SCIENTIFIC PAPERS
OF THE UNIVERSITY OF PARDUBICE
Series A
Faculty of Chemical Technology
8 (2002)

ELECTROCHEMICAL BIOSENSORS; A PROMISING TOOL IN CHEMICAL ANALYSIS

Stella GIROUSI[1]
Laboratory of Analytical Chemistry, The Aristotle University of Thessaloniki,
GR–54124 Thessaloniki

Received April 14, 2002

The scope of the present article is to provide some useful information to new beginners in the field of electrochemical biosensors. A biosensor exploits biological or biochemical mechanisms in the recognition and in the analysis of certain chemical compounds. Analytical advantages are specificity, sensitivity and cheap instrumentation. Regarding the analytical accuracy, they are proper in the development and application of screening methods of analysis. This article presents a short bibliographic survey of the applications of biosensors in environmental, drug and food analysis.

[1] Based on a lecture given, in the frame of Erasmus-Socrates Programme (staff mobility exchange), on March 21, 2002 at the Department of Analytical Chemistry of the Faculty of Chemical Technology at the University of Pardubice, Czech Republic

Introduction

Biosensors are devices which combine a biological recognition element (enzyme, antibody, DNA), which confers selectivity, with a transducer, which provides sensitivity and converts the recognition event into a measurable electronic signal [1]. A schematic representation of a biosensor is shown in Fig. 1.

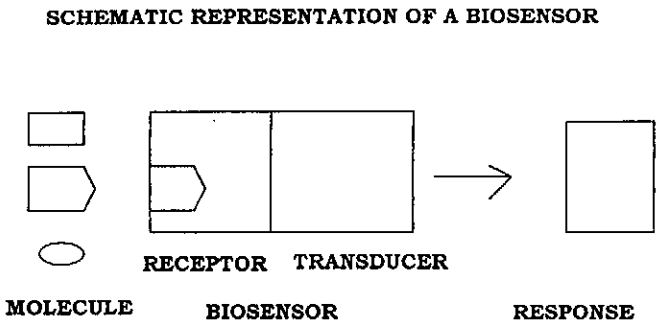


Fig. 1 Schematic representation of a biosensor

Concerning the combination of the bioelement with the transducer (which is based on different physicochemical principles) various biosensor configurations may result [2]. Such a combination cascade is demonstrated in Fig. 2.

In an electrochemical biosensor the transducer is an electrode and mainly the transduction is realized through amperometry or potentiometry

As far as it concerns the analytical importance there exist two main categories of electrochemical biosensors: enzyme and DNA biosensors.

Enzyme Biosensors

In an enzyme biosensor the enzyme, which is specific to certain substances known as substrates, is being immobilized at the electrode surface. The redox mechanism of a redox enzyme is shown in Fig. 3.

Concerning substrates, mainly carbon, occasionally mercury, platinum or gold are used in preparation of enzyme biosensors. Physical techniques such as adsorption and entrapment in gels or polymers, as well as chemical methods as covalent coupling and cross-linking are used for immobilization.

Electron transfer between the electrode surface and the substrate is realized in three main ways, (a) direct; (b) oxidation-reduction process of the enzymatic products generated; (c) reactant mediator

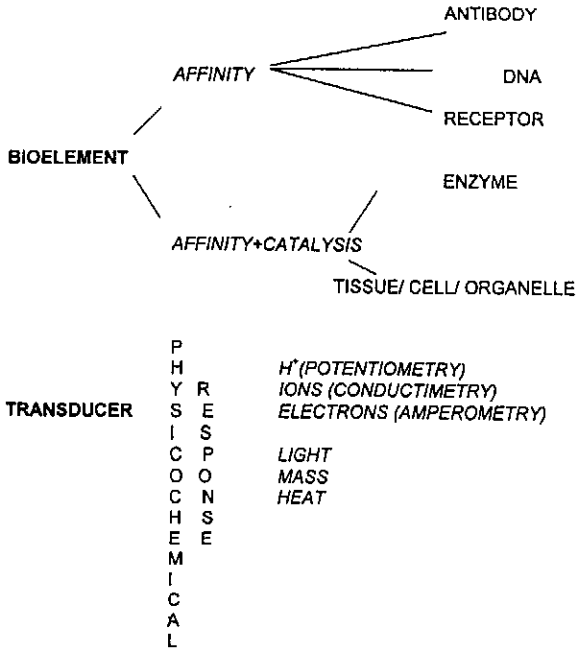
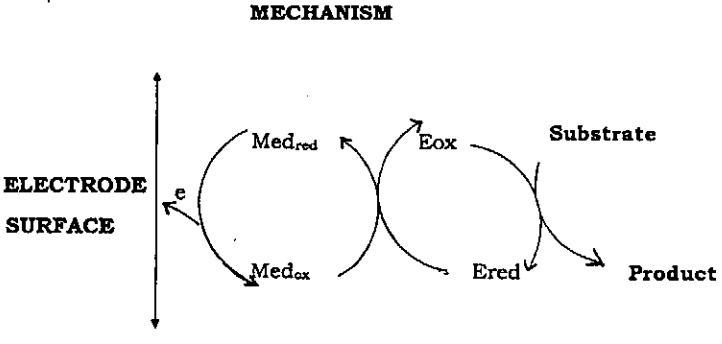


Fig. 2 Schematic diagram of possible biosensor analyte recognition cascade [2]



Med$_{red}$ / Med$_{ox}$ **reduced/oxidized mediator**

E$_{red}$ / E$_{ox}$ **reduced/oxidized enzyme**

Fig. 3 Redox mechanism

DNA Biosensors

In an electrochemical DNA biosensor, DNA is being immobilized at the electrode surface and its electrochemical behaviour is to studied in relation with compounds with affinity for DNA

Nucleic acids are electroactive species which produce well formed electrochemical signals. In Fig. 4 are shown the primary oxidation sites and/or reduction sites of adenine (A), cytosine (C) guanine (G) and thymine (T) [3].

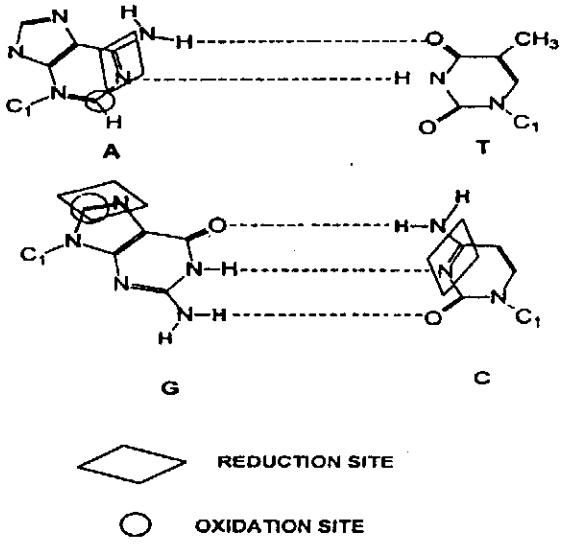


Fig. 4 Watson–Crick base pairing [3]: The primary reduction sites of adenine (A), cytosine (C) and guanine (G) are indicated by rectangles. Oxidation sites of A and G are denoted by circles thymine (T)

Depending on the effect of interactions of DNA with different agents, there exist two categories of electrochemical DNA biosensors [4–7]

i) Electrochemical biosensors for DNA damage
ii) Electrochemical biosensors for DNA hybridization

An electrochemical biosensor for DNA damage exploits phenomena such as:

1. Changes in the redox signals of base residues of DNA (especially guanine), immobilized at the electrode surface, can be used as a sign of the damage of DNA
2. Some compounds interacting with DNA produce their own signals on binding

3. Supercoiled DNA attached to HMDE surface can be used for the sensitive detection of agents cleaving the DNA sugar-phosphate backbone

The interaction between chemical compounds and DNA occurs in 4 different ways:

i) intercalation within DNA (i.e. actinomycin D, daunomycin)
ii) non intercalative DNA binding (i.e. triostin, netropsin)
iii) covalent binding to DNA (i.e. mitomycin)
iv) strand-breaking interactions (i.e. bleomycin, streptonigrin)[8]. This interaction results in inhibition of nucleic acid synthesis.

At present and to a large extent gel electrophoresis is performed in order to study the DNA damage due to physical and chemical environmental agents.

The main drawbacks of gel electrophoresis are the facts that it is time consuming and laborious technique; moreover, is not able to detect small damages to DNA induced by ionizing radiation, enzymatic digestion or treatment with chemicals.

Electrochemical methods offer an interesting alternative in the development of analytical methodology in the field of screening agents with affinity for DNA or agents that result in DNA damage. It is highly important to notice that different DNA forms result in different electrochemical profiles as is shown in Figs 5 and 6.

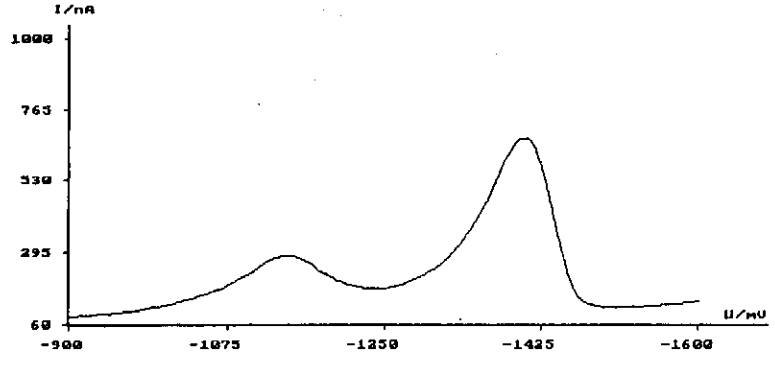


Fig. 5 Alternating current voltammogram of single stranded, ssDNA (40 mg l^{-1}) in phosphate buffer pH 8.5 + 0.3 M NaCl, on the hanging mercury drop electrode (HMDE)

An electrochemical biosensor for DNA damage can be applied in the...

- Determination of traces of DNA or RNA (ng levels)
- Electrochemical characterization of ssDNA, dsDNA or sc form
- Elucidation of the structural integrity of different DNA forms

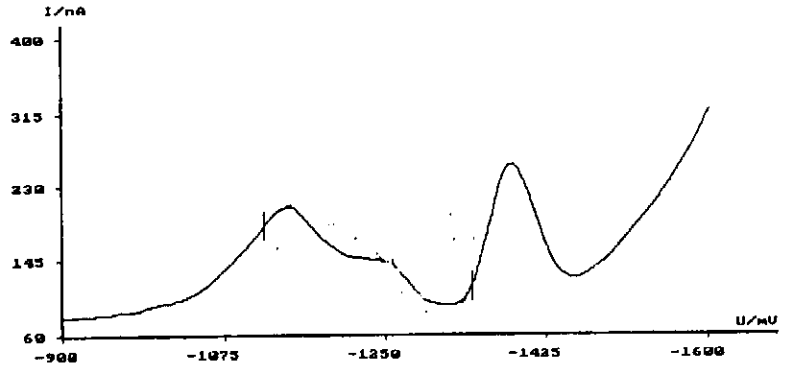


Fig. 6 Alternating current voltammogram of double stranded, dsDNA (80 mg l^{-1}) in phosphate buffer pH 8.5 + 0.3 M NaCl, on the HMDE

Electrochemical DNA Biosensor for DNA Hybridization

The principle on which it is based comprises the surface-confined hybridization probe which is incubated in a solution of target DNA. After washing the electrode is transferred into a solution of a redox indicator (e.g $[Co(phen_3)]^{3+}$) followed by transfer into blank background electrolyte solution and the electrochemical signal of the indicator is measured. When hybridization takes place the signal of the indicator is stronger since the indicator should be preferentially accumulated at the duplex form.

An electrochemical biosensor for DNA hybridization can be applied in the...

- Immobilization of the DNA sequence of a pathogen or a virus; a promising alternative to time consuming PCR protocols
- Gene detection

Applications

Considering the plethora of combinations that could be realized between the bioelement and the transducer it is obvious that a huge number of biosensors could result and be applied in chemical analysis.

In Tables I – IV a short bibliographic survey of the applications of biosensors in environmental, drug and food analysis is given.

Conclusion

The advantages of electrochemical biosensors include low cost, fast response, selectivity, sensitivity, small sample volumes and applicability in flow analysis. As far as it concerns the analytical accuracy, since they suffer to a certain extent in it, they are proper in the development in screening methods of chemical analysis.

Acknowledgements

The author wishes to thank Prof. Vytras, Prof. Jandera, Dr. Ivan Svancara and all their coworkers for their kind hospitality during her stay at the Department of Analytical Chemistry of the Faculty of Chemical Technology at the University of Pardubice, in the frame of the staff mobility exchange of the Erasmus-Socrates Programme.

References

[1] Gorton L.: Electroanalysis **7**, 23 (1995).
[2] Vadgama P., Crump P.W: Analyst **117**, 1657 (1992).
[3] Palecek E.: Electroanalysis **8**, 7 (1996).
[4] Palanti S., Marazza G, Mascini M: Anal. Let. **29**, 2309 (1996).
[5] Wang J., Rivas G., Cai X., Palecek E., Nielsen P., Shiraishi H., Dontha N., Luo D., Parrado C., Chicharro M., Farias P.A.M., Valena F.S., Grant D.H., Ozsoz M, Flair M.N.: Anal. Chim. Acta **347**, 1 (1997).
[6] Fojita M., Palecek E.: Anal. Chim. Acta **342**, 1 (1997).
[7] Palecek E., Fojta M., Tomschik M., Wang J.: Biosensors & Bioelectronics **13**, 621 (1998).
[8] Waring M.J.: Ann. Rev. Biochem. **50**, 159 (1981).
[9] Karube I., Y. Nomura and Y. Arikawa (1995) "Biosensors for environmental control", Trends in analytical chemistry, vol. 14, no. 7, 295 – 299.
[10] Karube I., Nakanishi K.: IEEE Engineering in Medicine and Biology, June/July, 364, (1994).
[11] Hyun C.K., Tamiya E., Takeuchi T., Karube I.: (1993) Biotechnol. Bioenerg., **41**, 1107 (1993).
[12] Karube I., Tamiya E.: Pure Appl. Chem. **59**, 545 (1987).
[13] Nomura Y., Ikebukuro K., Yokoyama K., Takeuchi T., Arikawa Y., Ohono S., Karube I.: Anal. Lett. **27**, 3095 (1994).
[14] Marty J.L., Mionetto N., Nogure T., Ortega F., Roy C.: Biosens. Bioelectron. **8**, 273 (1993).

[15] Hiroaki S., Tamiya E., Karube I.: Anal. Chim. Acta **199**, 85 (1987).
[16] Suzuki H., Kojima N, Sugama A., Takei F., Igekami K., Tamiya E., Karube I.: Electroanalysis **1**, 305 (1989).
[17] D'Urso E.M., Coulet P.R.: Anal. Chim. Acta **239**, 1 (1990).
[18] La Rosa C., Pariente F., Hernandez L, Lorenzo E.: Anal. Chim. Acta **295**, 273 (1994).
[19] Mc Ardle F., Persaud K.C.: Analyst **118**, 419 (1993).
[20] Wang J., Chicharro M., Rivas G., Cai X., Dontha N., Farias P.A.M., Shiraishi H.: Anal.Chem. **68**, 2251 (1996).
[21] Wang J., Rivas G., Luo D., Cai X., Valera F. S., Dontha N.: Anal. Chem. **68**, 4365 (1996).
[22] Marazza G., Chianella I., Mascini M.: Anal. Chim. Acta **387**, 297 (1999).
[23] Mikkelsen S.R., Electroanalysis **8**, 15 (1996).
[24] Wang J., Rivas G., Parrado C., Cai X., Flair M.N.: Talanta **44**, 2003 (1997).
[25] Wang J., Rivas G., Cai X.: Electroanalysis **9**, 395 (1997).
[26] Erdem A., Kerman k., Meric B., Akarca U.S., Ozsoz M.: Electroanalysis **11**, 586 (1999).
[27] J-M. Kauffmann: Proceedings of the 5th Int. Symp. on Pharm. Sciences (ISOPS-5), June 24 – 27, 1997, Ankara, pp 328 – 338.
[28] Frew J.E., Bayliff S.W., Gibbs P.N.B., Green M.: Anal. Chim. Acta **39**, 224 (1989).
[29] Jones A.F., Mc Aleer J. Braithwaite R.A., Scott L.D., Brown S.S., Vale J.A., Lancet.: **35**, 793 (1990).
[30] Vaughan P.A., Scott L.D., Mc Aleer J.: Anal. Chim. Acta **248**, 361 (1991).
[31] Foulds N.C., Wilshere J.M., Green M.: Anal. Chim. Acta **229**, 57 (1990).
[32] Wang J., Dempsey E., Ozsoz M., Smyth M.: Analyst **116**, 997 (1991).
[33] Wang J., Lin Chen L.: Analyst **118**, 277 (1993).
[34] Kaku S., Nakanishi S., Horiguchi K.: Anal. Chim. Acta **225**, 283 (1989).
[35] Simonian A.L., Rainina E.I., Lozinsky V.I., Badalian I.E., Khachatrian G.E., Tatikian S.S., Makhlis T.A., Varfolomeyev S.D.: Applied Biochem.& Biotechnol. **36**, 199 (1992).
[36] Hua C., Smyth M., Fagain C.O.: Analyst **116**, 922 (1991).
[37] Holt P-J., Gray Stephens L.D., Bruce N.C., Lowe C. R.: Biosensors & Bioelectronics **10**, 517 (1995).
[38] Brett A.M.O., Serrano S.H.P., Macedo T.A., Raimundo D., Marques M.H., La-Scalea M.A.: Electroanalysis 1996, 8, 992 (1996).
[39] Chu X., Shen G.L., Jiang J.H., Kang T.F., Xiong B., Yu.R.O.: Anal. Chim. Acta **373**, 29 (1998).
[40] Wang J., Rivas G., Chai X., Shiraishi H., Farias P., Dontha N., Luo D.:Anal. Chim. Acta **332**, 139 (1996).
[41] Brett A.M.O., Serrano S.H.P., Gutz I.G.R., La-Scalea M.A.: Electroanalysis

9, 110 (1997).
[42] Hale P.D., Boguslavsky L.I., Inagaki T., Lee H.S., Skotheim T.A., Karan H.I., Okamoto Y.: Mol. Cryst. Liq. Cryst. **190**, 251, 1990, 96.
[43] Hale P.D., Boguslavsky L.I., Inagaki T., Karan H.I., Lee H.S., Skotheim T. A., Okamoto Y.: Anal. Chem. **63**, 677, 1991, 116.
[44] Ikeda T., Matsushita F., Senda M.: Biosens. Bioelectron. **6**, 299 (1991).
[45] Miki K., Ikeda T., Todoriki S., Semda M.: Anal. Sci. **5**, 269, (1989).
[46] Lorenzo E., Gonzalez E., Pariente F., Hernández L.: Electroanalysis **3**, 319 (1991).
[47] Gorton L., Jonsson-Petersson G., Csoregi E., Johansson K., Dominguez E., Marko-Varga G.: Analyst **117**, 1235, (1992).
[48] Johansson K., Kacaniclic V., Marko-Varga G., Gorton L., Jonsson-Petersson G., Csoregi E.: Biosensors '92, Elsevier, Amsterdam, 119, 1992.
[49] Marko-Varga G., Gorton L., Dominguez E., Barcelo D.: Chromatographia **36**, 381 (1993).
[50] Kacaniclic V., Johansson K., Marko-Varga G., Gorton L., Jonsson-Petersson G., Csoregi E.: Electroanalysis **6**, 381 (1994).
[51] Skladal P.: Collect. Czech. Chem. Commun. **56**, 1427 (1991).
[52] Amine A., Kauffmann J.M., Palleschi G.: Anal. Chim. Acta **273**, 213 (1993).
[53] Yao T., Musha S.: Anal. Chim. Acta **110**, 203 (1979).

Table I Application in environmental analysis [9]

Analytical parameter	Bioelement	Remarks	Analytical characteristics	Ref.
BOD	microorganism, Trichosporon cutaneum	immobilization on a membrane, oxygen electrode	linearity: 3 – 60 mg l^{-1}	[10]
Nitrites	microorganism Nitrobacter	immobilization on a membrane, oxygen electrode	$NO_2^- + 1/2O_2 \rightarrow NO_3^-$	[11]
Ammonia	microorganism, Nitrosomonas sp., Nitrobacter sp.	immobilization on a membrane, oxygen electrode	$NH_3 + 3/2O_2 \rightarrow NO_2^- + H_2O + H^+$ (Nitrosomonas sp.) $NO_2^- + 1/2O_2 \rightarrow NO_3^-$ (Nitrobacter sp.)	[12]
Anionic surfactants	bacteria	immobilization on a membrane, oxygen electrode		[13]
Sulphites	microorganism, thiobacilus, thioparus	immobilization on a membrane, oxygen electrode		[14]
Carbon dioxide	bacteria	immobilization on a membrane, oxygen electrode	3 – 12 %	[15,16]
Phosphates	enzymes, nucleoside phosphorylase Xantine oxidase	detection of hydrogen peroxide	linearity: 10 – 250 μM	[17]
Pesticides	enzyme, acetylcholinesterase	enzyme inhibition	Paraoxon, C_L = 4 nM Carbanyl, C_L = 13 nM	[18]
Pesticides	enzymic, tyrisinase	enzyme inhibition	Antrazine, C_L = 5 μM	[19]

Table II Application in environmental analysis

Analytical parameter	Bioelement	Remarks	Analytical characteristics	Ref.
Hydrazine	DNA	carbon paste electrode DNA damage	C_L = 2 mg l^{-1}	[20]
Aromatic amines	DNA	carbon paste electrode DNA damage	2-aminonaphthalene C_L = 1.8 μM 2-anthramine, C_L = 80 nM 1-aminopyrene, C_L = 60 nM	[21]
Polychlorinated biphenyls	DNA	carbon paste electrode DNA damage	C_L = 0.2 mg l^{-1}	[22]
Aflatoxins	DNA	carbon paste electrode DNA damage	C_L = 10 mg l^{-1}	[22]
Pathogens (Chlamydia trachomatis, E. coli, cryptosporidium)) viruses (HIV-1)	DNA	carbon paste electrode DNA hybridization	indication of DNA sequences	[23–26]

Table III Application in drug analysis [27]

Analytical parameter	Bioelement	Remarks	Analytical characteristics	Ref.
Acetylsalicylic acid	aryl acyl amidase	carbon strip.	linearity: 1 – 7 mM, stability 3 months	[28]
Acetaminophen	aryl acyl amidase	carbon strip.	linearity: 0 – 3 mM, stability 6 months	[29,30]
Theophylline	theophilline oxidase	Pt electrode	linearity: 0 – 0.3 mM, C_L = 2 μM, life-time 35 d	[31,32]
Phenol, peroxides, antiseptics	tyrosinase	glassy carbon electrode	linearity: 0 – 40 μM	[33]
Insulin	anti-insulin IgG/GA?protein A	Pt electrode	linearity: 2 – 200 μIU	[34]
L-proline	pseodomonas	oxygen electrode	linearity: 0.01 – 1 mM	[35]
Glutathuion	glutathion peroxidase heroin esterase/morphine dehydrogenase	glassy carbon electriode	linearity: 0.2 – 2.4 mM	[36]
Opiates	DNA	Pt electrode	C_L = 23.7 μM	[37]
Carboplatin (anticancer drug)	DNA	glassy carbon electrode	C_L = 5.7 μM in serum	[38]
Daunomycin (anticancer drug)	DNA	glassy carbon electrode	C_L = 2.4 mgl^{-1}	[39]
Phenothiazine drugs	DNA	glassy carbon electrode	promethazine, C_L = 5 nM chlorpromazine, C_L = 7 nM phenothiazine, C_L = 12 nM	[40]
Metronidazole bacteriostaticaction	DNA	glassy carbon electrode	C_L = 1 μM	[41]

Table IV Application in food analysis

Analytical parameter	Bioelement	Remarks	Analytical characteristics	Ref.
Glucose *(tracking of fermentattion media)	glucose oxidase	carbon paste electrode	linearity: 0.1 – 10 mM glucose	[42,43]
Fructose	fructose PQQ-dehydrogenase	carbon paste electrode	linearity: 0.2 – 5 mM	[44]
Alcohols *(evaluation of the alcohol degree)	alcohol dehydrogenase	carbon paste electrode	linearity: 1 – 5 mM ethanol	[45]
Hypoxanthine *(fish freshness)	xanthine oxidase	carbon paste electrode	linearity: 0.6 – 700 μM xanthine	[46]
Amino acids *(control of nutritional quality)	amino acid oxidase	carbon paste electrode	all 20 common L-amino acids 20 – 100 μM	[47–50]
Phenols	tyrosinase	carbon paste electrode	linearity: 0.01 – 100 μM phenol, catechol	[51]
L-glutamate	L-glutamate oxidase	carbon paste electrode	linearity: 1 – 500 μM	[52]
L-lactate *(wines and yoghurts)	L-lactate dehydrogenase	carbon paste electrode	linearity: 0.05 -2×10^{-9} mol	[53]

* may find a possible application