

Non-Traditional Electrode Materials for Detection of Biomarkers

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Abstract: In this paper, new electrochemical methods suitable for detection of various types of biomarkers (biomarkers of exposition, tumor biomarkers, and biomarkers of medical treatment) are briefly reviewed. Attention is paid to the use of non-traditional electrode materials (various forms of amalgam electrodes, boron doped diamond film electrodes, carbon paste and carbon film electrodes, etc.) for voltammetric (batch analysis) and amperometric (flowing systems) detection of above mentioned biomarkers.

Keywords: Amalgam electrodes; Carbon paste electrodes; Boron doped diamond film electrodes; Differential pulse voltammetry; Amperometry; Biologically active organic compounds; Biomarkers; Tumor markers; Markers of Medical Treatment.

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Introduction

There is an ever increasing demand for analytical methods applicable for monitoring of various biomarkers (BM) in human organism. Electroanalytical methods, namely voltammetry and amperometry, allow large scale screening of selected electrochemically active BM because they are inexpensive, fast, simple, sufficiently sensitive and selective and

necessary equipment is easily operated, small, portable and simple [7]. They are not affected by sample turbidity, quenching or interference from absorbing and fluorescing compounds commonly occurring in biological samples and complicating spectrometric determinations. Easy miniaturization enables the development of disposable devices and methodologies for ultra-small samples.

Recently, different electroanalytical methods were developed in our laboratory for BM serving as markers of exposition (ME) to detrimental environmental pollutants (*e.g.* genotoxic or carcinogenic compounds [43-45], tumor markers (TM) serving for early diagnosis of cancer [47] and markers of medical treatment (MMT), *i.e.* applied drugs or their metabolites serving *e.g.* as markers of anti-cancer drugs application [51].

At the moment, there is an intensive research focused at finding new principles of the electrochemical identification and quantification of various BM. The new challenge in the field of electroanalytical methods is the rational design and development of surface chemistry and/or surface modification. Simple electrochemically active BM can be easily detected by direct, simple, selective and sensitive voltammetric and/or amperometric techniques. It is necessary to detect them directly in biological samples (whole blood, plasma, urine, *etc.*). The most troublesome problem in this field is passivation of the working electrode surface and interference of other electrochemically active (coincidence of signals) and electrochemically inactive (suppression of signals) substances.

The problems with electrode passivation were successfully addressed in our laboratory using two different approaches. The first approach is based upon a development of the new sophisticated non-traditional electrode materials. They can eliminate problems of passivation by a suitable surface structure resistant to passivation (*e.g.* boron doped diamond film electrode [21] or by a simple renewal of passivated electrode surface (*e.g.* carbon paste electrodes [22], amalgam paste electrodes [23,24], carbon film electrodes [9], meniscus modified amalgam electrodes [25]), or composite amalgam electrodes [26]). The second approach uses the insertion of suitable miniaturized electrode into a hollow fiber of porous polypropylene thus suppressing unspecific adsorption of large molecules from biological samples and simultaneously separating and preconcentrating electrochemically active analytes using so-called hollow fiber membrane liquid-phase microextraction (HF LPME) [15].

Even though HPLC techniques with fluorescent and/or MS detection are well established in the field of monitoring of TM in biological matrices [27], electrochemical detection (ED) using excellent but relatively expensive ESA Coulochem II Multi-Electrode electrochemical detector is successfully employed as well [28].

However, much cheaper tubular [1] or microcylindrical [29] electrochemical detectors developed in our laboratory can be obviously used for this purpose as well. Moreover, HPLC-ED can serve as more robust and reliable reference method for confirming positive results obtained by voltammetric screening methods. Simultaneously, electrochemical detection techniques in flowing systems can be useful for FIA-ED detection significantly increasing the sample throughput.

The present state of the art clearly shows that electrochemical approaches can compete with fascinating possibilities of modern separation and spectrometric methods from the point of view of low investment and running costs, simplicity, portability and easy operability. The key point of applicability of electrochemical methods is to increase the selectivity of electrochemical sensors towards BM in various biological matrices. Hence, it is necessary to design new recognition systems, to test their properties, to insight into mechanism and physical-chemical principles of BM recognition, to develop novel sophisticated approaches of chemical/electrochemical modification of electrode surface and immobilization of new recognition systems, and to build up reliable electrochemical biosensors. One of the most important questions in this filed is proper choice of electrode material for reliable voltammetric and amperometric sensor.

The article presented herein is aimed at solution of this crucial issue based on some novel electrode materials recently developed and/or applied in our laboratory.

Amalgam Electrodes

Various forms of non-toxic amalgam electrodes are successfully used in our laboratory since the beginning of this century- see reviews [16,25,34,37].

The silver solid amalgam electrodes are prepared using a drawn out glass tube, whose tip is packed with a fine silver powder, amalgamated by liquid mercury and connected to an electric contact. Mechanically stable and nontoxic amalgam electrodes thus prepared are mechanically polished to prepare a polished silver solid amalgam electrode (p-AgSAE). Mercury meniscus modified silver solid amalgam electrode (m-AgSAE) more suitable for analytical purposes can be prepared by mere immersing of p-AgSAE into a small volume of liquid mercury for 15 s. The m-AgSAE provide lower noise and better repeatability of the response. Silver solid amalgam paste electrodes with an organic pasting liquid (AgSA-PE) [23,24,30,38], silver amalgam composite electrodes (AgSCE) [11,26,39], single crystal silver

amalgam microelectrodes (SCAgAE) [37,40], silver solid amalgam tubular detector [46], or porous amalgam detector [48] represent other interesting alternatives.

In the field of biomarkers detection, various amalgam electrodes were used for determination of micromolar and submicromolar concentrations of anticancer drugs (MMT) Carmustine [31], Lomustine [31], and Flutamide and its metabolites [17]. Interesting example is detection of Sarcosine (an N-methyl derivative of glycine being identified as a differential metabolite highly increased during prostate cancer progression to metastasis) in flowing system at tubular detector based on silver solid amalgam [47]. For this type of biosensor the polished silver solid amalgam electrode was used. First, the 0.5 mm thick layer of polysaccharide chitosan was formed at the surface of the electrode (silver solid amalgam/chitosan). Then, the chitosan layer was modified by –CHO groups by reaction with glutaraldehyde.

Finally, the enzyme sarcosine oxidase was covalently immobilized on the silver solid amalgam/chitosan/glutaraldehyde. The proper determination of Sarcosine is based on the decrease of oxygen concentration during enzymatic reaction. The compound was measured amperometrically in the wall-jet arrangement under FIA conditions. It can be reasonably expected that various forms of amalgam electrodes will be applicable to determine electrochemically reducible ME, too. Further research on this topic is in progress in our laboratory.

Boron Doped Diamond Film Electrodes

Boron doped diamond film electrodes are fascinating devices with extremely broad potential window, very low noise, resistant towards passivation via adsorption of reaction products and/or interferants in the sample, chemically and electrochemically stable and biocompatible [18,21,32, 41]. They are applicable for the determination of both electrochemically reducible and electrochemically oxidizable compounds.

Typical examples of their applications for the determination of various biomarkers developed in our laboratory are HPLC-ED determination of aminonaphthalenes and aminobiphenyls (metabolites of carcinogenic parent nitrocompounds) [3], voltammetric determination of 1-nitropyrene and its metabolite 1-aminopyrene [35], voltammetric determination of carcinogenic derivatives of pyrene and its metabolites [44], determination of 1-hydroxypyrene in human urine by HPLC-ED at BDDFE [45]. Further research is in progress on the development of DPV and HPLC-ED methods for determination of trace

amounts of electrochemically active biomarkers, especially of ME to various PAH which usually contain electrochemically easily oxidizable hydroxy group on aromatic skeleton or NPAH metabolites which usually contain NH_2 group readily transformable as well. Both DPV — either direct or after preliminary separation and preconcentration using SPE or HPLC-ED — have given promising preliminary results in this field.

Carbon Paste Electrodes

Carbon paste electrodes are frequently used sensors for the determination of trace amounts of electrochemically oxidizable organic compounds [2,19,22,36]. Numerous ME contain electrochemically oxidisable functional groups and thus they can be determined using CPE. The following aromatic or heterocyclic amino derivatives resulting from metabolic transformation of corresponding NPAHs and thus suitable as ME of exposition to those genotoxic NPAHs can be named here: 2-amino-fluorene and 2,7-diaminofluorene [4], 1-aminopyrene [6], polycyclic aromatic amines [5], 8-aminoquinoline [8] and other selected aminoquinolines [10].

Voltammetry at CPE based on glassy carbon microbeads can be used for the determination of N,N-di-methyl-4-amino-4'-hydroxyazobenzene (metabolite of carcinogenic N,N-dimethyl-4-aminoazobenzene) in the presence of parent compound [12]. Aminonaphthalenes, which are ME to nitronaphthalenes, can be determined by HPLC-ED at CPE in wall-jet arrangement [13]. CPEs can be also used as MMT sensors, e.g. for detection of anticancer drug Doxorubicin [33] and Methotrexate [14]. More details on MMT determinations can be found in review [14] devoted to possibilities and limitations of carbon paste electrodes in electroanalysis of pharmaceuticals.

Carbon Film Electrodes

Another non-traditional electrode material suitable for voltammetric and/or amperometric determination of various electrochemically active biomarkers is based on carbon films on traditional solid electrodes [9]. These carbon film electrodes (CFE) can be viewed as a thick film analogue of composite electrodes which, in dependence on polymer: conductive powder ratio, can behave like a microelectrode array or like a classical electrodes with homogeneous

surface and they involve a “disposable electrode principle” without the need to dispose the whole electrode. The main advantages of these easily renewable conductive carbon films are substantial broadening of available potential window of classical solid electrodes, the possibility to return to classical application of solid electrodes after easy removal of the film, fast and simple film renewal (up to 3 min by wiping off the old film with filter paper and forming a new one by immersing the electrode into an ink or applying an ink on its surface), fast exchange of films with different composition, good reproducibility of measurements and elimination of problems connected with the “electrode (surface) history”, the possibility to use different conductive powders (from carbon or other conductive materials) and negligible price of the film renewal.

Moreover, the film can be easily modified by the addition of a modifier into the conductive ink and the behavior of the electrode can be easily changed from classical configuration (giving peak shaped response) to a “microelectrode array” (giving S-shaped response) by changing the content of conductive particles in the ink. If we use single inexpensive solid amalgam electrode (either bare or covered with the film) both the determination of reducible (bare amalgam electrode) and oxidizable (film covered amalgam electrode) substance is simple and straightforward. In the field of biomarkers, CFEs were used *e.g.* for voltammetric determination of 5-nitroquinoline [42], 5-aminoquinoline as ME to 5-nitroquinoline [20] or Paracetamol (MMT) [50].

Conclusions

From the above given facts it can be seen that the above mentioned non-traditional electrode materials can be successfully used for the determination of various biomarkers even in rather complicated biological matrices.

Undoubtedly, further research in this field and the combination of these new sensors with a preliminary separation and/or preconcentration using HPLC, TLC, SPE, or HF LPME can further broaden their possibilities and their applicability in this field.

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