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Review Study On Electrochemical-Based Biosensors

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Abstract
Biosensors based on electrochemical sensors for multiple analytes were the first scientifically proposed as well as successfully commercialized biosensors.A long time study has been done for electrochemical biosensors.A typical platform for the construction of biosensors is represented by transducers based on semiconductors and screen printed electrodes.The most common biorecognition components of biosensors are enzymes or enzyme labeled antibodies.In this review, the description of the principles of and the most typical applications for electrochemical biosensors is done. Potentiometric, amperometric and conductimetric are the three types of relevant systems according to the operating principles governing their method of the measurement and for each group some representatives devices are described. In the text, some of the most typical essays are also mentioned.

Keywords – enzymes electrode-immunosensor-potentiometric-amperometric-impedimetric transducer

I. Introduction

Electrochemical biosensor have been the subject of basic as well as applied research for nearly fifty years[1]. It has been stated that more than half of the biosensors reported in the literature are based on electrochemical transducers. A recent survey of the literature has revealed that the electrochemical-based sensor platform is the most common and in many cases the most frequently cited in the literature [2, 3, 4, 5]. A review by Stefan *et al.* (2000) has

revealed that electrochemical immunosensors are gradually increasing in popularity in clinical analysis and this is partly due to improved sensor design [6]. Similarly, Warsinke *et al.* (2000) demonstrated that the electrochemical immunosensor is a promising alternative compared to existing laboratory-based immunochemical assays [7]. Wang (2002) suggests in his review of nucleic acid biosensors that the electrochemical-based device will be responsible for achieving future large-scale genetic testing [8]. This may not be surprising considering that electrochemical transduction spossesses the following advantages: low cost; high sensitivity; independence from solution turbidity; easily miniaturized / well suited to microfabrication; low power requirements; and relatively simple instrumentation [9, 7]. These characteristics make electrochemical transduction methods highly compatible for implantable and/or portable hand-held devices. In general, there are several approaches that can be used to detect electrochemical changes during a biorecognition event and these can be classified as follows: amperometric; potentiometric; impedance; and conductometric.

1.1 Amperometric

In the amperometric approach, the signal transduction process is accomplished by controlling the potential of the working electrode (i.e., usually an inert metal) at a fixed value relative to a reference electrode (usually silver / silver chloride), and monitoring the current as a function of time. The applied potential serves as the driving force for the electron transfer reaction, and the current produced is a direct measure of the rate of electron transfer. Amperometric biosensors take advantage of the fact that certain molecules can be oxidised or reduced at the working electrode (i.e., gold, carbon, platinum, etc). If the working electrode is driven to a positive potential an oxidation reaction occurs, and the current flow depends on the concentration of the electroactive species (analyte) diffusing to the surface of the working electrode. Similarly, if the working electrode is driven to a negative potential then a reduction reaction occurs. A third electrode called the counter (or auxiliary) electrode is often used to help measure the current flow. In most cases the bioreceptor molecule is immobilized on the working electrode, and as the analyte diffuses to the electrode surface the current generated reflects the rctionea occurring between the bioreceptor molecule and analyte. A recent review by Habermuller et al. (2000) discusses various electron transfer mechanisms [10]. It is important to note that at least 150 articles have been published over the past decade on improving the electron transfer mechanism in amperometric biosensors [10].

The amperometric sensor for glucose is the most studied of all biosensors, noting that it employs an enzyme (glucose oxidase) to catalyse the conversion of glucose to gluconic acid [3, 11,12]. Similarly, the amperometric approach has become widely used for the detection of nucleic acid and antigens for disease identification / diagnosis [7, 15, 14 15]. In fact, amperometric transduction is the most suitable and common electrochemical detection method in immunosensors [6] Another important application of the amperometric biosensor has been in environmental monitoring of pesticides [16]. These biosensors are highly sensitive, rapid and inexpensive [17]. In addition, they display a high degree of reproducibility, which removes the need for repeated calibration [18]. A possible limitation with amperometric transduction is the interferences that arise from electroactive compounds / species, and this can sometimes generate a false current reading [18]. However, these problems have been largely eliminated by the use of electrodes coated with various polymers [19,20].

1.2. Potentiometric

In this method the analytical information is obtained by converting the biorecognition process into a potential signal. A permselective ion-conductive membrane is normally used to measure the potential signal, which occurs when the analyte molecule interacts with the surface. A high impedance voltmeter is used to measure the electrical potential difference or electromotive force (EMF) between two electrodes as shown in Figure 1, noting that potential measurements are made at near zero current. One of the electrodes develops a change in potential as a function of analyte activity or concentration in solution and this electrode is known as the indicator electrode or sometimes called an ion-selective electrode (ISE). The potential response of an ISE is described by the Nernst equation (i.e., the potential is proportional to the logarithm of the concentration of the substance being measured). The second electrode is the reference and is used to complete the electrochemical cell by providing a constant half-cell potential, which is independent of the analyte concentration. ISEs are chemical sensors with the longest history and with the largest number of applications [4, 5, 21]. In fact, billions of measurements are performed each year in nearly every hospital all over the world [21]. This comes as no surprise considering that these devices are well known for providing direct, rapid, maintenance-free and non-expensive measurements [18, 21].

Most of the work that is reported in the literature on the potentiometric sensor for antigen and DNA detection has employed the indirect approach. This involves measuring a change in either the pH or changes in the ionic concentration of an elemental species, which occur during a biorecognition event. A common strategy that has been employed is the use of enzymes to catalyse the consumption or production of protons and/or charged elemental species [18]. Uithoven et al. (2000) demonstrated that this detection platform can rapidly (<15 mins) monitor biological warfare (BW) agents using an enzyme-immunoassay approach [22]. In this process, a BW agent forms an immunocomplex with both a fluorescein-labelled antibody and a biotin-streptavidin- labelled antibody [22]. The fluorescein-labelled immunocomplex undergoes a further complexation reaction with an anti-fluorescein urease conjugated antibody and the enzymatic breakdown of urea causes a change in pH, which is detected potentiometrically [22]. More importantly, the biosensor employs an eight-channel instrument, and has been designed to assay up to eight BW agents simultaneously [22]. A similar but much simpler approach developed recently by Purvis et al. (2003) involves the formation of an enzyme labeled immuno-complex at the surface of a polypyrrole-coated gold electrode [23]. Detection is achieved by a secondary reaction that produces charged products (i.e., changes in the redox state, pH and/or ionic strength), and the potential shift is measured at the sensor surface [23].

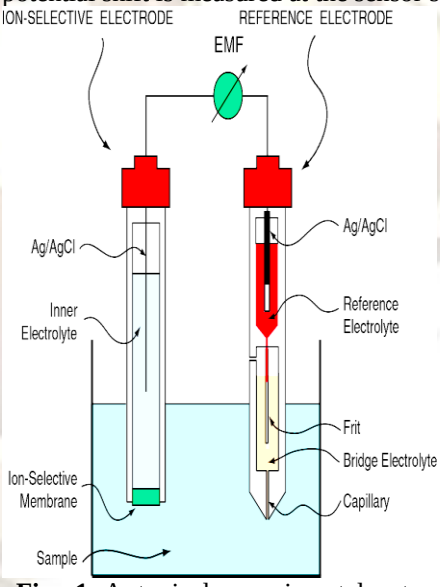

Fig. 1 A typical experimental set-up using potentiometric-based sensing.

Some reports suggest that potentiometric transducers cannot provide the required sensitivity for the detection of antibody-antigen reactions [6]. However, a light addressable potentiometric sensor (LAPS) based on field effect transistor (FET) technology has proved to be highly successful for immunoassay of various pathogens. A LAPS device consists of n-type silicon doped with

phosphorus and an insulating layer. The FET is used to detect changes in the potential at the silicon-insulator surface [17,24]. A LAPS measures an alternating photocurrent generated when a light source, such as a light emitting diode (LED), flashes rapidly. Lee et al. (2000) developed a LAPS biosensor to detect Newcastle disease virus and report a detection limit of ~2 ng/ml [25]. Likewise, Ercole and coworkers (2003) used LAPS for the detection of Escherichia coli in various foods [26]. It is reported that Escherichia coli can be detected down to 10 cells/ml, which represents a significant improvement in sensitivity compared to conventional methods [26]. More recently, LAPS was used to identify the virus Venezuelan equine encephalitis [27]. In this study, an immunofiltration enzyme assay was used in conjunction with the LAPS device, and a limit of detection of~30ng/ml was achieved[27].

1.3. Electrical Impedance Spectroscopy

Electrochemical impedance spectroscopy (EIS) is a method that has only recently become a popular tool for bioreceptor transduction [31,32]. A review by Katz and Willner (2003), which has cited almost 200 references, suggests that this technique has played an important role in biosensor development over the past decade and will continue to play a significant role in the future [33]. Impedance spectroscopy has been widely used by many research groups to detect DNA hybridisation [34, 42,35], antibody- antigen reactions [36, 37], and enzyme reactions [32, 33, 38]. In EIS measurements, a controlled AC electrical stimulus of between 5-10 mV is applied over a range of frequencies, and this causes a current to flow through the biosensor, which depends on various processes. During a biorecognition event the interfacial characteristics (i.e., capacitance and resistance) of the biosensor change, and the application of a voltage perturbation allows the interfacial capacitance and resistance to be evaluated. Typically, a conventional three-electrode system (i.e., counter, reference and the working electrode) is used to monitor the current variations, noting that a potentiostat / galvanostat and a lock-in-amplifier or a frequency response analyzer (FRA) are used in the detection process. The role of the lock-in-amplifier or FRA is to supply the excitation waveform over a range of frequencies and measure the AC current and voltage waveforms. A potentiostat is normally incorporated into to provide high input impedance, and is also used when precise control of the electrode potential is required. An important feature of EIS is that it is able to provide 'reagentless' or 'label-free' sensing, which makes it highly attractive for real-time monitoring.

1.4. Conductometric

Conductometric-based biosensors harness the relationship between conductance and a biorecognition event. Most reactions involve a change in the ionic species concentration and this can lead to a change in the solution electrical conductivity or current flow [18]. Essentially, a conductometric biosensor consists of two metal electrodes (usually platinum or silver) separated by a certain distance. Normally an AC (alternating current) voltage is applied across the electrodes, which causes a current flow to be sustained between them. During a biorecognition event the ionic composition changes and an Ohmmeter (or multimeter) is used to measure the change in conductance between the metal electrodes. Some recent studies have shown that this technique is capable of rapidly detecting (<10 mins) various food borne pathogens (i.e., Escherichia coli O157:H7, Salmonella) [28,29]. Alocilja and coworkers used a conductive polyaniline label in the sandwich immunoassay scheme, which significantly improved the sensitivity via the formation of a conductive molecular bridge between the two electrodes [30, 29]. Unfortunately, one of the major issues with this technique is that the sensitivity is generally inferior compared to other electrochemical methods [18].

1.5. Electrode Materials

Gold and carbon are the most common materials used to carry current / charge during an electrochemical event . However, the development of nanomaterials as electrodes for electrochemical-based detectors represents an exciting area of research. The ability of carbon-nanotube modified electrodes to promote electron-transfer reactions of important biomolecules has been recently repo reported by Wang and coworkers [40,41]. Carbon nanotubes represent a new class of materials, which are composed of graphitic carbon with one or several concentric tubules, and have shown promising results in DNA- and enzyme-based biosensors [42, 40,41]. Likewise, gold and alumina nanotubules have been exploited as membranes for biomimetic ion channels and sensing applications [43, 44]. Consequently, the development of nanomaterial sensors has the potential to revolutionise the bioanalytical, biomedical and pharmaceutical fields [44].

There has also been considerable interest in the development of biosensors that use conductive polymers (e.g. polyaniline, polypyrrole) as an electrochemical transducer [23, 30, 45, 46]. The conductive polymer is usually prepared by electropolymerisation of the monomer onto a metal surface such as gold. The growth in the use of conductive polymers has primarily been stimulated by improved response characteristics such as increased sensitivity, stability, and reproducibility. The response

characteristics (i.e., sensitivity, stability, reproducibility, etc) of polymer-based sensors are greatly dependent on the mode of polymerization, the monomer concentration, and the counterions used during polymerization [23]. By varying these parameters, it allows the surface properties of the biosensor to be modified, and this feature can be used to optimise the transduction signal

Screen printed electrodes have also attracted a great deal of attention recently [47, 48, 49, 23, 52, 146, 50]. This technology, as shown in Figure 2, is a particularly attractive procedure for the mass production of disposable electrodes [51]. It is well known that "memory effects" and "membrane fouling", which are sometimes observed with electrochemical-based biosensors can be alleviated when using disposable sensors. Disposable biosensors prepared by screen-printing technology are characterised by high reproducibility, low cost and require no calibration. More importantly, this technology has been widely used as a platform in DNA-, immuno and enzyme-based biosensors [46, 56, 69,70, 88, 105, 110, 111].

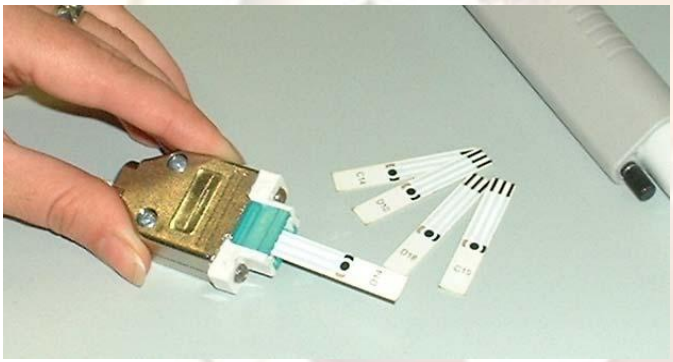

Fig. 2 Disposable screen-printed electrodes

1.6. Microelectrodes

Microelectrodes represent a major area of biosensor research and development [54]. The use of microelectrodes offers many advantages such as imparting stir independent response characteristics, lower limits of detection, and increased sensitivity [19, 55]. These advantages make microelectrodes very attractive for in vivo biosensor studies. Recently, Higson et al. (2004) developed a novel sonochemical approach in the fabrication of a microelectrode array enzyme-based glucose biosensor [56]. When a polymer-modified electrode is sonochemically ablated it exposes localized areas on the electrode surface, which act as individual microelectrodes and collectively as a microelectrode array [56]. It was shown that this approach generates a biosensor with significantly improved response characteristics [56].

2. Conclusion

Electrochemical biosensors have existed for nearly fifty years and seem to passess great potential for the future. This technology gains pratical usefulness from a combination of selective biochemical recognition with the high sensitivity of electrochemical detection. Thanks to current technological progress, such biosensors project from miniaturized electrochemical instrumentation and are thus very advantageous for some sophisticated applications requiring portability, rapid measurement and use with a small volume of samples. Numerous commercial applications confirm the attractive advantages of electrochemical biosensors

Refrence

[1] Meadows, D., Recent developments with biosensing technology and applications in the pharmaceutical industry, *Advanced Drug Delivery Reviews, 1996,* ***21****(3),* 179-189.

[2] Tothill, I.E., Biosensors developments and potential applications in the agricultural diagnosis sector, *Computers & Electronics in Agriculture, 2001,* ***30***, 205-218.

[3] D'Orazio, P., Biosensors in clinical chemistry, *Clinica Chimica Acta, 2003,* ***334***, 41-69.

[4] Bakker, E. & Telting-Diaz, M., Electrochemical sensors, *Analytical Chemistry, 2002,* ***74****(12),* 2781-2800.

[5] Bakker, E., Electrochemical sensors, *Analytical Chemistry, 2004,* ***76****(12),* 3285-3298.

[6] Stefan, R.I., van Staden, J.F. & Aboul-Enein, H.Y., Immunosensors in clinical analysis, Fresenius *Journal of Analytical Chemistry, 2000,* ***366***, 659-668.

[7] Warsinke, A., Benkert, A. & Scheller, F.W., Electrochemical immunoassays, *Fresenius Journal of Analytical Chemistry, 2000,* ***366***, 622-634.

[8] Wang, J., Electrochemical nucleic acid biosensors, *Analytica Chimica Acta, 2002,* **469**, 63-71.

[9] Gooding, J.J., Electrochemical DNA

hybridization biosensors, Electroanalysis, *2002*, ***14****(17)*, 1149-1156.

[10] Habermuller, K., Mosbach, M. & Schuhmann, W., Electron-transfer mechanisms in amperometric biosensors, *Fresenius Journal of Analytical Chemistry, 2000*, ***366***, 560-568.

[11] Atanasov, P. & Wilkins, E., In vivo rechargeable glucose biosensors, *Sensors & Actuators B Chemical, 1996*, ***35-36***, 435-447.

[12] Hrapovic, S. & Luong, J.H.T., Picoamperometric detection of glucose at ultrasmall platinum-based biosensors: preparation and characterization, *analytical Chemistry, 2003*, ***75****(14)*, 3308-3315.

[13] Babkina, S.S., Ulakhovich, N.A. & Zyavkina, Y.I., Amperometric DNA biosensor for the determination of auto-antibodies using DNA interaction with Pt(II) complex, *Analytica Chimica Acta, 2004*, ***502***, 23-30.

[14] Wang, J., Ibanez, A., Chatrathi, M.P. & Escarpa, A., Electrochemical enzyme immunoassays on microchip platforms, *Analytical Chemistry, 2001*, ***73****(21)*, 5323-5327.

[15] Ivnitski, D., Sitdikov, R. & Ivnitski, N., Non-invasive electrochemical hand-held biosensor as diagnostic indicator of dental diseases, Electrochemistry Communications, 2003, **5**, 225- 229.

[16] Palchetti, I., Cagnini, A., Del Carlo, M., Coppi, C., Mascini, M. & Turner, A.P.F., Determination of anticholinesterase pesticides in real samples using a disposable biosensor, *Analytica Chimica Acta, 1997*, ***337***, 315-321.

[17] Ivnitski, D., Abdel-Hamid, I., Atanasov, P. & Wilkins, E., Biosensors for detection of pathogenic bacteria, *Biosensors & Bioelectronics, 1999*, ***14***, 599-624.

[18] Rogers, K.R. & Mascini, M., Biosensors for field analytical monitoring, *Field Analytical Chemistry & Technology, 1998*, ***2****(6)*, 317-331.

[19] Wang, J., *Analytical Electrochemistry, 2nd ed., 2000*, New York, Wiley-VCH.

[20] Wisniewski, N. & Reichert, M., Methods for reducing biosensor membrane biofouling, Colloids & Surfaces B: *Biointerfaces, 2000*, ***18***, 197-219.

[21] Bakker, E., Diamond, D., Lewenstam, A. & Pretsch, E., Ion sensors: current limits and new trends, *Analytica Chimica Acta, 1999*, ***393****(1-3)*, 11-18.

[22] Uithoven, K.A., Schmidt, J.C. & Ballman, M.E., Rapid identification of biological warfare agents using an instrument employing a light addressable potentiometric sensor and a flow-through immunofiltration-enzyme assay system, *Biosensors & Bioelectronics, 2000*, ***14***, 761-770.

[23] Purvis, D., Leonardova, O., Farmakovsky, D. & Cherkasov, V., An ultrasensitive and stable potentiometric immunosensor, *Biosensors & Bioelectronics, 2003*, ***18****(11)*, 1385-1390.

[24] Cattrall, R.W., *Chemical Sensors*, 1997, Oxford University Press, New York.

[25] Lee, W.E., Thompson, G.H., Hall, J.G. & Bader, D.E., Rapid detection and identification of biological and chemical agents by immunoassay, gene probe assay and enzyme inhibition using a silicon-based biosensor, *Biosensors & Bioelectronics, 2000*, ***14***, 795-804.

[26] Ercole, C., Del Gallo, M., Mosiello, L., Baccella, S. & Lepidi, A., Escherichia coli detection in vegetable food by a potentiometric biosensor, *Sensors & Actuators B Chemical, 2003*, ***91***, 163-168.

[27] Hu, W.G., Thompson, G.H., Alvi, A.Z., Nagata, L.P., Suresh, M.R. & Fulton, E.R., Development of immunofiltration assay by light addressable potentiometric sensor with genetically biotinylated recombinant antibody for rapid identification of Venezuelan equine encephalitis virus, *Journal of Immunological Methods, 2004*, ***289****(1-2)*, 27-35.

[28] Louie, A.S., Marenchic, I.G. & Whelan, R.H., A fieldable modular biosensor for use in detection of foodborne pathogens, *Field Analytical Chemistry & Technology, 1998*, ***2****(6)*, 371-377.

[29] Muhammad-Tahir, Z. & Alocilja, E.C., A

disposable biosensor for pathogen detection in fresh produce samples, *Biosystems Engineering, 2004,* ***88****(2),* 145-151.

[30] Muhammad-Tahir, Z. & Alocilja, E.C., A conductometric biosensor for biosecurity, *Biosensors & Bioelectronics, 2003,* ***18****,* 813-819.

[31] Lillie, G., Payne, P. & Vadgama, P., Electrochemical impedance spectroscopy as a platform for reagentless bioaffinity sensing, *Sensors & Actuators B Chemical, 2001,* ***78****,* 249-256.

[32] Guan, J.G., Miao, Y.Q. & Zhang, Q.J., Impedimetric Biosensors, *Journal of Bioscience & Bioengineering, 2004,* ***97****(4),* 219-226.

[33] Katz, E. & Willner, I., Probing biomolecular interactions at conductive and semiconductive surfaces by impedance spectroscopy: *routes to* impedimetric immunosensors, DNA-sensors, and enzyme biosensors, *Electroanalysis, 2003,* ***15****(11),* 913-947.

[34] Cai, W., Peck, J. R., van der Weide, D. W. & Hamers, R. J., Direct electrical detection of hybridization at DNA-modified silicon surfaces, *Biosensors & Bioelectronics, 2004,* ***19****,* 1013-1019.

[35] Zhao, F.Q., Huang, L. J., Zeng, B. Z. & Pang, D. W., Electrochemical investigation on DNA-gemini complex films immobilized on gold electrode, Electrochemistry Communications, 2004, **6**, 319-324.

[36] Radke, S.M. & Alocilja, E.C., A high density micro-electrode array biosensor for detection of E.coli., The Eighth World Congress on *Biosensors*, 2004, Granada, Spain.

[37] Wang, M., Wang, L., Wang, G., Ji, X., Bai, Y., Li, T., Gong, S. & Li, J., Application of impedance spectroscopy for monitoring colloid Au-enhanced antibody immobilization and antibody-antigen reactions, *Biosensors & Bioelectronics, 2004,* ***19****,* 575-582.

[38] Takhistov, P., Electrochemical synthesis and impedance characterization of nano-patterned biosensor substrate, *Biosensors & Bioelectronics, 2004,* ***19****,* 1445-1456.

[39] Liedberg, B., Nylander, C. and Lundstrm, I. Sensors and Actuators 4, 299-304 (1983).

[40] Wang, J., Liu, G. & Jan, R.M., Ultrasensitive electrical biosensing of proteins and DNA: carbon-nanotube derived amplification of the recognition and transduction events, *Journal of American Chemical Society, 2004,* ***126****,* 3010-3011.

[41] Deo, R.P. & Wang, J., Electrochemical detection of carbohydrates at carbon- nanotube modified glassy-carbon electrodes, Electrochemistry *Communications, 2004,* ***6****,* 284-287.

[42] Guo, M., Chen, J., Nie, L. & Yao, S., Electrostatic assembly of calf thymus DNA on multi-walled carbon nanotube modified gold electrode and its interaction with chlorpromazine hydrochloride, *Electrochimica Acta, 2004,* ***49****(16),* 2637-2643.

[43] Gyurcsanyi, R.E., Vigassy, T. & Pretsch, E., Biorecognition-modulated ion fluxes through functionalized gold nanotubules as a novel label-free biosensing approach, *Chemical Communications, 2003,* ***20****,* 2560-2561.

[44] Kohli, P., Wirtz, M. & Martin, C.R., Nanotube membrane based biosensors, *Electroanalysis, 2004,* ***16****(1-2),* 9-18.

[45] Sargent, A., Loi, T., Gal, S. & Sadik, O.A., The electrochemistry of antibody- modified conducting polymer electrodes, *Journal of Electroanalytical Chemistry, 1999,* ***470****,* 144-156.

[46] Killard, A.J., Micheli, L., Grennan, K., Franek, M., Kolar, V., Moscone, D., Palchetti, I. & Smyth, M.R., Amperometric separation-free immunosensor for real-time environmental monitoring, *Analytica Chimica Acta, 2001,* ***427****,* 173-180.

[47] Susmel, S., Guilbault, G.G. & O'Sullivan, C.K., Demonstration of labeless detection of food pathogens using electrochemical redox probe and screen printed electrodes, *Biosensors & Bioelectronics, 2003,* ***18****,* 881-889.

[48] Chiti, G., Marrazza, G. & Mascini, M., Electrochemical DNA biosensor for environmental monitoring, *Analytica Chimica Acta, 2001,* ***427****,* 155-164.

[49] Lucarelli, F., Marrazza, G., Palchetti, I., Cesaretti, S. & Mascini, M., Coupling of an indicator-free electrochemical DNA biosensor with polymerase chain reaction for the detection of DNA sequences, *Analytica Chimica Acta, 2002, **469**,* 93-99.

[50] Schmidt, J.C., Enzyme-based electrodes for environmental monitoring applications, *Field Analytical Chemistry & Technology, 1998, **2**(6),* 351-361.

[51] Laschi, S., Franek, M. & Mascini, M., Screen-printed electrochemical immunosensors for PCB detection, *Electroanalysis, 2000, **12**(16),* 1293-1298

[52] Lin, Y., Lu, F. & Wang, J., Disposable carbon nanotube modified screen- printed biosensor for amperometric detection of organophosphorus pesticides and nerve agents, *Electroanalysis, 2004, **16**(1-2),* 145-149.

[53] Lucarelli, F., Kicela, A., Palchetti, I., Marrazza, G. & Mascini, M., Electrochemical DNA biosensor for analysis of wastewater samples, *Bioelectrochemistry, 2002, **58**,* 113-118.

[54] Koudelka-Hep, M. & van der Wal, P.D., Microelectrode sensors for biomedical and environmental applications, *Electrochimica Acta, 2000, **45**,* 2437-2441

[55] Fisher, A.C., *Electrode Dynamics,* 1998, Oxford University Press, New York.

[56] Barton, A.C., Collyer, S.D., Davis, F., Gornall, D.D., Law, K.A., Lawrence, E.C.D., Mills, D.W., Myler, S., Pritchard, J.A., Thompson, M. & Higson, S.P.J., Sonochemically fabricated microelectrode arrays for biosensors offering widespread applicability: Part 1, *Biosensors & Bioelectronics, 2004, **20**,* 328-337.