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Electrochemical Study of DNA Damaged by Oxidation Stress

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Abstract: Many compounds can interact with DNA leading to changes of DNA structure as point mutation and bases excision, which could trigger some metabolic failures, which leads to the changes in DNA structure resulting in cancer. Oxidation of nucleic acid bases belongs to the one of the mostly occurred type of DNA damaging leading to the above mentioned phenomena. The investigation of processes of DNA oxidation damage is topical and electrochemical methods include a versatile and sensitive tool for these purposes. 8-hydroxydeoxyguanosine (8-OHdG) is the most widely accepted marker of DNA damage. Oxidative damage to DNA by free radicals and exposure to ionizing radiation generate several other products within the double helix besides mentioned oxidation products of nucleic acid bases. The basic electrochemical behaviour of nucleic acids bases on various types of carbon electrodes is reviewed. Further, we address our attention on description of oxidation mechanisms and on detection of the most important products of nucleic bases oxidation. The miniaturization of detector coupled with some microfluidic devices is suggested and discussed.

The main aim of this review is to report the advantages and features of the electrochemical detection of guanine oxidation product as 8-OHdG and other similarly produced molecules as markers for DNA damage.

Keywords: Miniaturization, nucleic acid bases, oxidation damage of DNA, voltammetry.

REACTIVE OXYGEN SPECIES

Reactive oxygen species (ROS) are strictly regulated by compounds with radical scavenging and antioxidant activities called antioxidants. Disruption of this delicate balance between reactive oxygen species and their scavengers leads to the induction of oxidative stress, which may induce various pathological processes ended by death of an organism. The oxidative stress is associated with the ageing processes, carcinogenesis and a number of diseases, such as Alzheimer's disease, atherosclerosis and Parkinson's disease [1-4]. Oxidation stress is induced by both free radicals and compounds of non-radical type (H_2O_2, ozone, $^1O_2$, HOCl). We can differentiate two basic groups of radicals inducing oxidative stress as reactive oxygen species – ROS ($HO^{\cdot}$, H_2O_2, $^1O_2$, O_3, $ROO^{\cdot}$, $HO_2^{\cdot}$) and as reactive nitrogen species – RNS ($NO^{\cdot}$, $NO_2^{\cdot}$) [5]. Thiol and carbon centred radicals belong to additional possible sources of oxidative stress [6].

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EFFECT OF ROS ON THE CELLULAR LEVEL

Increased levels of ROS directly damage DNA and can induce cascade of reactions, which can be terminated by apoptosis. This cascade has been intensively studied in numerous types of cells including neuronal ones, as it is shown in Fig. (**1**). Depending on the initial stress stimulus, activation of p53 occurs *via* various pathways that may intersect with each other upstream of p53 activation. Amount of this 393-aminoacid protein is kept at low concentration level by ubiquitin system degradation in unstressed cells [7, 8]. During cell stress (e.g. DNA damage induced by UV rays and/or alkylating agents) the degradation process of protein p53 is blocked and its intracellular concentration significantly increases. After that, the protein p53 is posttranslationally modified and subsequently oligomerized to tetramer, which has high affinity to DNA [9, 10]. Protein p53 is able to bind onto DNA specifically (by sequence specific bond) or nonspecifically e.g. *via* protein C-terminal domain [11]. Moreover, p53 exerts its deadly function by transactivation of pro-apoptotic target genes including those encoding Bax, the BH3-only proteins PUMA and Noxa, which translocate to mitochondria where they mediate disruption of the mitochondrial membrane potential and release of apoptotic factors including cytochrome c and AIF. Many transcriptional targets such as Peg3/Pw1, Siah-1a and SIVA act in a similar

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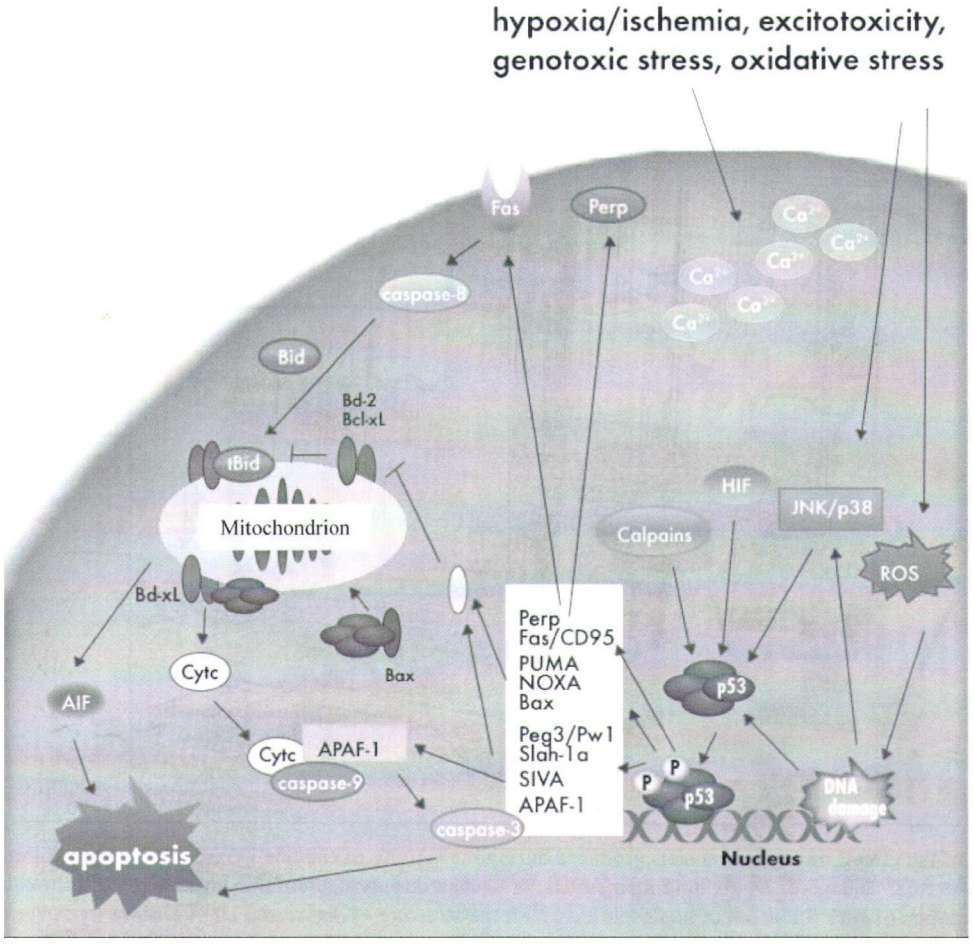

Fig. (1). Scheme of development of cell death (in neurons) *via* the p53 protein by influence of metabolic signal or by oxidative damage (adapted from the [130]). The scheme shows the relationship between p53 protein and its overexpression in the case of oxidative damage or in the connection with the induction of other signalling molecules. The overexpressed protein p53 influences expression of gene of Bax, the BH3-only proteins PUMA and Noxa. Moreover, there are affected Fas or up-regulation of APAF-1 by this protein.

way by interacting with pro-apoptotic members of the Bcl-2 family at the level of mitochondria. In addition, p53 may promote cell death *via* transactivation of the death receptor Fas or up-regulation of APAF-1, which promotes caspase-dependent apoptosis after formation of the apoptosome with cytochrome c and caspase-9 (Fig. **1B**). In addition to such transcriptional control of the cell death machinery, p53 can directly trigger apoptosis after translocation to mitochondria, a process that can occur in synapses (synaptic apoptosis) and may involve interactions with Bax or Bcl-xL. The origination of mutations, translational mistakes or subsequent inhibition of proteosynthesis is the results of these damages.

Free radicals can damage not only DNA but also proteins and lipids, which are essential components of biomembranes [12]. Scheme of such damaging is shown in Fig. (**2A**). A model for iron misregulation and reactive oxygen species generation followed by gyrase inhibition and DNA damage formation is introduced. (a) Gyrase inhibitors (red triangles), such as norfloxacin and CcdB, target DNA-bound gyrase (yellow circles). Resulting complex induces double-stranded breakage and loss of chromosomal supercoiling by preventing strand rejoining by the gyrase enzyme. (b) Gyrase poisoning promotes the generation of superoxide anion radical (O_2-.), which (c) oxidatively attacks iron–sulphur clusters (three-dimensional cube depicts [4Fe–4S] cluster; iron and sulphur are shown as orange and blue circles, respectively); sustained superoxide attacks the iron–sulphur-containing proteins (light blue), which leads to functional inactivation (dark blue), destabilization and iron leaching. (d) Repetitious oxidation and repair of clusters, or redox cycling, promote iron misregulation and may serve to generate a cytoplasmic pool of 'free' ferrous (Fe^{2+}) iron. (e) Ferrous iron, *via* the Fenton´s reaction, rapidly catalyses the formation of deleterious hydroxyl radicals (OH), which readily damage DNA, lipids and proteins; the Fenton´s reaction can thus take place at the destabilized iron–sulphur clusters or where free ferrous iron has accumulated. It can be suggested that reactive oxygen species are generated *via* an oxygen-dependent death pathway that amplifies the primary effect of gyrase inhibition and contributes to cell death following gyrase poisoning.

OXIDATION OF DNA

Based on the above-mentioned facts that ROS represent one of the most important sources of oxidative DNA damage. Study of the oxidative DNA damage is one of

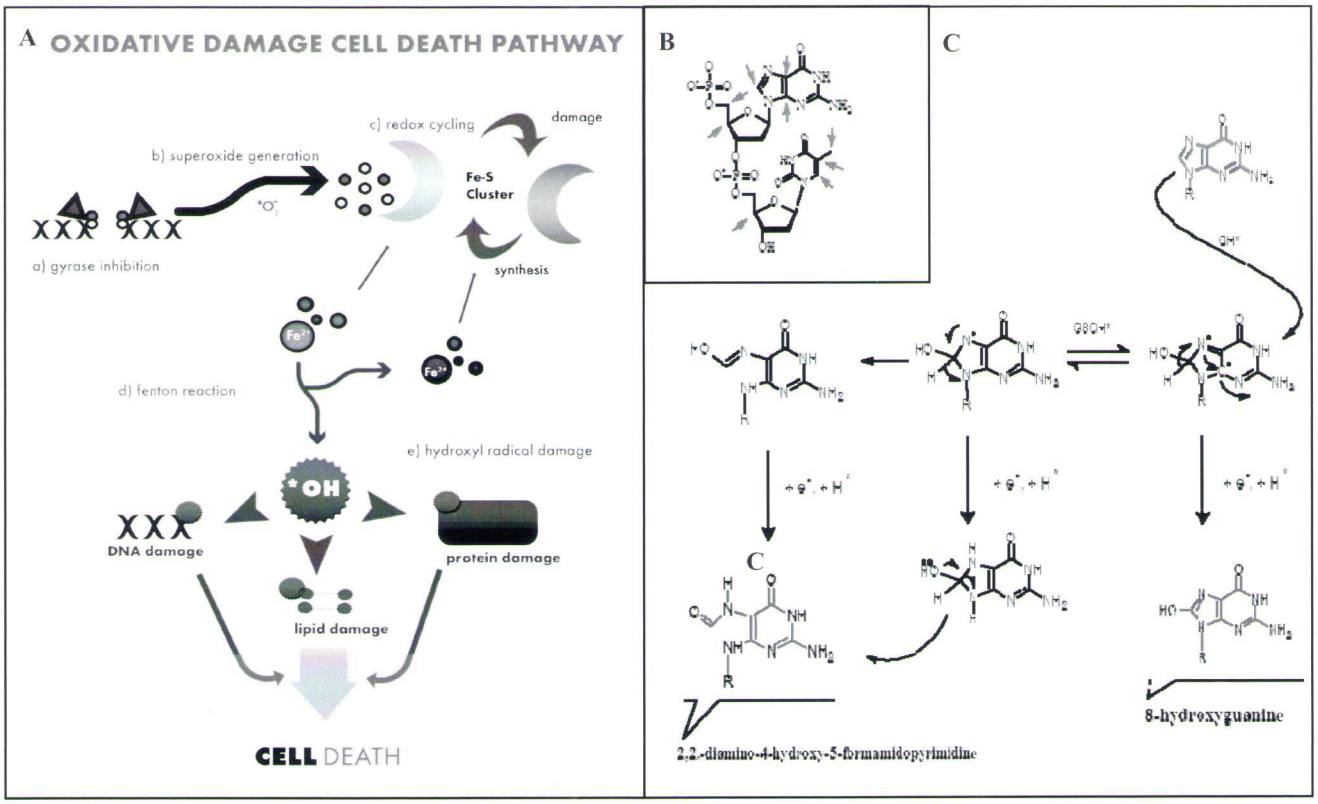

Fig. (2). (**A**) Scheme of the effects of oxygen radicals produced during the activity of enzyme gyrase *via* a cascade of mechanisms including Fenton´s reaction to the final damage of DNA, lipid membranes, or protein through the unforced hydroxyl radical oxidation. Adapted from the [12]. (**B**) Representation of sites for attack of nucleophiles within the structure of one-strand DNA (shown by red arrows). (**C**) Schematic representation of the formation of two types of oxidation products of guanine. The first one is 8-hydroxyguanine, 2,6 – diamino – 4 oxo – 5 - formamidopyrimidine, which gives the possibility of the opening of the ring.

topical scientific fields with the fact that markers of the oxidative stress are still looked for. More than 20 various oxidative modifications of DNA bases have been described including 8-hydroxyguanine, 8,5'-cyclo-2'-deoxyguanosine, 7,8-dihydro-8-oxoguanine, 2,6 – diamino – 4 oxo – 5 – formamidopyrimidine, xanthine, hypoxanthine, further oxidation products of adenine as 7,8-dihydro-8-oxoadenine, 8,5′-cyclo-2′-deoxyadenosine, 2-hydroxyade-nine, 4,6-diamino-5-formamidopyrimidine, cytosine oxidat-ion products as 5-hydroxycytosine, 5,6-dihydoxycytosine, uracil, 5-hydroxyuracil, isodialuric acid and alloxan and thymine derived lesions as thymine glycol, 5-hydroxy-methyluracil, 5-hydroxymethyluracil, 5-formyluracil, 5-hydroxy-5-methylhydantoin and urea [13-15]. During the reaction with purine bases, hydroxyl radical is added into the position 4, 5, or 8 (Fig. **2B**). Addition into the C8 position of guanine leads to the formation of 8-hydroxyguanine radical, which can be subsequently oxidized under formation of 8-hydroxyguanine. This product may be reduced under the opening the purine ring and 2,6-diamino-4-hydroxy-5-formaminopyrimidine formation (Fig. **2C**). Structure of adenine is modified by the same mechanism. From the mentioned oxidative products, 8-oxo-7,8-dihydro-2'-deoxyguanosine belongs to the promising ones. Progress in the number of papers containing "8-oxo-7,8-dihydro-2'-deoxyguanosine (8-OHdG)" in their abstracts, titles and keywords listed in the Web of Science is from 1991 to 2011 is almost exponential.

Single strand breaks are formed as a result of direct action of radicals together with the chemical modification of purine and pyrimidine bases or the phosphate-sugar skeleton. An example of indirect effect is the activation of Ca^{2+}-dependent endonucleases in the nucleus leading to the DNA fragmentation and generally induction of processes of programmed cell death/apoptosis. In addition to DNA damage, nuclear proteins having important structural and regulatory (e.g. transcription) functions may be damaged. The protein radicals can subsequently react with nucleic acid bases generating DNA-protein cross-products. As a result of DNA damage, the formation of mutations (e.g. GC ↔ AT transition, GC ↔ TA transversion) occurs with subsequent translational mistakes in the protein synthesis, which can lead to inhibiting proteosynthesis. The presence of 8-oxoG (O) in a DNA chain can cause GC ↔ T(A) transversion as it is shown in Fig. (**3**). Formation of DNA-protein cross-products is involved in the prevention of chromatin unfolding, its subsequent replication and transcription. Products of hydroxyl radical attack on thymine base include formation of thymine glycol, 5-hydroxy-5-methylhydantoin, 5-formyluracil and 5-(hydroxymethyl)uracil. The most common reaction products with cytosine are cytosine glycol and 5,6-dihydroxycytosine. Action of hydroxyl radical on deoxyribose leads to the cleavage of the hydrogen atom and hydrocarbon radicals (each C with the same preference) formation. In the presence of O_2-. radicals are formed

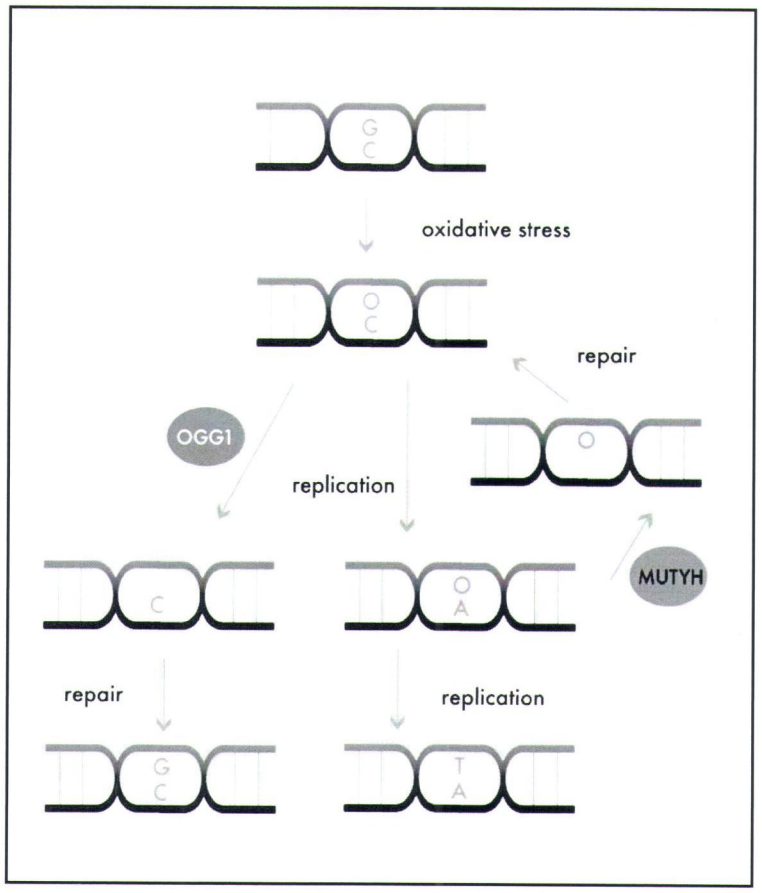

Fig. (3). Scheme of the repair of damaged DNA strain by the excision of the DNA base with the participation of BER glycosylase MutM (OGG1) and DNA glycosylase MUTYH. Modified in accordance with [131]. The presence of 8-oxoG (O) in DNA causes G-to-T transversions, as illustrated in the central pathway. The human DNA glycosylases OGG1 and MUTYH are involved in excision of bases from the DNA. OGG1 removes 8-oxoG from 8-oxoG•C base pairs, and MUTYH removes A from 8-oxoG•A base pairs, both generating AP sites in the DNA. The corresponding bacterial enzymes are MutM and MutY. The steps labelled 'repair' summarize the actions of Apurinic/apyrimidinic AP endonuclease, deoxyribophosphate lyase, DNA polymerase and DNA ligase. Notably, OGG1 also has AP lyase activity. MutT and its human homologue MTH1 have an important role in preventing the incorporation of 8-oxoG, through hydrolysis of free 8-oxo-dGTP.

peroxyl radicals subsequently undergoing a number of reactions including disproportionation, elimination of water, C-C bond cleavage and rearrangements. In the case of low O_2-. concentration intramolecular cyclization occurs and the final product is 8,5'-cyclopurine-2'-deoxynucleotide. $^1O_2$ reacts mainly with guanine, giving rise to unstable endoperoxide, which gives raise a number of decomposition products. The most common final product of this reaction is 8-OHdG [16].

DETERMINATION OF 8-OHdG

8-OHdG as one of the most frequently monitored derivatives of guanine oxidation is not digested, thus, its level is not affected by diet and is well determinable. Therefore, the 8-OHdG levels are considered to be a valid marker of oxidative DNA damage. This compound can be determined directly or indirectly in selected biological matrixes, mainly in the urine [16-22]. From the direct ones, high performance liquid chromatography (HPLC) with electrochemical detection (ED) [23, 24] belongs to the most commonly used method for determination of 8-OHdG. In addition, new techniques have been introduced into this field as gas chromatography with mass spectrometry [17, 25], electrospray ionization mass spectrometry (MS) [26], liquid chromatography with mass spectrometry [27-30] and capillary electrophoresis [31-36]. Using a highly sensitive analytical methods such as HPLC-MS-MS in connection with solid phase extraction for the determination of 8-OHdG in human urine we can detect one damage per 10^6 DNA nucleotides [37].

Immunohistochemistry are often used as indirect method for determination of DNA oxidation [38-40]. Interaction of antibodies, which have low specificity, or DNA reparation enzyme, is used [39]. Formamidopyrimidine-DNA N-glycosylase catalyses breaks of the double helix at the places of 8-OHdG presence. They can be determined using the Comet assay (single-cell gel electrophoresis) or alkaline elution analysis [41-43]. Generally, it can be concluded that results of individual analyses are different (by two or three orders) based on used method [44]. Such differences may be caused by inaccuracies of the experiment itself, however, the isolation technique and especially unwanted oxidation of DNA during the experiment must be also carefully considered [45, 46].

Amounts of 8-OHdG, which can be detected using different methods expressed by both limits of detection and quantification, are introduced in Table **1**. The table shows that the sensitivities of methods for the determination of 8-OHdG are as follows: GC–MS 2471 pM (700 pg 8-OHdG /ml; ELISA 441 pM (125 pg 8-OHdG /ml); HPLC–MS 3530 pM (1000 pg 8-OHdG /ml); LC–MS–MS 706 pM (200 pg 8-OHdG /ml), HPLC-ED 353 – 35306 pM (100–10000 pg 8-OHdG /ml) and HPLC-EC-SPE 46 – 17653 pM (13–5000 pg 8-OHdG /ml). The best sensitivity using the electrochemical determination has been achieved by LSV method with glassy carbon electrode coated by multi-walled carbon nanotubes [47]. This sensitivity is absolutely comparable with results obtained for HPLC-ED [24]. The lowest limit of detection expressed as 3 fM (0.85 fg 8-OHdG per ml) has been achieved using the HPLC with Pt nanocarbon electrode [48].

Table 1. Comparison of Various Methods Used for Detection of 8-OHdG

Method	LD	LQ	Ref.
CE-ED, carbon fibre	0.5 nM	1.6 nM	[31]
GCE	8.10^{-7} M	26.10^{-7} M	[117]
DPASV, MWNT	1.10^{-7} M	3.10^{-7} M	[118]
LSV, GCE-MWNT	9 nM	30 nM	[47]
HPLC, amperometric Au	1.2 nM	3.7 nM	[23]
HPLC MS/MS	0.018 μM	0.056 μM	[25]
HPLC MS/MS	141 pM	282 pM	[26]
HPLC, nanocarbon	3 nM	9.9 nM	[24]
HPLC, GCE	7.2 nM	23.8 nM	[24]
HPLC – ED-SPE	4.59 pM	15.18 pM	[72]
ELISA	441 pM	1455 pM	[119]
ELISA	0.35 – 35.3 nM	1.16 – 116.5 nM	[120]
HPLC-ED	282 pM	931 pM	[39]
GC-MS	2.47 nM	2.5 nM	[121]
LC-MS	3530 pM	11649 pM	[122]
LC-MS/MS	0.71 nM	2.34 nM	[123]
LC-MS/MS	0.01 μM	0.03 μM	[124]
LC-MS/MS	0.71 nM	2.34 nM	[37]
LC-MS/MS	0.48 nM	1.58 μM	[125]
HPLC-ED-SPE	24.7 – 74.1 pM	81.5 – 244.5 pM	[126, 127]
HPLC-ED	0.4 nM	1.32 nM	[128]
HPLC-ED-SPE	17.7 nM	58.4 nM	[75]
HPLC-ED-SPE	47.1 pM	157 pM	[129]
HPLC, Pt nanocarbon	3 fM	9.9 fM	[48]
SFIA LSV-GCE	17.65 pM	58.25 pM	[40]

CE-ED – capillary electrophoresis with electrochemical detection, DPASV – differential pulse anodic stripping voltammetry, ED – with electrochemical detection, ELISA – enzyme-linked immunoSorbent assay, GC-MS – gas chromatography with mass spectrometric detection, GCE – glassy carbon electrode, HPLC – high performance liquid chromatography, LD – limit of detection, LQ – limit of quantification, LSV – linear sweep voltammetry, MS – mass spectrometry, MWNT – multi-walled carbon nanotubes, SPE – solid phase extraction, SFIA – segmental flow injection analysis.

Based on the mentioned facts HPLC-ED is excellent from the point of view of sensitivity and selectivity in the determination of compounds that can undergo oxidation or reduction processes, such as bases of nucleic acids or products of oxidative DNA damage. Thanks to technological advances, miniaturization and the possibilities in electrodes modification these methods have become favourable and beneficial, mainly due to the elimination of noise, lower detection limit, wider linear range, rapid response and low-volume measuring cell [49, 50]. Analysis of DNA oxidation products, i.e. oxidized bases such as 8-OHdG, most frequently takes place using the chromatographic reverse phase columns, however, immunoaffinity chromatography can be used for purification of samples with subsequent HPLC-ED detection too. Yin *et al.* used the immunoaffinity chromatography for purification of samples and subsequent determination of 8-OHdG in the urine of smokers and non-smokers [39]. Analysis of the chromatogram of real sample of patient's urine, where signal of 8-OHdG is well evident, is shown in Fig. (**4A**). For more sensitive electrochemical detection Samcova *et al.* have suggested the special geometry and arrangement of the electrochemical detector for 8-OHdG determination. Compared to typical detector, auxiliary electrode is constituted by Pt tube, which is in the contact with electrolyte at the one end and at the second end it is connected with chromatographic column and as a reference electrode Ag/AgCl is used. Optimal detection potential of 800 mV was determined voltammetrically (Fig. **4B**). Limit of detection was estimated as 0.85 fg/ml (S/N = 3) [33]. HPLC with amperometric detection is applicable also for samples containing strong interfering agent (uric acid) under achievement of very promising detection limit of 8-OHdG [51]. On the other hand, separation techniques with electrochemical detection are still under intense investigation and represent very promising direction with extensive field of applications.

STATIONARY ELECTROCHEMICAL ANALYSIS OF NUCLEIC BASES OXIDATION

Electroactivity of nucleic acids (NA) is well known for many years. Mercury electrodes are suitable for the study of molecules originated in oxidative DNA damage. These electrodes are adapted for studying redox processes, especially those ongoing on nucleic acids [52-54], whereas solid electrodes, especially carbon electrodes, are suitable for the monitoring the oxidation processes, which are important for the understanding the processes of oxidation DNA and generally NA damage. When studying the signal of NA using the method of cyclic voltammetry (CV), hanging mercury drop electrode (HMDE) is the working electrode and Ag/AgCl/3M KCl is the reference electrode. The reduction signals of cytosine (C) and adenine (A) at the potential of about -1.45 V and oxidation peak of guanine (G) at the potential of app 0.3 V are detectable [55-58], as it is shown in Fig. (**5A**). Carbon electrodes are the most commonly used electrodes for studying the oxidation processes taking place on the nucleic bases and their nucleotides, respectively nucleosides. Oxidation of purine bases takes place at lower potentials and significantly depends on pH [59-61]. Differential pulse voltammetry on glassy carbon electrode (GCE) was used for studying of the oxidation of adenine (A), guanine (G), thymine (T) and

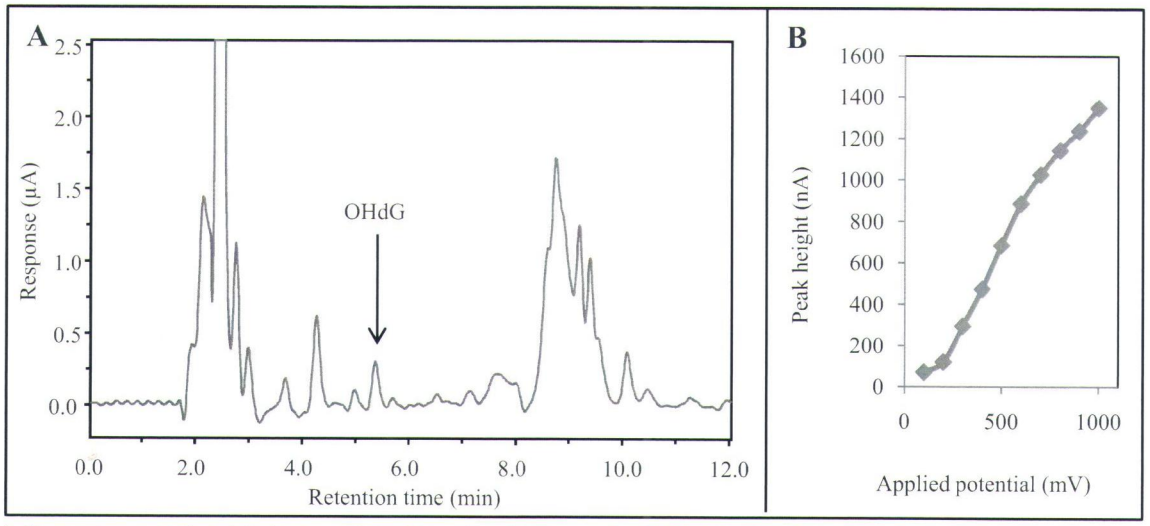

Fig. (4). (**A**) Chromatogram of real (urine sample) with well-evident signal of 8-OHdG (RT 5.4). The chromatogram was obtained using the HPLC-ED CoulArray apparatus. (**B**) Summation hydrodynamic voltammogram (HDV) for the determination of optimal applied potential (800 mV) using the HPLC-ED CoulArray apparatus.

cytosine (C). Limit of detection lower than 1 μM was achieved [62]. Similar limit of detection was determined using square wave voltammetry (SWV) at carbon paste electrode (CPE). At the neutral pH and Ag/AgCl/3 M KCl as a reference electrode the signals at 1.00, 1.28, 1.47 and 1.53 V for G, A, T and C were observed [63].

To enhance the sensitivity of the electrochemical determination of NA the adsorptive stripping technique (AdS) may be advantageously used. It exploits the strong adsorption ability of NA to bind onto the electrode surface, mainly onto HMDE. Stirring enables faster transfer and accumulation of NA on the surface of HMDE, which leads to an increase in redox or oxidation signals of the adsorbed NA. Increase the sensitivity by two orders was achieved in the determination of signal G using the AdS cyclic voltammetry (CV AdS) [64], respectively. Application an adsorption technique (AdS) enabled us to obtain similar sensitivity as by using gel electrophoresis, however, large sample volume (1 ml) was needed. Due to this fact, adsorptive transfer stripping technique (AdTS) has been suggested and developed by Palecek [65]. This technique is based on the adsorption of (sample volume of 3-5 μl) on the electrode surface, which is subsequently washed with water (Fig. **5B**). A measurement itself takes place in the pure electrolyte [65, 66]. The advantage of this method is also in the removal of interfering substances. Like this way modified DNA electrode may also be used as an electrochemical biosensor, which can be used for the study the DNA damaging agents. Solid electrodes were used in the detection of oxidative DNA damage as the fragmentation of longer DNA molecules, releasing the individual bases, etc. Oxidative damage is often induced by ionizing radiation or free radicals. The resulting oxidation products are analysed by optical or electrochemical methods with the preceding separation by capillary electrophoresis or liquid chromatography. To save time and costs, electroc-hemical methods that would not require pre-preparation of a sample are developed. Guanine is the base that can be easily oxidized and is also the most common reaction site of both damage and DNA repair. Oxidation peak of guanine at carbon electrodes is observed in the connection with an effect of many genotoxic compounds on DNA, such as platinum-based drugs [59], hydrazine derivatives [67], benzene derivatives [68, 69], and oxides of arsenic and chromium [70].

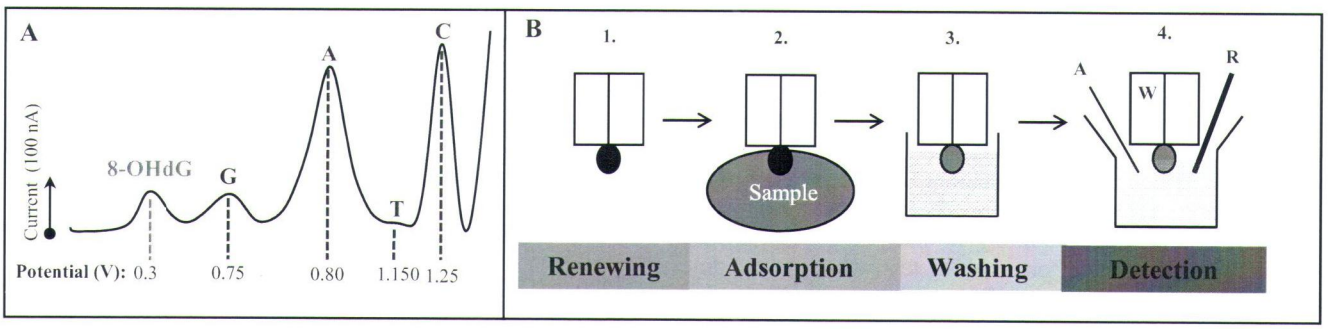

Fig. (5). (**A**) Square wave voltammogram of individual DNA bases (in the concentration of 50 μg/ml). Letters represent individual bases - Cytosine, Adenine, Thymine and Guanine processed using the Baseline correction (GPES) measured in 0.1 M phosphate buffer (0.1 M NaH_2PO_4 + Na_2HPO_4) pH 6.00. Initial potential 0 V, end potential 1.4 V, frequency 100 Hz, potential step 1.05mV [57]. (**B**) Scheme of adsorptive transfer technique according to [132]. 1) hanging mercury drop electrode (HMDE). 2) accumulation of drop of sample (5-20 μl) onto HMDE surface. 3) Washing of excess of unaccumulated sample from the surface of the electrode in distilled water. 4) Transfer into measuring dish – course of the analysis. Method is designated for the lowering of the limits of detection.

Much better sensitivity is achieved if the DNA damage leads to the formation of new substances, which is 8-OHdG in the case of DNA oxidative damage. Oxidation signal of 8-OHdG is detected at significantly lower potential of 0.4 V compared to other nucleic acids [47], which is caused by the presence of hydroxyl (-OH) group. $[Os(bipy)_3]^{2+}$ complex is able to selectively oxidize 8-OHdG, so, it is possible to determine it in the presence of guanine in DNA [71]. However, if we consider the concentration of 8-OHdG in plasma of healthy humans, which is 29.2 ± 21.6 nM [72], LSV voltammetry, which requires no special sample treatment and is inexpensive and time-saving, is quite sufficient [47].

8-OHdG signal can be observed on carbon or platinum electrodes. For the detection of DNA damage by the drug Adriamycin (doxorubicin) [73], flavonoid quercetin [74] or chromium [70], the 8-OHDG signal was monitored. Limit of detection are 5 ng/ml when using the SPE extraction and carbon paste electrode as the working one [75]. The possibility of carbon glassy electrodes modification and thus, achievement better limits of detection and improvement of the sensitivity of detection belongs to - its most important advantages. This also makes the sample preparation easier. Modification of the gold (Au) electrode by monolayers of thiol-terminated dendrimers with two terminal functional $-NH_2$ and –COOH groups that strongly bind to the amide bridges enables reduction of the limit of detection to 0.4 pg/ml at the applied potential of 1100 mV. This procedure makes an analysis faster, and, in addition, eliminates possible interfering substances. The literature describes the preparation and use of Pt electrode modified by sputtered nanocarbon, which was used as a working electrode and installed in a radial-flow electrochemical cell. Ag/AgCl was used as a reference electrode and Pt as a working electrode. Compared to glassy carbon electrode, the nanocarbon-modified electrode demonstrates excellent reproducibility for a plenty of biomolecules [48]. These advantages are based on its homogeneous and stable structure, which consists of nanocrystalline sp^2 and sp^3 carbon hybrids that cause high electrode activity [48].

PERSPECTIVES OF 8-OhDG DETERMINATION IN MEDICINE

Recently, the term "personalised medicine" is intensely discussed not only in medical applications, but also in the technological sciences. The miniaturization of various instruments for detection of biologically active substances is of great interest in relation to various diseases. Moving an examination device from the catchment area directly into the general practitioner surgery or directly to the patient shortens not only the time from sampling to analysis itself, but also reduces the waiting time for result of test, which is in some "critical" cases very necessary. For the detection of oxidative DNA damage, there are also developed methods, which highly contribute to the possibility of redirecting into the area of personalized medicine due to their characteristics in the near future. Point-of-care (POC) genetic diagnostics critically depends on miniaturization and integration of sample processing, nucleic acid amplification, and detection systems. Polymerase chain reaction (PCR) assays have extensively applied for the diagnosis of genetic markers of disease. Microfluidic chips for microPCR with different materials and designs have been reported. Temperature cycling systems with varying thermal masses and conductivities, thermal cycling times, flow-rates, and cross-sectional areas, have also been developed to reduce the nucleic acid amplification time [76]. It is essential especially for two groups of patients.

One group consists of patients who were exposed to the toxic effect of metals or organic compounds in the environment. Many experiments in the field of environmental toxicology deal with the effects of environmental pollutants on the degree of oxidative damage of the organism, which can be assessed by numerous markers including 8-OHdG. A correlation of increased levels of 8-OHdG levels and environmental pollution has been evidenced [17, 77]. A group of probands living in Mexico City, which was exposed to ozone, dust particles, aldehydes, heavy metals and oxides, showed elevated levels of 8-OHdG in cells of the nasal respiratory epithelium [78]. Increased oxidative stress has also been demonstrated in people working under conditions with an increased occurrence of volatile organic compounds [79], at which levels of muconic acid, hippuric acid, mandelic acid, and methyl hippuric acid as urinary exposure biomarkers for benzene, toluene, ethylbenzene, and xylene, malondialdehyde and 8-OHdG as oxidative stress biomarkers were determined in all subjects, and in children exposed to arsenic, chromium, and nickel [80], in which levels of 8-OHdG were determined. Much higher concentrations of 8-OHdG in the urine of people working in factories for the production of galvanic cells have been experimentally proved too [81], as well as in the people living in the environment with elevated levels of arsenic [82]. Incomplete combustion generates polycyclic aromatic hydrocarbons, which are important pro-cancerogenes. It was confirmed that prolonged exposure to increased levels leads to the increase of 8-OHdG in blood serum and urine [83, 84]. The presence of polyaromatic hydrocarbons has been demonstrated also in the processing of coke and iron and thus contributes to higher oxidative stress of workers, who have an increased presence of 8-OHdG in the urine compared to control groups [85, 86]. Oxidative stress is manifested also in other fields of occupational sector, such as pharmacy. Detection of 8-OHdG using Enzyme-Linked ImmunoSorbent Assay (ELISA) in urine of people working as a pharmacy technicians preparing antineoplastic drugs demonstrated increased levels of this biomarker correlated with the increased concentrations of 5-fluorouracil in the working environment [87].

The second group represents patients suffering from increased oxidative stress in relation to serious illness. Increased concentrations of 8-OHdG in the urine or blood correlate with the occurrence of breast cancer [88], prostate cancer [89], bladder cancer and lung cancer [90], lymphoma [91], diabetes [4], atherosclerosis [4], chronic hepatitis [82, 92], colitis [90], atopic eczema [93] and others. 8-OHdG is the major oxidative stress biomarker of diagnostic procedures analysed in the urine or blood. In the case of periodontitis patients, saliva was used for analysis [94].

PERSPECTIVES OF 8- OHdG DETERMINATION USING MICROFLUIDICS AND MINIATURIZATION DEVICES

Prabhulkar and Li focused on the application of carbon fibre electrodes for the detection of 8-OHdG in real time on the surface of epithelial cell to study the effect of nicotine contribute to the development of miniaturized analytical devices [95]. Those electrodes can then be implemented into the microfluidic system that can be produced using Micro-Electro-Mechanical Systems technology likewise technology lab-on-a-chip or biochips. In addition to these direct approaches, microfluidic immunoassay offers a combination of simplicity, sensitivity and specificity as well as possibility of automation and miniaturization [96].

Using of an electrochemical detector for analysis of 8-OHdG brings an indisputable advantage in the possibility of a plenty of utilizing of various working electrodes for this purpose. Using flow injection analysis (FIA) with the amperometric detector and ruthenium oxide hexacyano-ferrate-modified electrode, 8-OHdG was determined model DNA in good agreement with high-performance liquid chromatographic analysis. The limit of detection was 26.6 fg/ml [97]. It was shown that the repeatability of the determination of 8-OHdG by FIA-ED with poly(3-methylthiophene)-modified glassy carbon electrode is very good with RSD = 0.77 % [98].

There is a great research in coupling of miniaturized detectors with some separation techniques [99]. Para-magnetic or superparamagnetic particles/beads (MPs) represent promising tool in this field [100-102]. MPs, which size is ranging from nm to mm, respond to external magnetic field and facilitate bioactive molecules binding because of their affinity for the MPs modified surface made of biologically components [103-106]. The paramagnetic properties of the particles enable us to use magnetic force for transferring of the beads or for rinsing of nonbinding, otherwise commonly interfering substances. Among other advantages of MPs belong easy-to-use, non-laborious relatively rapid sample preparation without centrifugation and dialysis compared to conventional purification techniques. The time needed to get target biomolecule is also reduced due to of the fact that binding of the biomolecule by MPs can protect it against physical and biological damage, e.g. denaturation [107]. The mostly used MPs in biosensors applications are superparamagnetic nanoparticles composed of ferrous oxide or ferric oxide [108]. Nanoparticles have a lot of physico-chemical advantages [109, 110]. Their size can be adapted to the extension and kind of a biological sample which is a source of target biomolecules (e.g. proteins 5–50 nm, viruses 20–450 nm, cells 10–100 μm) [111-115] and their surface can be modified by numerous substances including antibodies. 8-OHdG has not necessarily been detected by electrochemical detector directly. Indirect detection with 1-naphthol generated after 8-OHdG immunoseparation using paramagnetic particles from biological sample has successfully been implemented [40] and it is shown in Fig. (**6A**). The electrochemical detection of formed 1-naphthol subsequently proceeded using the microfluidic system (Fig. **6B**). The advantage of this arrangement consists in the low usage of sample (10 μl) and very satisfactory detection limit (Table **1**). For the characterization of the system, number of parameters has been optimized [40]. Minimal volume of dosed sample in the electrolyte was tested. Here, already volume of 8 μl led to the achievement of maximum signal. The influence of the time of the sample accumulation on the surface of GC electrode, namely on the height of the signal was also investigated. The best values were determined for the 10 s of accumulation; in addition, longer time of accumulation led to the shift of signal into more positive values. The change in

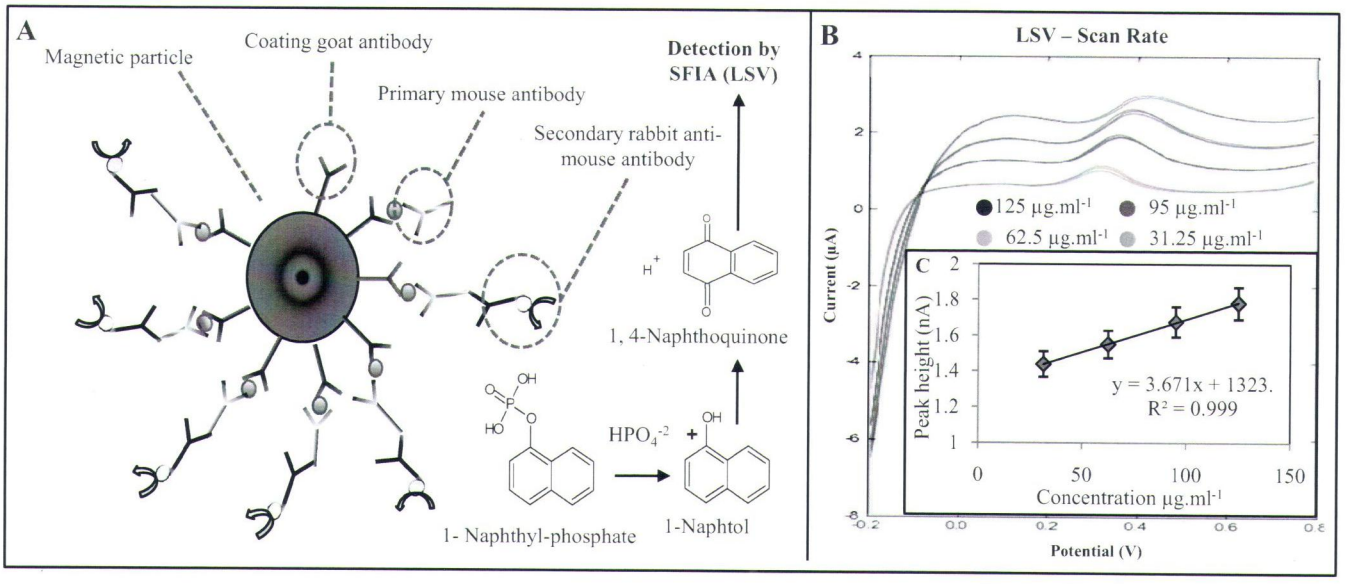

Fig. (6). (**A**) The recombinant G protein that was covalently bound to the nanoparticles was connected with antibody conjugated with alkaline phosphatase. Alkaline phosphatase, which acts as an enzyme in reaction, splits naphthyl phosphate on naphthol and hydrogenphosphate. Product naphthol was detected electrochemically (linear scan voltammetry) [40]. (**B**) Calibration dependence of measured voltage and potential on the different naphthol concentrations measured using the SFIA. (**C**) Calibration dependence of 1- naphthol in the range from 31.25 $\mu g.ml^{-1}$ to 125 $\mu g.ml^{-1}$.

the signal height and course of the cyclic voltammogram at different scan rates (0.05, 0.1 and 0.15 (V/s)) were analysed for the characterization system. They were compared with earlier works, where similar results were obtained [116]. Although the studies focused on the determination of the oxidative products of DNA using flow analyses are almost missing, these techniques provide many advantages such as sensitive detection and excellent repeatability.

ABBREVIATIONS

8-OHdG	=	8-Oxo-7,8-dihydro-2'-deoxyguanosine
AdS	=	Adsorptive stripping technique
AdTS	=	Adsorptive transfer stripping technique
CE-ED	=	Capillary electrophoresis with electrochemical detection
CV	=	Cyclic voltammetry
DPASV	=	Differential pulse anodic stripping voltammetry
ED	=	With electrochemical detection
ELISA	=	Enzyme-linked immunoSorbent assay
FIA	=	Flow injection analysis
GC-MS	=	Gas chromatography with mass spectrometric detection
GCE	=	Glassy carbon electrode
HMDE	=	Hanging mercury drop electrode
HPLC	=	High performance liquid chromatography
LD	=	Limit of detection
LQ	=	Limit of quantification
LSV	=	Linear sweep voltammetry
MPs	=	Paramagnetic or superparamagnetic particles/beads
MS	=	Mass spectrometry
MWNT	=	Multi-walled carbon nanotubes
PCR	=	Polymerase chain reaction
POC	=	Point-of-care
ROS	=	Reactive oxygen species
SPE	=	Solid phase extraction
SFIA	=	Segmental flow injection analysis
SWV	=	Square wave voltammetry

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest by any means with respect to the instant research manuscript.

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REFERENCES

[1] Dandona, P.; Thusu, K.; Cook, S.; Snyder, B.; Makowski, J.; Armstrong, D.; Nicotera, T. Oxidative damage to DNA in diabetes mellitus. *Lancet*, **1996**, *347*(8999), 444-445.

[2] Kikuchi, A.; Takeda, A.; Onodera, H.; Kimpara, T.; Hisanaga, K.; Sato, N.; Nunomura, A.; Castellani, R. J.; Perry, G.; Smith, M. A.; Itoyama, Y. Systemic increase of oxidative nucleic acid damage in Parkinson's disease and multiple system atrophy. *Neurobiol. Dis.*, **2002**, *9*(2), 244-248.

[3] Markesbery, W. R.; Carney, J. M. Oxidative alterations in Alzheimer's disease. *Brain Pathol.*, **1999**, *9*(1), 133-146.

[4] Wu, L. L.; Chiou, C. C.; Chang, P. Y.; Wu, J. T. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. *Clin. Chim. Acta*, **2004**, *339*(1-2), 1-9.

[5] Valko, M.; Leibfritz, D.; Moncol, J.; Cronin, M. T. D.; Mazur, M.; Telser, J. Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.*, **2007**, *39*(1), 44-84.

[6] Halliwell, B. The wanderings of a free radical. *Free Radic. Biol. Med.*, **2009**, *46*(5), 531-542.

[7] Elledge, R. M.; Lee, W. H. Life and death by p53. *BioEssays*, **1995**, *17*(11), 923.

[8] Lane, D. P. Cancer - p53, guardian of the genome. *Nature*, **1992**, *385*(15).

[9] Meek, D. W. Posttranslation modification of p53. *Semin. Cancer Biol.*, **1994**, *5*(3), 203-210.

[10] Potesil, D.; Mikelova, R.; Adam, V.; Kizek, R.; Prusa, R. Change of the protein p53 electrochemical signal according to its structural form - quick and sensitive distinguishing of native, denatured, and aggregated form of the "Guardian of the Genome". *Protein J.*, **2006**, *25*(1), 23-32.

[11] McLure, K. G.; Lee, P. W. How p53 binds DNA as a tetramer. *EMBO J.*, **1998**, *17*, 3342-3350.

[12] Dwyer, D. J.; Kohanski, M. A.; Hayete, B.; Collins, J. J. Gyrase inhibitors induce an oxidative damage cellular death pathway in Escherichia coli. *Mol. Syst. Biol.*, **2007**, *3*, 91.

[13] Evans, M. D.; Dizdaroglu, M.; Cooke, M. S. Oxidative DNA damage and disease: induction, repair and significance. *Mutat. Res.-Rev. Mutat. Res.*, **2004**, *567*(1), 1-61.

[14] Lloyd, D. R.; Phillips, D. H. Oxidative DNA damage mediated by copper(II), iron(II) and nickel(II) Fenton reactions: evidence for site-specific mechanisms in the formation of double-strand breaks, 8-hydroxydeoxyguanosine and putative intrastrand cross-links. *Mutat. Res.-Fundam. Mol. Mech. Mutagen.*, **1999**, *424*(1-2), 23-36.

[15] Collins, A. R.; Cadet, J.; Moller, L.; Poulsen, H. E.; Vina, J. Are we sure we know how to measure 8-oxo-7,8-dihydroguanine in DNA from human cells? *Arch. Biochem. Biophys.*, **2004**, *423*(1), 57-65.

[16] Lagorio, S.; Tagesson, C.; Forastiere, F.; Iavarone, I.; Axelson, O.; Carere, A. Exposure to benzene and urinary concentrations of 8-hydroxydeoxyguanosine, a biological marker of oxidative damage to DNA. *Occup. Environ. Med.*, **1994**, *51*(11), 739-743.

[17] Pilger, A.; Rudiger, H. W. 8-Hydroxy-2'-deoxyguanosine as a marker of oxidative DNA damage related to occupational and environmental exposures. *Int. Arch. Occup. Environ. Health*, **2006**, *80*(1), 1-15.

[18] Valavanidis, A.; Vlachogianni, T.; Fiotakis, C. 8-hydroxy-2' -deoxyguanosine (8-OHdG): A Critical Biomarker of Oxidative Stress and Carcinogenesis. *J. Environ. Sci. Health Pt. C-Environ. Carcinog. Ecotoxicol. Rev.*, **2009**, *27*(2), 120-139.

[19] Barroso, M. F.; de-los-Santos-Alvarez, N.; Delerue-Matos, C.; Oliveira, M. Towards a reliable technology for antioxidant capacity and oxidative damage evaluation: Electrochemical (bio)sensors. *Biosens. Bioelectron.*, **2011**, *30*(1), 1-12.

[20] Rusling, J. F. Sensors for toxicity of chemicals and oxidative stress based on electrochemical catalytic DNA oxidation. *Biosens. Bioelectron.*, **2004**, *20*(5), 1022-1028.

[21] Fojta, M. Electrochemical sensors for DNA interactions and damage. *Electroanalysis*, **2002**, *14*(21), 1449-1463.

[22] Hocek, M.; Fojta, M. Nucleobase modification as redox DNA labelling for electrochemical detection. *Chem. Soc. Rev.*, **2011**, *40*(12), 5802-5814.

[23] Gutierrez, A.; Osegueda, S.; Gutierrez-Granados, S.; Alatorre, A.; Garcia, M. G.; Godinez, L. A. Amperometric Detection and Quantification of 8-Hydroxy-2'-deoxyguanosine (8-OHdG) Using

Dendrimer Modified Electrodes. *Electroanalysis*, **2008**, *20*(21), 2294-2300.

[24] Kato, D.; Komoriya, M.; Nakamoto, K.; Kurita, R.; Hirono, S.; Niwa, O. Electrochemical Determination of Oxidative Damaged DNA with High Sensitivity and Stability Using a Nanocarbon Film. *Anal. Sci.*, **2011**, *27*(7), 703-707.

[25] Harri, M.; Kasai, H.; Mori, T.; Tornaeus, J.; Savela, K.; Peltonen, K. Analysis of 8-hydroxy-2 '-deoxyguanosine in urine using high-performance liquid chromatography-electrospray tandem mass spectrometry. *J. Chromatogr. B*, **2007**, *853*(1-2), 242-246.

[26] Hu, J. J.; Zhang, W. B.; Ma, H. M.; Cai, Y. M.; Sheng, G. Y.; Fu, J. M. Simultaneous determination of 8-hydroxy-2'-deoxyguanosine and 5-methyl-2'-deoxycytidine in DNA sample by high performance liquid chromatography/positive electrospray ionization tandem mass spectrometry. *J. Chromatogr. B*, **2010**, *878*(28), 2765-2769.

[27] Collins, A. R.; Brown, J.; Bogdanov, M.; Cadet, J.; Cooke, M.; Douki, T.; Dunster, C.; Eakins, J.; Epe, B.; Evans, M.; Farmer, P.; Gedik, C. M.; Halliwell, B.; Herbert, K.; Hofer, T.; Hutchinson, R.; Jenner, A.; Jones, G. D. D.; Kasai, H.; Kelly, F.; Lloret, A.; Loft, S.; Lunec, J.; McEwan, M.; Moller, L.; Olinski, R.; Podmore, I.; Poulsen, H.; Ravanat, J. L.; Rees, J. F.; Reetz, F.; Shertzer, H.; Spiegelhalder, B.; Turesky, R.; Tyrrell, R.; Vina, J.; Vinicombe, D.; Weimann, A.; de Wergifosse, B.; Wood, S. G.; Escodd. Comparison of different methods of measuring 8-oxoguanine as a marker of oxidative DNA damage. *Free Radic. Res.*, **2000**, *32*(4), 333-341.

[28] Kasai, H. A new automated method to analyze urinary 8-hydroxydeoxyguanosine by a high-performance liquid chromatography-electrochemical detector system. *J. Radiat. Res.*, **2003**, *44*(2), 185-189.

[29] Serrano, J.; Palmeira, C. M.; Wallace, K. B.; Kuehl, D. W. Determination of 8-hydroxydeoxyguanosine in biological tissue by liquid chromatography electrospray ionization mass spectrometry mass spectrometry. *Rapid Commun. Mass Spectrom.*, **1996**, *10*(14), 1789-1791.

[30] Wang, C. J.; Yang, N. H.; Chang, C. C.; Liou, S. H.; Lee, H. L. Rapid and simple one-step membrane extraction for the determination of 8-hydroxy-2'-deoxyguanosine in human plasma by a combination of on-line solid phase extraction and LC-MS/MS. *J. Chromatogr. B*, **2011**, *879*(30), 3538-3543.

[31] Arnett, S. D.; Osbourn, D. M.; Moore, K. D.; Vandaveer, S. S.; Lunte, C. E. Determination of 8-oxoguanine and 8-hydroxy-2'-deoxyguanosine in the rat cerebral cortex using microdialysis sampling and capillary electrophoresis with electrochemical detection. *J. Chromatogr. B*, **2005**, *827*(1), 16-25.

[32] Li, M. J.; Zhang, J. B.; Li, W. L.; Chu, Q. C.; Ye, J. N. Capillary electrophoretic determination of DNA damage markers: Content of 8-hydroxy-2'-deoxyguanosine and 8-nitroguanine in urine. *J. Chromatogr. B*, **2011**, *879*(32), 3818-3822.

[33] Samcova, E.; Marhol, P.; Opekar, F.; Langmaier, J. Determination of urinary 8-hydroxy-2'-deoxyguanosine in obese patients by HPLC with electrochemical detection. *Anal. Chim. Acta*, **2004**, *516*(1-2), 107-110.

[34] Kvasnicova, V.; Samcova, E.; Jursova, A.; Jelinek, I. Determination of 8-hydroxy-2'-deoxyguanosine in untreated urine by capillary electrophoresis with UV detection. *J. Chromatogr. A*, **2003**, *985*(1-2), 513-517.

[35] Weiss, D. J.; Lunte, C. E. Detection of a urinary biomaker for oxidative DNA damage 8-hydroxydeoxyguanosine by capillary electrophoresis with electrochemical detection. *Electrophoresis*, **2000**, *21*(10), 2080-2085.

[36] Xu, G. W.; Yao, Q. H.; Weng, Q. F.; Su, B. L.; Zhang, X.; Xiong, J. H. Study of urinary 8-hydroxydeoxyguanosine as a biomarker of oxidative DNA damage in diabetic nephropathy patients. *J. Pharm. Biomed. Anal.*, **2004**, *36*(1), 101-104.

[37] Sabatini, L.; Barbieri, A.; Tosi, M.; Roda, A.; Violante, F. S. A method for routine quantitation of urinary 8-hydroxy-2'-deoxyguanosine based on solid-phase extraction and micro-high-performance liquid chromatography electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.*, **2005**, *19*(2), 147-152.

[38] Chiou, C. C.; Chang, P. Y.; Chan, E. C.; Wu, T. L.; Tsao, K. C.; Wu, J. T. Urinary 8-hydroxydeoxyguano sine and its analogs as DNA marker of oxidative stress: development of an ELISA and measurement in both bladder and prostate cancers. *Clin. Chim. Acta*, **2003**, *334*(1-2), 87-94.

[39] Yin, B. Y.; Whyatt, R. M.; Perera, F. P.; Randall, M. C.; Cooper, T. B.; Santella, R. M. Determination of 8-hydroxydeoxyguanosine by an immunuaffinity chromatography-monoclonal antibody-based ELISA. *Free Radic. Biol. Med.*, **1995**, *18*(6), 1023-1032.

[40] Zitka, O.; Krizkova, S.; Krejcova, L.; Hynek, D.; Gumulec, J.; Masarik, M.; Sochor, J.; Adam, V.; Hubalek, J.; Trnkova, L.; Kizek, R. Microfluidic tool based on the antibody-modified paramagnetic particles for detection of 8-hydroxy-2'-deoxyguanosine in urine of prostate cancer patients. *Electrophoresis*, **2011**, *32*(22), 3207-3220.

[41] Collins, A. R.; Duthie, S. J.; Dobson, V. L. Direct enzymatic detection of endogenous oxidative base damage in human lymphocyte DNA. *Carcinogenesis*, **1993**, *14*(9), 1733-1735.

[42] Epe, B.; Hegler, J. Oxidative DNA-damage - endonuclease fingerprinting. *Methods Enzymol.-Oxygen Radicals in Biological Systems, Pt D*, **1994**, *234*(122-131.

[43] Hartwig, A.; Dally, H.; Schlepegrell, R. Sensitive analysis of oxidative DNA damage in mammalian cells: Use of the bacterial Fpg protein in combination with alkaline unwinding. *Toxicol. Lett.*, **1996**, *88*(1-3), 85-90.

[44] Cadet, J.; D'Ham, C.; Douki, T.; Pouget, J. P.; Ravanat, J. L.; Sauvaigo, S. Facts and artifacts in the measurement of oxidative base damage to DNA. *Free Radic. Res.*, **1998**, *29*(6), 541-550.

[45] Finnegan, M. T. V.; Herbert, K. E.; Evans, M. D.; Griffiths, H. R.; Lunec, J. Evidence for sensitisation of DNA to oxidative damage during isolation. *Free Radic. Biol. Med.*, **1996**, *20*(1), 93-98.

[46] Ravanat, J. L.; Turesky, R. J.; Gremaud, E.; Trudel, L. J.; Stadler, R. H. Determination of 8-oxoguanine in DNA by gas chromatography mass-spectrometry and HPLC-electrochemical detection - overestimation of the background level of the oxidized base by the gas-chromatography mass-spectrometry assay. *Chem. Res. Toxicol.*, **1995**, *8*(8), 1039-1045.

[47] Wan, C. D.; Liu, T.; Wei, S.; Zhang, S. H. Electrochemical determination of 8-hydroxydeoxyguanosine using a carbon nanotube modified electrode. *Russ. J. Electrochem.*, **2008**, *44*(3), 327-331.

[48] Inokuchi, H.; Kato, D.; Ueda, A.; Niwa, O. Development of a Sputtered Nanocarbon Film Based Microdisk Array Electrode for the Highly Stable Detection of Serotonin. *Electroanalysis*, **2011**, *23*(4), 827-831.

[49] Kurita, R.; Tabei, H.; Liu, Z. M.; Horiuchi, T.; Niwa, O. Fabrication and electrochemical properties of an interdigitated array electrode in a microfabricated wall-jet cell. *Sens. Actuator B-Chem.*, **2000**, *71*(1-2), 82-89.

[50] Stulik, K. Electrochemical detection for flow-analysis and liquid-chromatography - present status and some roads to the future. *Anal. Chim. Acta*, **1993**, *273*(1-2), 435-441.

[51] Rebelo, I. A.; Piedade, J. A. P.; Oliveira-Brett, A. M. Development of an HPLC method with electrochemical detection of femtomoles of 8-oxo-7,8-dihydroguanine and 8-oxo-7,8-dihydro-2'-deoxyguanosine in the presence of uric acid. *Talanta*, **2004**, *63*(2), 323-331.

[52] Palecek, E. Oszillographische Polarographie der Nucleinsauren und Ihrer Bestandteile. *Naturwissenschaften*, **1958**, *45*(8), 186-187.

[53] Palecek, E. Oscillographic polarography of highly polymerized deoxyribonucleic acid. *Nature*, **1960**, *188*(4751), 656-657.

[54] Palecek, E.; Frary, B. D. A highly sensitive pulse-polarographic estimation of denatured deoxyribonucleic acid in native deoxyribonucleic acid samples. *Arch. Biochem. Biophys.*, **1966**, *115*(3), 431-436.

[55] Palecek, E.; Bartosik, M. Electrochemistry of Nucleic Acids. *Chem. Rev.*, **2012**, *112*(6), 3427-3481.

[56] Palecek, E.; Tomschik, M.; Stankova, V.; Havran, L. Chronopotentiometric stripping of DNA at mercury electrodes. *Electroanalysis*, **1997**, *9*(13), 990-997.

[57] Shestivska, V.; Adam, V.; Prasek, J.; Macek, T.; Mackova, M.; Havel, L.; Diopan, V.; Zehnalek, J.; Hubalek, J.; Kizek, R. Investigation of the Antioxidant Properties of Metallothionein in Transgenic Tobacco Plants using Voltammetry at a Carbon Paste Electrode. *Int. J. Electrochem. Sci.*, **2011**, *6*(7), 2869-2883.

[58] Tomschik, M.; Jelen, F.; Havran, L.; Trnkova, L.; Nielsen, P. E.; Palecek, E. Reduction and oxidation of peptide nucleic acid and DNA at mercury and carbon electrodes. *J. Electroanal. Chem.*, **1999**, *476*(1), 71-80.

[59] Brabec, V.; Dryhurst, G. Electrochemical behaviour of natural and biosynthetic polynucleotides at pyrolytic-graphite electrode A new probe for studies of polynucleotide structure and reactions. *J. Electroanal. Chem.*, **1978**, *89*(1), 161-173.

[60] Dryhurst, G.; Elving, P. J. Electrochemical oxidation of adenine reaction products and mechanisms. *J. Electrochem. Soc.*, **1968**, *115*(10), 1014-&.

[61] Dryhurst, G.; Pace, G. F. Electrochemical oxidation of guanine at pyrolytic graphite electrode. *J. Electrochem. Soc.*, **1970**, *117*(10), 1259-&.

[62] Oliveira-Brett, A. M.; Piedade, J. A. P.; Silva, L. A.; Diculescu, V. C. Voltammetric determination of all DNA nucleotides. *Anal. Biochem.*, **2004**, *332*(2), 321-329.

[63] Stempkowska, I.; Liga, M.; Jasnowska, J.; Langer, J.; Filipiak, M. Electrochemical response of oligonucleotides on carbon paste electrode. *Bioelectrochemistry*, **2007**, *70*(2), 488-494.

[64] Palecek, E.; Boublikova, P.; Jelen, F. Cyclic voltammetry of nucleic-acids and determination of submicrogram quantities of deoxyribonucleic acids by adsorptive stripping voltammetry. *Anal. Chim. Acta*, **1986**, *187*, 99-107.

[65] Palecek, E.; Postbieglova, I. Adsorptive stripping voltammetry of biomacromolecules with transfer of the adsorbed layer. *J. Electroanal. Chem.*, **1986**, *214*(1-2), 359-371.

[66] Palecek, E. Adsorptive transfer stripping voltammetry - determination of nanogram quantities of DNA immobilized at the electrode surface. *Anal. Biochem.*, **1988**, *170*(2), 421-431.

[67] Wang, J.; Chicharro, M.; Rivas, G.; Cai, X. H.; Dontha, N.; Farias, P. A. M.; Shiraishi, H. DNA biosensor for the detection of hydrazines. *Anal. Chem.*, **1996**, *68*(13), 2251-2254.

[68] Chiti, G.; Marrazza, G.; Mascini, M. Electrochemical DNA biosensor for environmental monitoring. *Anal. Chim. Acta*, **2001**, *427*(2), 155-164.

[69] Lucarelli, F.; Kicela, A.; Palchetti, I.; Marrazza, G.; Mascini, M. Electrochemical DNA biosensor for analysis of wastewater samples. *Bioelectrochemistry*, **2002**, *58*(1), 113-118.

[70] Oliveira, S. C. B.; Oliveira-Brett, A. M. In situ evaluation of chromium-DNA damage using a DNA-electrochemical biosensor. *Anal. Bioanal. Chem.*, **2010**, *398*(4), 1633-1641.

[71] Holmberg, R. C.; Tierney, M. T.; Ropp, P. A.; Berg, E. E.; Grinstaff, M. W.; Thorp, H. H. Intramolecular electrocatalysis of 8-oxo-guanine oxidation: Secondary structure control of electron transfer in osmium-labeled oligonucleotides. *Inorg. Chem.*, **2003**, *42*(20), 6379-6387.

[72] Koide, S.; Kinoshita, Y.; Ito, N.; Kimura, J.; Yokoyama, K.; Karube, I. Determination of human serum 8-hydroxy-2'-deoxyguanosine (8-OHdG) by HPLC-ECD combined with solid phase extraction (SPE). *J. Chromatogr. B*, **2010**, *878*(23), 2163-2167.

[73] Oliveira-Brett, A. M.; Vivan, M.; Fernandes, I. R.; Piedade, J. A. P. Electrochemical detection of in situ adriamycin oxidative damage to DNA. *Talanta*, **2002**, *56*(5), 959-970.

[74] Oliveira-Brett, A. M.; Diculescu, V. C. Electrochemical study of quercetin-DNA interactions - Part II. In situ sensing with DNA biosensors. *Bioelectrochemistry*, **2004**, *64*(2), 143-150.

[75] Matayatsuk, C.; Wilairat, P. Quantitative determination of 8-hydroxy-2'-deoxyguanosine as a biomarker of oxidative stress in thalassemic patients using HPLC with an electrochemical detector. *J. Anal. Chem.*, **2008**, *63*(1), 52-56.

[76] Ahmad, F.; Hashsham, S. A. Miniaturized nucleic acid amplification systems for rapid and point-of-care diagnostics: A review. *Anal. Chim. Acta*, **2012**, *733*(1-15.

[77] Lai, C. H.; Liou, S. H.; Lin, H. C.; Shih, T. S.; Tsai, P. J.; Chen, J. S.; Yang, T.; Jaakkola, J. J. K.; Strickland, P. T. Exposure to traffic exhausts and oxidative DNA damage. *Occup. Environ. Med.*, **2005**, *62*(4), 216-222.

[78] Calderon-Garciduenas, L.; Lian, W. W.; Zhang, Y. J.; Rodriguez-Alcaraz, A.; Osnaya, N.; Villarreal-Calderon, A.; Santella, R. M. 8-hydroxy-2'-deoxyguanosine, a major mutagenic oxidative DNA lesion, and DNA strand breaks in nasal respiratory epithelium of children exposed to urban pollution. *Environ. Health Perspect.*, **1999**, *107*(6), 469-474.

[79] Kim, J. H.; Moon, J. Y.; Park, E. Y.; Lee, K. H.; Hong, Y. C. Changes in Oxidative Stress Biomarker and Gene Expression Levels in Workers Exposed to Volatile Organic Compounds. *Ind. Health*, **2011**, *49*(1), 8-14.

[80] Wong, R. H.; Kuo, C. Y.; Hsu, M. L.; Wang, T. Y.; Chang, P. I.; Wu, T. H.; Huang, S. Increased levels of 8-hydroxy-2'-deoxyguanosine attributable to carcinogenic metal exposure among schoolchildren. *Environ. Health Perspect.*, **2005**, *113*(10), 1386-1390.

[81] Kuo, H. W.; Chang, S. F.; Wu, K. Y.; Wu, F. Y. Chromium(VI) induced oxidative damage to DNA: increase of urinary 8-hydroxydeoxyguanosine concentrations (8-OHdG) among electroplating workers. *Occup. Environ. Med.*, **2003**, *60*(8), 590-594.

[82] Fujino, Y.; Guo, X. J.; Liu, J.; Matthews, I. P.; Shirane, K.; Wu, K. G.; Kasai, H.; Miyatake, M.; Tanabe, K.; Kusuda, T.; Yoshimura, T.; Japan Inner Mongolia Arsenic, P. Chronic arsenic exposure and urinary 8-Hydroxy-2'-deoxyguanosine in an arsenic-affected area in Inner Mongolia, China. *J. Exp. Anal. Environ. Epidem.*, **2005**, *15*(2), 147-152.

[83] Jeng, H. A.; Pan, C. H.; Diawara, N.; Chang-Chien, G. P.; Lin, W. Y.; Huang, C. T.; Ho, C. K.; Wu, M. T. Polycyclic aromatic hydrocarbon-induced oxidative stress and lipid peroxidation in relation to immunological alteration. *Occup. Environ. Med.*, **2011**, *68*(9), 653-658.

[84] Lee, M. W.; Chen, M. L.; Lung, S. C. C.; Tsai, C. J.; Lai, C. F. S.; Yang, S. C.; Mao, I. F. Increase of urinary concentrations of 8-hydroxy-2'-deoxyguanosine in diesel exhaust emission inspector exposed to polycyclic aromatic hydrocarbons. *Int. Arch. Occup. Environ. Health*, **2012**, *85*(3), 273-282.

[85] Liu, A. L.; Lu, W. Q.; Wang, Z. Z.; Chen, W. H.; Lu, W. H.; Yuan, J.; Nan, P. H.; Sun, J. Y.; Zou, Y. L.; Zhou, L. H.; Zhang, C.; Wu, T. C. Elevated levels of urinary 8-hydroxy-2'-deoxyguanosine, lymphocytic micronuclei, and serum glutathione S-transferase in workers exposed to coke oven emissions. *Environ. Health Perspect.*, **2006**, *114*(5), 673-677.

[86] Zhang, J.; Ichiba, M.; Hanaoka, T.; Pan, G.; Yamano, Y.; Hara, K.; Takahashi, K.; Tomokuni, K. Leukocyte 8-hydroxydeoxyguanosine and aromatic DNA adduct in coke-oven workers with polycyclic aromatic hydrocarbon exposure. *Int. Arch. Occup. Environ. Health*, **2003**, *76*(7), 499-504.

[87] Huang, Y. W.; Jian, L.; Zhang, M. B.; Zhou, Q.; Yan, X. F.; Hua, X. D.; Zhou, Y.; He, J. L. An Investigation of Oxidative DNA Damage in Pharmacy Technicians Exposed to Antineoplastic Drugs in Two Chinese Hospitals Using The Urinary 8-OHdG Assay. *Biomed. Environ. Sci.*, **2012**, *25*(1), 109-116.

[88] Matsui, A.; Ikeda, T.; Enomoto, K.; Hosoda, K.; Nakashima, H.; Omae, K.; Watanabe, M.; Hibi, T.; Kitajima, M. Increased formation of oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine, in human breast cancer tissue and its relationship to GSTP1 and COMT genotypes. *Cancer Lett.*, **2000**, *151*(1), 87-95.

[89] Miyake, H.; Hara, I.; Kamidono, S.; Eto, H. Oxidative DNA damage in patients with prostate cancer and its response to treatment. *J. Urolog.*, **2004**, *171*(4), 1533-1536.

[90] Dincer, Y.; Erzin, Y.; Himmetoglu, S.; Gunes, K. N.; Bal, K.; Akcay, T. Oxidative DNA damage and antioxidant activity in patients with inflammatory bowel disease. *Dig. Dis. Sci.*, **2007**, *52*(7), 1636-1641.

[91] Honda, M.; Yamada, Y.; Tomonaga, M.; Ichinose, H.; Kamihira, S. Correlation of urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG), a biomarker of oxidative DNA damage, and clinical features of hematological disorders: a pilot study. *Leuk. Res.*, **2000**, *24*(6), 461-468.

[92] Kato, J.; Kobune, M.; Nakamura, T.; Kuroiwa, G.; Takada, K.; Takimoto, R.; Sato, Y.; Fujikawa, K.; Takahashi, M.; Takayama, T.; Ikeda, T.; Niitsu, Y. Normalization of elevated hepatic 8-hydroxy-2'-deoxyguanosine levels in chronic hepatitis C patients by phlebotomy and low iron diet. *Cancer Res.*, **2001**, *61*(24), 8697-8702.

[93] Tsukahara, H.; Shibata, R.; Ohshima, Y.; Todoroki, Y.; Sato, S.; Ohta, N.; Hiraoka, M.; Yoshida, A.; Nishima, S.; Mayumi, M. Oxidative stress and altered antioxidant defenses in children with acute exacerbation of atopic dermatitis. *Life Sci.*, **2003**, *72*(22), 2509-2516.

[94] Takane, M.; Sugano, N.; Iwasaki, H.; Iwano, Y.; Shimizu, N.; Ito, K. New biomarker evidence of oxidative DNA damage in whole saliva from clinically healthy and periodontally diseased individuals. *J. Periodont.*, **2002**, *73*(5), 551-554.

[95] Prabhulkar, S.; Li, C. Z. Assessment of oxidative DNA damage and repair at single cellular level *via* real-time monitoring of 8-OHdG biomarker. *Biosens. Bioelectron.*, **2010**, *26*(4), 1743-1749.

[96] Lin, C. C.; Wang, J. H.; Wu, H. W.; Lee, G. B. Microfluidic Immunoassays. *Jala*, **2010**, *15*(3), 253-274.

[97] Paixao, T.; Garcia, C. C. M.; Medeiros, M. H. G.; Bertotti, M. Flow injection amperometric detection of 2'-deoxyguanosine at a ruthenium oxide hexacyanoferrate modified electrode. *Anal. Chem.*, **2007**, *79*(14), 5392-5398.

[98] Li, T. H.; Jia, W. L.; Wang, H. S.; Liu, R. M. Electrochemical performance of 8-hydroxy-2'-deoxyguanosine and its detection at poly(3-methylthiophene) modified glassy carbon electrode. *Biosens. Bioelectron.*, **2007**, *22*(7), 1245-1250.

[99] Pumera, M.; Merkoci, A.; Alegret, S. New materials for electrochemical sensing VII. Microfluidic chip platforms. *Trac-Trends Anal. Chem.*, **2006**, *25*(3), 219-235.

[100] Becker, C.; Hodenius, M.; Blendinger, G.; Sechi, A.; Hieronymus, T.; Muller-Schulte, D.; Schmitz-Rode, T.; Zenke, M. Uptake of magnetic nanoparticles into cells for cell tracking. *J. Magn. Magn. Mater.*, **2007**, *311*(1), 234-237.

[101] Cao, Z. J.; Li, Z. X.; Zhao, Y. J.; Song, Y. M.; Lu, J. Z. Magnetic bead-based chemiluminescence detection of sequence-specific DNA by using catalytic nucleic acid labels. *Anal. Chim. Acta*, **2006**, *557*(1-2), 152-158.

[102] Chiou, C. H.; Huang, Y. Y.; Chiang, M. H.; Lee, H. H.; Lee, G. B. New magnetic tweezers for investigation of the mechanical properties of single DNA molecules. *Nanotechnology*, **2006**, *17*(5), 1217-1224.

[103] Jaffrezic-Renault, N.; Martelet, C.; Chevolot, Y.; Cloarec, J. P. Biosensors and bio-bar code assays based on biofunctionalized magnetic microbeads. *Sensors*, **2007**, *7*(4), 589-614.

[104] Kim, D. K.; Zhang, Y.; Voit, W.; Rao, K. V.; Muhammed, M. Synthesis and characterization of surfactant-coated superparamagnetic monodispersed iron oxide nanoparticles. *J. Magn. Magn. Mater.*, **2001**, *225*(1-2), 30-36.

[105] Kinoshita, T.; Seino, S.; Mizukoshi, Y.; Nakagawa, T.; Yamamoto, T. A. Functionalization of magnetic gold/iron-oxide composite nanoparticles with oligonucleotides and magnetic separation of specific target. *J. Magn. Magn. Mater.*, **2007**, *311*(1), 255-258.

[106] Palecek, E.; Fojta, M. Magnetic beads as versatile tools for electrochemical DNA and protein biosensing. *Talanta*, **2007**, *74*(3), 276-290.

[107] Yeung, S. W.; Hsing, I. M. Manipulation and extraction of genomic DNA from cell lysate by functionalized magnetic particles for lab on a chip applications. *Biosens. Bioelectron.*, **2006**, *21*(7), 989-997.

[108] Ngomsik, A. F.; Bee, A.; Draye, M.; Cote, G.; Cabuil, V. Magnetic nano- and microparticles for metal removal and environmental applications: a review. *C. R. Chim.*, **2005**, *8*(6-7), 963-970.

[109] Lund-Olesen, T.; Dufva, M.; Hansen, M. F. Capture of DNA in microfluidic channel using magnetic beads: Increasing capture efficiency with integrated microfluidic mixer. *J. Magn. Magn. Mater.*, **2007**, *311*(1), 396-400.

[110] Lunov, O.; Bespalova, S.; Zablotskii, V. A model for magnetic bead microrheometry. *J. Magn. Magn. Mater.*, **2007**, *311*(1), 162-165.

[111] Hsing, I. M.; Xu, Y.; Zhao, W. T. Micro- and nano-magnetic particles for applications in biosensing. *Electroanalysis*, **2007**, *19*(7-8), 755-768.

[112] Huska, D.; Adam, V.; Trnkova, L.; Kizek, R. The dependence of adenine isolation efficiency on the chain length evidenced using paramagnetic particles and voltammetry measurements. *J. Magn. Magn. Mater.*, **2009**, *321*(10), 1474-1477.

[113] Huska, D.; Hubalek, J.; Adam, V.; Vajtr, D.; Horna, A.; Trnkova, L.; Havel, L.; Kizek, R. Automated nucleic acids isolation using paramagnetic microparticles coupled with electrochemical detection. *Talanta*, **2009**, *79*(2), 402-411.

[114] Xu, C. J.; Sun, S. H. Monodisperse magnetic nanoparticles for biomedical applications. *Polym. Int.*, **2007**, *56*(7), 821-826.

[115] Yu, C. H.; Lo, C. C. H.; Tam, K.; Tsang, S. C. Monodisperse binary nanocomposite in silica with enhanced magnetization for magnetic separation. *J. Phys. Chem. C*, **2007**, *111*(22), 7879-7882.

[116] Palecek, E.; Kizek, R.; Havran, L.; Billova, S.; Fojta, M. Electrochemical enzyme-linked immunoassay in a DNA hybridization sensor. *Anal. Chim. Acta*, **2002**, *469*(1), 73-83.

[117] Brett, A. M. O.; Piedade, J. A. P.; Serrano, S. H. P. Electrochemical oxidation of 8-oxoguanine. *Electroanalysis*, **2000**, *12*(12), 969-973.

[118] Gutierrez, A.; Gutierrez, S.; Garcia, G.; Galicia, L.; Rivas, G. A. Determinatiom of 8-Hydroxy 2'-Deoxyguanosine Using Electrodes Modified with a Dispersion of Carbon Nanotubes in Polyethylenimine. *Electroanalysis*, **2011**, *23*(5), 1221-1228.

[119] Pan, H. Z.; Chang, D.; Feng, L. G.; Xu, F. J.; Kuang, H. Y.; Lu, M. J. Oxidative damage to DNA and its relationship with diabetic complications. *Biomed. Environ. Sci.*, **2007**, *20*(2), 160-163.

[120] Watanabe, E.; Matsuda, N.; Shiga, T.; Kajimoto, K.; Ajiro, Y.; Kawarai, H.; Kasanuki, H.; Kawana, M. Significance of 8-hydroxy-2'-deoxyguanosine levels in patients with idiopathic dilated cardiomyopathy. *J. Card. Fail.*, **2006**, *12*(7), 527-532.

[121] Lin, H. S.; Jenner, A. M.; Ong, C. N.; Huang, S. H.; Whiteman, M.; Halliwell, B. A high-throughput and sensitive methodology for the quantification of urinary 8-hydroxy-2'-deoxyguanosine: measurement with gas chromatography-mass spectrometry after single solid-phase extraction. *Biochemical Journal*, **2004**, *380*(541-548.

[122] Moriwaki, H. Determination of 8-hydroxy-2'-deoxyguanosine in urine by liquid chromatography-electrospray ionization-mass spectrometry. *Anal. Sci.*, **2000**, *16*(2), 105-106.

[123] Renner, T.; Fechner, T.; Scherer, G. Fast quantification of the urinary marker of oxidative stress 8-hydroxy-2'-deoxyguanosine using solid-phase extraction and high-performance liquid chromatography with triple-stage quadrupole mass detection. *J. Chromatogr. B*, **2000**, *738*(2), 311-317.

[124] Orhan, H.; Vermeulen, N. P. E.; Tump, C.; Zappey, H.; Meerman, J. H. N. Simultaneous determination of tyrosine, phenylalanine and deoxyguanosine oxidation products by liquid chromatography-tandem mass spectrometry as non-invasive biomarkers for oxidative damage. *J. Chromatogr. B*, **2004**, *799*(2), 245-254.

[125] Crow, B.; Bishop, M.; Kovalcik, K.; Norton, D.; George, J.; Bralley, J. A. A simple and cost effective method for the quantification of 8-hydroxy-2'-deoxyguanosine from urine using liquid chromatography tandem mass spectrometry. *Biomed. Chromatogr.*, **2008**, *22*(4), 394-401.

[126] Bogdanov, M.; Brown, R. H.; Matson, W.; Smart, R.; Hayden, D.; O'Donnell, H.; Beal, M. F.; Cudkowicz, M. Increased oxidative damage to DNA in ALS patients. *Free Radic. Biol. Med.*, **2000**, *29*(7), 652-658.

[127] Bogdanov, M. B.; Beal, M. F.; McCabe, D. R.; Griffin, R. M.; Matson, W. R. A carbon column-based liquid chromatography electrochemical approach to routine 8-hydroxy-2'-deoxyguanosine measurements in urine and other biologic matrices: A one-year evaluation of methods. *Free Radic. Biol. Med.*, **1999**, *27*(5-6), 647-666.

[128] Lengger, C.; Schoch, G.; Topp, H. A high-performance liquid chromatographic method for the determination of 8-oxo-7,8-dihydro-2'-deoxyguanosine in urine from man and rat. *Anal. Biochem.*, **2000**, *287*(1), 65-72.

[129] Bolin, C.; Cardozo-Pelaez, F. Assessing biomarkers of oxidative stress: Analysis of guanosine and oxidized guanosine nucleotide triphosphates by high performance liquid chromatography with electrochemical detection. *J. Chromatogr. B*, **2007**, *856*(1-2), 121-130.

[130] Giaccia, A. J.; Kastan, M. B. The complexity of p53 modulation: emerging patterns from divergent signals. *Genes Dev.*, **1998**, *12*(19), 2973-2983.

[131] David, S. S.; O'Shea, V. L.; Kundu, S. Base-excision repair of oxidative DNA damage. *Nature*, **2007**, *447*(7147), 941-950.

[132] Adam, V.; Baloun, J.; Fabrik, I.; Trnkova, L.; Kizek, R. An electrochemical detection of metallothioneins at the zeptomole level in nanolitre volumes. *Sensors*, **2008**, *8*(4), 2293-2305.

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