ref. 34) have also been used. Here concentration of the antibody enzyme-labelled antigen complex in the vicinity of the electrode surface can be achieved by applying a magnetic field following immunoreaction in bulk solution. Enzyme retained within antigen-coated liposomes have allowed sensitive immunoassay; rupture of the coated liposome in the presence of complement and appropriate antibody serves as a reporter system for the amount of free antigen in solution competing for the antibody; theophylline has been assayed in this way (ref. 35). Hitherto, considerable ingenuity has been shown by basic researchers in developing immunosensor strategies; it is now likely that with equivalent advances in the formatting of such devices with a simplified sample presentation and automation, simple commercial near-patient immunosensors will result.

CONCLUSIONS

From the earlier phase of delineation and optimisation of basic chemical/biosensor chemistries, we have now moved into an era of practical, multidisciplinary research. This has begun to critically address interfacing and biocompatibility issues (ref. 36), in alliance with materials scientists, and the rationalisation of fabrication and formatting in conjunction with instrument designers. As a result, the transfer of biosensor devices from the academic to the industrial sector has begun. Sensors are, thereby, set to follow the development trends exemplified by dry reagent chemistries. Undoubtedly, there are deficiencies with many individual sensor systems with regard to achieving exciting analytical targets in the clinical environment. However, even for systems with severe limitations, advances in electronic signal processing and associated development of the smart sensor (ref. 37) will bring about practical application.

Acknowledgements

IC and ZK acknowledge generous support from SERC and ICI plc during preparation of this manuscript.

REFERENCES

1. V. Marks, K.G.M.M. Alberti eds Clinical Biochemistry Nearer the Patient, Edinburgh, Churchill Livingstone (1985).
2. M. Fleisher and M.K. Schwartz, Clin. Chem. 36, 1557-1561 (1990).
3. P. Vadgama, Anal. Proc. 25, 274-276 (1988).
4. J.W. Severinghaus, P.B. Astrup, Int. Anesthesiol. Clin. 25, 1-224 (1987).
5. D.S. North, J.F. Steiner, K.M. Woodhouse and J.A. Maddy, Diabetes Care 10, 360-366 (1987).
6. W.J. Sibbald, M. Escat and J.E. Calvin, Clin. Chem. 36, 1604 (1990).
7. L.C. Clark, US Patent 2,913,386,1959.
8. H. Suzuki, E. Tamiya and I. Karube, Anal. Chem. 60, 1078-1080 (1988).
9. J.W. Severinghaus, A.F. Bradley, J. Appl. Physiol. 13, 515-520 (1958).
10. H. Wöltjen, Anal. Chem. 56, 87A (1984).
11. R.A. Strickland, T.R. Hill and G.P. Zaloga, J. Clin. Anaesth. 1, 248-250 (1989).
12. D.W. Lübbers, Crit. Care. Med. 9, 721-723 (1981).
13. G. Cassady, J. Pediatr. 103, 837-848 (1983).
14. J.A. Pino, G. Bashin and M.A. Kenny, J. Clin. Monit. 4, 186-194 (1988).
15. D.J. Claremont and T.M. Pagdin, J. Med. Eng. Technol. 9, 174-179 (1985).
16. P. Rubin, S. Bradbury and K. Prowse, Br. Med. J. 1, 156-158 (1979).
17. S.M. Cobbe and P.A. Poole-Wilson, Br. Heart J. 47, 369-374 (1982).
18. M.F. Burritt, K.D. Fallon, G. Graham et al, NCCLS Proposed Standard C29-P Villanova PA, NCCLS (1989).
19. W.H. Mullen, F.H. Keedy, S.J. Churchouse and P. Vadgama, Anal. Chim. Acta 183, 59-66 (1986).
20. G.S. Calabrese, K.M. O'Connell, Topics Current Chem. 143, 51-58 (1988).
21. M. Fleisher, M. Gladstone, D. Crystal and M.K. Schwartz, Clin. Chem. 35, 1532-1535 (1989).
22. E.J. Fogt, L.M. Dodd, E.M. Jennings et al, Clin. Chem. 24, 1366-1372 (1978).
23. S.J. Churchouse, C. Battersby, W. H. Mullen and P. Vadgama, Biosensors 2, 325-342 (1986).
24. N. Ito, A. Saito, S. Miyamoto et al, Sensors and Actuators B1, 488-490 (1990).
25. W. Olthuis, J. Luo, B.H. van der Schoot et al, Sensors and Actuators B1, 416-420 (1990).
26. B. Danielsson and K. Mosbach, Methods Enzymol. 137, 181-187 (1988).
27. M.A. Arnold, GBF Monographs 10, 223 (1987).
28. J. Wangsa and M.A. Arnold, Anal. Chem. 60, 1080-1082 (1988).
29. S.M. Gautier, L.J. Blum and P.R. Coulet, Sensors and Actuators B1, 580-584 (1990).
30. N. Yamamoto, Y. Nagasawa, S. Shuto et al, Clin. Chem. 26, 1569-1572 (1980).
31. J.E. Roederer and G.T. Bastiaans, Anal. Chem. 55, 2333 (1983).
32. T. Vodinh, B.J. Tromberg, G.D. Griffin et al, Appl. Spectrosc. 41, 735-738 (1987).
33. M. Aizawa, A. Morioka and S. Suzuki, Anal. Chim. Acta 115, 61 (1980).
34. H.H. Weetall and T. Hotaling, Biosensors 3, 57-63 (1987).
35. Haga M, Sugawara S and H. Itagaki, Anal Biochem 118, 286-293 (1981).
36. P. Vadgama, Sensors and Actuators B1, 1-7 (1990).
37. W. Sansen, D. De Wachter, M. Callewaert et al, Sensors and Actuators B1, 298-302 (1990).