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**Editorial**

# Recent trends in electrochemical biosensors design for pharmaceutical and biomedical analysis

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Detection of pharmaceuticals and residues in biological, environmental and food matrices has become a priority for researchers during the last decades. However, current analytical methods capable of detecting pharmaceuticals at very low levels require time-consuming sample preparation, concentration and/or extraction prior to analysis. (Bio)Sensors offer several advantages over existing techniques (e.g., less time, high-throughput screening, improved sensitivity, real-time analysis and the possibility of developing label-free detection methods and devices). By incorporating the nanotechnology achievements into biosensor design, it is increasing the capability of the system as diagnostic methods, point-of-care systems or multiplexed devices [1].

Electrochemical sensors are an important domain of modern analytical chemistry. Understanding sensor devices requires knowledge from a variety of expertise domains such as chemistry, biology, electronics, materials, pharmacology, immunology, biomedical engineering etc. This leads to a very interdisciplinary field populated by physicists, chemists, engineers, biologists and biochemists, materials scientists, electrochemists and others. An electrochemical biosensor converts the modification of the physical or chemical properties of a biomatrix, which occurs as a result of biochemical interactions, into an electric signal whose amplitude depends on the concentration of the analytes in the solution. In fact, the device consists of two parts: a detecting layer of immobilised material (enzymes, antibodies, receptors, organelles, microorganisms, entire cell, tissues etc.) and a transducer (potentiometric, impedimetric, amperometric, conductometric) [2].
One of the key steps in case of electrochemical biosensors design is the optimal immobilization of the biocomponent at the surface of the electrode. By optimum immobilization we are thinking of having a maximum quantity of bioreceptor immobilized at the surface of the transducer or, more appropriately, a maximum number of

functional reactive active sites immobilized in a unity of immobilization substrate as well as its stability and its efficacy.

The trend of using novel materials in electrochemical sensing systems for improved selectivity and sensitivity is constant, with their success largely due to the continuous design and development that meets the needs of modern electrochemical (bio) sensor technology. Materials ranging from carbon composites, beads or microspheres, molecular imprinted polymers or quantum dots are playing an important role in these sensing systems [4]. Nanomaterials (e.g., magnetic or metallic nanoparticles, carbon nanotubes CNTs, graphene) are the core of an emerging technological revolution. The main advantages of these materials are unique thermal, mechanical, electronic and biological properties not found in conventional materials. Combining these unique properties with their remarkable recognition capabilities, significantly improved performance for analytical applications was achieved. Most of the exceptional characteristics of nanomaterials are linked to their surface properties (area, roughness, energetic and electron distributions) which enable improved interactions with many chemical and biological entities. These characteristics result in improved stability and selectivity of nanomaterials and finally of the whole electrochemical sensor.

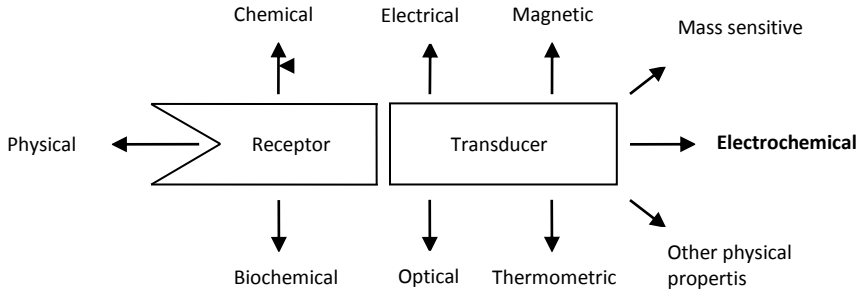


Fig. 1. Sensors classification [5].

Different type of carbon nanostructures are very popular due to their specific structures and properties as well as for the possibility to be used for many applications such as: dispersions and coatings (wear resistant coatings, optical coatings, coatings with medical applications), high surface area materials (used for drug delivery, energy storage, molecular sieves, chemical sensors and biosensors fabrication), consolidated materials (ultrahigh-strength structural materials, MEMS components), functional nanostructures (cold cathodes, transistors, proximal probe tip), magnetic recording systems, electrodes for fuel cells, etc. [6]. Regarding the carbon-based materials, great varieties are available such as: nanoparticles, nanodiamonds, nanoonions, peapods, nanofibers, nanorings, fullerenes and nanotubes and have been extensively used in sensors construction. The configuration of each type of carbon based material is responsible for the enhanced electrical conductivity and the capability to form charge-transfer complexes when in contact with electron donor groups [7]. In the field of pharmaceutical and biomedical analysis several bio/immunosensors were developed but no one has such huge success as the biosensor for glucose monitoring. With a market exceeding several billion dollars this analytical device remain today as the leading commercial success of all biosensors. Besides glucose, other compounds were detected by the mean of electrochemical sensors. Several examples are given in Table1.

**Table 1**. Examples of electrochemical sensors reported in the literature in the last years.

| Analyte | Sensor configuration | Reference |
|---|---|---|
| Acetaminophen | HRP entrapped into a porous alcoxide gel of zirconium and PEI at the surface GCE<br>HRP immobilized in CNT-PEI and CNT-Ppy nanocomposite film at the surface of two types of transducer GCE and SPEs | [8, 9] |
| Ascorbic acid | GCE-βCD-PEI films | [ 10, 11] |
| Uric acid | PANI-PB/uricase/Pt<br>Uricase on a PANI-Ppy film | [12]<br>[13] |

| | | |
|---|---|---|
| Dopamine | Patternable gold nanowire | [14] |
| | MIP of *o*-phenylenediamine with sulfonated graphene onto Au electrode | [15] |
| Serotonine | MWCNT, PPy and colloidal silver NP on the Pt electrode surface | [16] |
| | Covalently bond GO - 5,15- pentafluorophenyl-10,20-p-aminophenylporphyrin | [17] |
| $H_2O_2$ | PEI/MWCNT | [18] |
| Cholesterol | Cholesterol oxidase immobilised with PB/ PPy on the surface of a GCE | [19] |
| Ureea | Conductimetric detection on interdigital silver transducer | [20] |
| Xanthine | XO - poly-TTCA | [21] |
| L-lactate | Lactate oxidase and platinized carbon | [22] |
| | LO-ZnO nanorods glutaraldehyde | [23] |
| Creatinine | ZnO-NPs/CHIT/MWCNT/PANI | [24] |

*Abreviations:* HRP- horse radish peroxidase; PEI- poly(ethyleneimine); GCE-galssy carbon electrodes; SPE-screen printed electrodes; NP-nanoparticles; CHIT-chitosan; SWCNT-single carbon nanotubes; MWCNT-multiwall carbon nanotubes; PANI-polyaniline; GO- graphene oxide; MIP- molecular imprinted polymers; β-CD- β cyclodextrine; PB-Prussian blue; Ppy-polypyrrole; XO-xanthine oxidase; Poly TTCA-Poly-5, 2′: 5′; 2″-terthiophine-3-carboxylic acid; LO- lactate oxidase.

The detection of pharmaceuticals is performed more and more often from environment matrices (drinking and tap water, soil, food) due to the trace levels of human prescription and over-the-counter pharmaceuticals with the possibility of adverse effects on humans and animals [25]. For example, contraception hormones, which can disrupt the endocrine system at ng/L levels, are commonly found in municipal wastewater as well as antibiotics, analgesic, β blockers etc [26]. The characteristics of CNTs (such as promoting different electron transfer reactions and increasing the active surface) have been exploited in the electronic detection of anabolic steroids [27], antibiotics, anti-inflamatory/ analgesics, beta-blockers, diuretics, antiepileptics [5].

Another fascinating application of sensors is the detection of circulating proteins which act as cancer biomarkers with the aid of immunosensors. Their major application consists in the detection of proteins involved in tumor pathogenesis but lately they are applied also in drug abuse control, food analysis and environmental analysis.

During the last decades the incidence of cancer increased dramatically especially in developed countries. In spite of the fact that the immunochemical methods allow the diagnosis in early stages, the biopsies are generally invasive methods that create discomfort to patients. The need for fast, sensitive, easy to use and noninvasive or minimal invasive diagnosis tools is actually of great interest for many research groups all over the world.

Immunosensors (ISs) are miniaturized measuring devices, which selectively detect their targets by means of antibodies (Abs) and provide concentration-dependent signals. Ab binding leads to a variation in electric charge, mass, heat or optical properties, which can be detected directly or indirectly by a variety of transducers.

A great number of proteins could be considered as recognition element. The development of electrochemical immunoassays that could be used in cancer diagnosis, prognosis and therapy monitoring was described by several authors [28, 30-31].

Without doubt, in the group of electrochemical methods applied for immunosensors, the dominant position has amperometry. There are at least two reasons of such state. The enzymes commonly applied as labels (for examples horseradish peroxidase and alkaline phosphatase) catalyze several reactions forming electroactive products easily detectable with voltamperometric techniques. Moreover, amperometry offers lower detection limits and higher sensitivity, so the resulting amperometric immunoenzymatic assays exhibit significantly better analytical characteristics, especially detection limits.

The main disadvantage of the label-based immunoassays is the multistep character of the analytical procedures consisting of the consecutive incubations of sensor with sample, conjugate, substrate, washing and regeneration steps, etc. Additionally, in case of enzyme labels rather expensive and sometimes unstable immunoenzymatic conjugates are required. To

bypass these drawbacks, label-free bioaffinity biosensors are intensively investigated. For this purpose some non-specific effects connected with bioaffinity events are monitored. Recently, some electrochemical immunosensors for the detection of some tumor biomarkers involved in breast and ovarian cancer were reported (see Table 2) [28].

**Table 2**. A list of biomarkers and their application in the development of electrochemical immunoassays for ovarian and breast cancer [28].

| Biomarker | Type of malignancy | Type of immunoassay |
|---|---|---|
| CA 125 | Ovarian | $[Co(bpy)_3]^{3+}$/MWNTs–Nafion |
| | | Au microspheres and porous |
| | | Polythionine modified GCEs |
| | | SPE with Au nanoparticles |
| CA 15-3 | Ovarian | (Fc-COOH)-doped silica nanoparticles (SNPs) |
| | | CNTs and core-shell organosilica and chitosan nanospheres |
| | | Graphene |
| | | Sandwich type with MNP and AP/HRP |
| | Breast | Au NP with aptamers |
| HE4 | Ovarian | Chitosan–titanium carbide (TiC) film that was first electrodeposited onto ITO |
| CEA | Breast | Nano-Au and $SiO_2$/Thionine nanocomposite |
| | | Nano-Au and nickel hexacyanoferrates nanoparticles |
| | | Au MP attached on a CPE |
| | | Nanosilver-doped DNA polyion complex membrane (PIC) |
| | | Au NP –graphene |
| | | Protonated L-cysteine entrapped in Nafion (Nf) membrane |
| | | Au NP functionalized with GOD and ferrocene monocarboxylic-grafted secondary antibodies |
| | | HRP-anti-CEA-NCAuPt |
| | | MIP |
| | | Au–$TiO_2$ nanoparticles and multiple HRP-labeled antibodies (HRP-$Ab_2$) functionalized hollow Pt nanospheres (HPtNPs) |
| | | Polyethylene-MWCNT- ferrocene Carboxylic acid encapsulated liposomes |
| | | Enzyme LBL-SWCNT |
| | | CEA/Au/thionine/Nafion-modified glassy carbon electrode |
| | | Au NP – azure I-MWCNT |
| | | Au NP and aminophenol label free |
| | | Multi layer of Prussian Blue and multiwalled-carbon nanotube/polyethylenimine/Au |
| BRCA 1 | Breast/ovarian | IL-HRP |
| Her2 | Breast | Piezoelectroc cantilever |
| | | Au NP with trastuzumab |
| | | Protein A magnetic beads |

*Abbreviations:* CA 125 – cancer antigen 125, Mucine 16; CA 15-3 – cancer antigen 15-3, Mucine 1; HE 4- Human epididymis protein; CEA- Carcinoembryonic antigen; BRCA 1- breast cancer type 1 susceptibility protein; Her2- Human Epidermal growth factor Receptor 2; MWCNT-multiwall carbon nanotubes; GCEs-glassy carbon electrodes; SPE-screen printed electrodes; Fc-ferrocene; MP-mangnetic nanoparticles; AP-alkalyne phosphatase; HRP-horse radish peroxidase; ITO-tin-doped indium oxide; CPE- carbon paste electrode; GOD-glucose oxide; LBL-layer by layer; SWCNT-single wall carbon nanotubes; IL-ionic liquid.

**Future trends:** The general strategies involving the design and the development of electrochemical biosensors will probably cover the discovery of new biological molecules and systems, the aspects regarding their immobilization and stabilization, nano fabrication technologies, issues associated with miniaturization and integrating technologies in order to produce innovative point- of -care devices. Furthermore, interfacing the technical challenges such as sample introduction and handling, will manage to deal with aspects of commercialization and acceptance of bio-sensing technology into chosen markets. Unquestionably, it is one of the most dynamic areas of research with multiple applications in the diagnosis, therapy control and monitoring, quantification of the analytes of interest in medical and pharmaceutical industry.

## REFERENCES


1. J. Wang, Amperometric biosensors for clinical and therapeutic drug monitoring: a review. J Pharm. Biomed. Anal., 19, 47–53, 1999.
2. N. J. Renault, V. S. Dzyadevych, Conductometric Microbiosensors for Environmental Monitoring. Sensors, 8, 2569-2588, 2008.
3. A. Chaubey, B. D. Malhotra, Mediated biosensors. Biosens. Bioelectron., 17, 441–456, 2002.
4. C. Cristea, V. Harceaga, R. Sandulescu, Chapter Electrochemical Biosensors. Environmental Analysis by Electrochemical Sensors and Biosensors, Editors K. Kalcher & L. Moretti, Ed. Springer, 155-165, 2014**.**
5. C.Cristea, B. Feier, R. Sandulescu, Chapter 2.2. Environmental Analysis by Electrochemical Sensors and Biosensors, Editors K. Kalcher & L. Moretti, Ed. Springer, 167-191, 2014.
6. O. A. Shenderova, V. V. Zhirnov, D. W. Brenner, Carbon Nanostructures :critical reviews. Solid State and Materials Sciences, 27, 227–356, 2002.
7. T. M. Barnes, J. Van de Lagemaat, D. Levi, G. Rumbles, T. J. Coutts, C. L. Weeks, D. A. Britz, I. Levitsky, J. Peltola, P. Glatkowski. Phys. Rev. B, 75, 23541001- 22354110, 2007.
8. V. Sima, C. Cristea, F. Lapadus, I. O. Marian, A. Marian, R. Sandulescu, Electroanalytical properties of a novel biosensor modified with zirconium alcoxide porous gels for the detection of acetaminophen. Journal of Pharmaceutical and Biomedical Analysis, 48, 1195–1200, 2008.
9. M. Tertis, A. Florea, R. Sandulescu, C. Cristea, Carbon Based Electrodes Modified with Horseradish Peroxidase Immobilized in Conducting Polymers for Acetaminophen Analysis. Sensors, 13, 4841-4854, 2013.
10. L. Fritea, M. Tertiş, C. Cristea, R. Săndulescu, New β-Cyclodextrin Entrapped in Polyethyleneimine Film-Modified Electrodes for Pharmaceutical Compounds Determination. Sensors, 13, 16312-16329, 2013.
11. L. Fritea, M. Tertiş, C. Cristea, S. Cosnier, R. Săndulescu, Simultaneous Determination of Ascorbic and Uric Acids in Urine Using an Innovative Electrochemical Sensor Based on β-Cyclodextrin. Analytical Letters, 48, 89–99, 2015.
12. B. Thakur, S. N. Sawant, Polyaniline/Prussian-Blue-Based Amperometric Biosensor for Detection of Uric Acid. ChemPlusChem, 78, 166–174, 2013.
13. F. Arslan, An Amperometric Biosensor for Uric Acid Determination Prepared From Uricase Immobilized in Polyaniline-Polypyrrole Film. Sensors, 8, 5492-5500, 2008.
14. P. Tyagi, D. Postetter, D. L. Saragnese, C. L. Randall, M. A. Mirski, D. H. Gracias, Patternable Nanowire Sensors for Electrochemical Recording of Dopamine. Anal. Chem., 81, 9979–9984, 2009.
15. D. Wu, H. Li, X. Xue, H. Fan, Q. Xin, Q. Wei, Sensitive and selective determination of dopamine by electrochemical sensor based on molecularly imprinted electropolymerization of *o*-phenylenediamine. Anal. Methods, 5, 1469-1473, 2013.
16. I. Cesarino, H. V. Galesco, A. Sergio, S. Machado, Determination of serotonin on platinum electrode modified with carbon nanotubes/polypyrrole/silver nanoparticles nanohybrid. Materials Science and Engineering C, 40, 49–54, 2014.
17. H. S. Han, H. K. Lee, J. M. You, H. Jeong, S. Jeon, Electrochemical biosensor for simultaneous determination of dopamine and serotonin based on electrochemically reduced GO-porphyrin. Sensors and Actuators B, 190, 886–895, 2014.
18. S. Laschi E. Bulukin, I. Palchetti, C. Cristea, M. Mascini, Disposable electrodes modified with multi-wall carbon nanotubes for biosensor applications. IRBM, 29, 202–207, 2008.
19. J. P. Li , H. N. Gu, A Selective Cholesterol Biosensor Based on Composite Film Modified Electrode for Amperometric Detection. Journal of the Chinese Chemical Society, 53, 575–582, 2006.
20. U. Bilitewski, W. Drewes, R. D. Schmid, Thick film biosensors for urea. Sensors and Actuators B, 7, 321-326, 1992.
21. A. Rahman, M. S. Won, Y. B. Shim, Xanthine Sensors Based on Anodic and Cathodic Detection of Enzymatically Generated Hydrogen Peroxide. Electroanalysis, 19, 631–637, 2007.
22. W. A. Collier, D. Janssen, A. L. Hart, Measurement of soluble L-lactate in dairy products using screen-printed sensors in batch mode. Biosens Bioelectron.,11, 1041-1049, 1996.
23. Z. H. Ibupoto, S. M. Shah, K. Khun, M. Willander, Electrochemical L-Lactic Acid Sensor Based on Immobilized ZnO Nanorods with Lactate Oxidase. Sensors, 12, 2456-2466, 2012.
24. S. Yadav, R. Devi, A. Kumar, C. S. Pundir, Tri-enzyme functionalized ZnO-NPs/CHIT/c-MWCNT/PANI composite film for amperometric determination of creatinine. Biosens. Bioelectron., 28, 64–70, 2011.
25. N. Sanvicens, I. Mannelli, J. P. Salvador, E. Valera, M. P. Marco, Biosensors for pharmaceuticals based on novel technology. Trends in Analytical Chemistry, 30, 541-553, 2011.
26. A. J. Bard, L. R. Faulkner, Electrochemical Methods: Fundamentals and Applications. John Wiley & Sons, 2nd Ed., New York, 2001.
27. R. Koncki, Recent developments in potentiometric biosensors for biomedical analysis. Analytica Chimica Acta, 599, 7–15, 2007.
28. I. Diaconu, C. Cristea, V. Hârceagă, G. Marrazza, I. B. Neagoe, R. Săndulescu, Electrochemical immunosensors in breast and ovarian cancer. Clinica Chimica Acta, 425 128–138, 2013.
29. T. R. J. Holford, F. Davis, S. P. J. Higson, Recent trends in antibody based sensors. Biosensors and Bioelectronics, 34, 12– 24, 2012.
30. A. Florea, Z. Taleat, C. Cristea, M. M. Ardakani, R. Săndulescu, Label free MUC1 aptasensors based on electrodeposition of gold nanoparticles on screen printed electrodes. Electrochemistry Communications, 33, 127–130, 2013.
31. Z. Taleat, C. Cristea, G. Marrazza, M. M. Ardakani, R. Săndulescu, Electrochemical immunoassay based on aptamer–protein interaction and functionalized polymer for cancer biomarker detection. Journal of Electroanalytical Chemistry, 717-718, 119–124, 2014.