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Electrochemical Biosensors; A Promising Tool in Pharmaceutical Analysis

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Introduction

One of the main challenges in analytical chemistry concerns the fast and simultaneous detection of compounds in clinical, environmental and food samples. For this purpose biosensors are promising tools for detecting directly many chemical and biological parameters. Biosensors consist of a biocomponent that performs the molecular recognition of an element, a transducer and an electronic signal processor. The main goal of biochemical sensors is the selective molecular recognition of the analyte which can be accomplished using enzymes, antibodies, nucleic acids, germs, cells and tissues. During the reaction between the biocomponent and the analyte a physicochemical change occurs, which is converted into electrical signal with the transducer. A major group of the biosensors reported in the literature are electrochemical and they are divided into amperometric, potentiometric and conductive. In electrochemical biosensors the transducer is an electrode and the transduction is mainly realized through amperometry or potentiometry. Regarding the analytical importance the two main categories of electrochemical biosensors are the enzyme and DNA biosensors.

An enzyme electrode consists of a thin layer of the biocomponent, immobilized on the electrode's surface. The enzyme is suitably selected to catalyze a reaction, which forms detectable products or consumes reactants. The applied techniques for the enzyme immobilization are the following:

1. Physical or chemical adsorption, this technique is normally applied when the enzyme has a strong affinity for the solid support.
2. Macromolecular or cellular entrapment into polymer matrices, the monomer polymerization can be caused electrochemically or by UV light.
3. Covalent coupling, the cross linking agent that is most commonly used is glutaraldehyde although it frequently inactivates the immobilized enzyme. In order to avoid this phenomenon several methods have been developed allowing site-directed covalent binding.

Enzymes immobilized by these methods show enhanced stability in contrast to enzymes dissolved in solutions. Finally another technique involves the direct mixing of the enzyme with carbon paste, which is both the electrode material and the enzyme immobilization medium.

Amperometry is an electrochemical technique that involves the oxidation or reduction of electroactive compounds on the electrode's surface while a constant potential is being applied.

Oxidase enzymes are frequently immobilized for the construction of amperometric biosensors. The liberated hydrogen peroxide is finally oxidized and detected amperometrically by poising the working electrode approximately at +0.5 V (vs Ag/AgCl).

$$\text{Substrate} + O_2 \xrightarrow{\text{Oxidase}} \text{Product} + H_2O_2$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$$

It is easily noticeable that the measurement depends on the oxygen concentration, which causes a problem since a sufficient amount of oxygen must be present to support the enzyme catalyzed reaction. This problem can be avoided by several approaches, including the use of semi-permeable membranes to limit the diffusion of the analyte to the enzyme layer, avoiding saturation of the enzyme. A different approach is the use of mediators to serve as electron acceptors. Mediators are normally co-immobilized with the enzyme. Mediators transfer electrons to the anode surface, where they are re-oxidized forming a cyclic mechanism. Mediators also allow the lessening of the applied potential compared to the one applied when H_2O_2 is oxidized. The application of high potential for the oxidation of H_2O_2 causes the oxidation of various other interfering substances that are present in the blood, such as ascorbic acid, uric acid or therapeutic drugs. Size exclusion or charge exclusion membranes, such as cellulose acetate or Nafion, are widely used allowing the transport of H_2O_2 and rejecting the interfering substances. Use of electrochemical mediators is another strategy to avoid the problem.

Apart from oxidase enzymes, dehydrogenases can also be immobilized on the electrode's surface for the construction of amperometric biosensors.

$$\text{Substrate} + NAD^+ \xrightarrow{\text{Dehydrogenase}} \text{Product} + NADH$$

$$NADH \rightarrow NAD^+ + H^+ + 2e^-$$

The product nicotinamide adenine dinucleotide (NADH) can be detected by poising the working electrode approximately at +0.5 V - + 0.8 V (vs Ag/AgCl).

In potentiometry the analytical information is obtained by the potential difference between the indicator and the reference electrode while the cell current is zero. The potential change of the indicator's electrode depends on the concentration or activity of the analyte and it is related in a logarithmic manner. In potentiometric biosensors the enzyme can be immobilized on a pH electrode, a gas electrode or an ion-selective electrode.

DNA Biosensors

Nucleic acids in combination with electrochemical transducers produce a type of biosensors and they are based on the interactions between the surface linked DNA and the target drug. The interaction between drugs and DNA occurs mainly in three different ways:

1. The drugs interact with proteins that bind to DNA.
2. RNA binds to DNA double helix forming a triple helical structure or by RNA hybridization to exposed DNA

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3. Aromatic ligand molecules bind to DNA with the following mechanism:

a) electrostatic interaction with the sugar-phosphate structure

b) intercalation of planar aromatic rings between the base pairs

c) groove binding interaction

A great interest occurs in electrochemical investigation of interactions between DNA and DNA-targeted molecules, such as anticancer drugs. These investigations are based on the difference of the electrochemical behaviour of the drugs in the absence and in the presence of DNA. The changes occurring are:

1. Changes in the oxidation or the reduction peak current of the drug.
2. Changes in the peak current of the electroactive DNA bases, such as guanine and adenine.
3. Shift of the potential

Applications in pharmaceutical analysis

Salicylates

Salicylates are the main metabolite from the hydrolysis of acetyl-salicylic acid. Several amperometric biosensors have been found in the literature for the determination of salicylate and its derivatives. The sensors are constructed by immobilizing salicylate hydroxylase, which catalyses the hydroxylation of salicylate to catechol in the presence of NADH and oxygen. The produced catechol is detected amperometrically.

Acetaminophen

Acetaminophen (paracetamol) is an analgesic and antipyretic drug that is alternatively used instead of acetyl-salicylic acid. A biosensor has been constructed for the determination of acetaminophen using the enzyme aryl acylamidase. This enzyme hydrolyses the target compound to 4-aminophenol, which is amperometrically detected at +0.25 V (vs Ag/AgCl).

Paracetamol has also been determined chronoamperometrically using polyphenol oxidase. Avocado tissue was used as the enzyme source and it was incorporated into carbon paste.

Fluoxetine

Fluoxetine is a substance that is used for the treatment of depression and bulimia. Monoamine oxidase was immobilized by electropolymerization of pyrrole on a platinum electrode. The enzyme oxidizes monoamines to aldehydes generating hydrogen peroxide which is finally monitored at +0.7 V (vs Ag/AgCl).

Theophylline

Theophylline is a xanthine drug and is used as a bronchodilator and respiratory stimulant. Theophylline's ability to inhibit alkaline phosphatase has been exploited for the construction of a disposable and electrochemical strip which was a carbon-based electrode system. Alkaline phosphatase catalyses the hydrolysis of *p*-aminophenyl phosphate to *p*-aminophenol, while theophylline is an uncompetitive inhibitor of this interaction and the enzymic reaction proceeds to an extend inversely dependent of the amount of theophylline.

Opiates

An electrochemical biosensor has been reported in the literature for the detection and determination of diacetylmorphine, morphine and their major metabolite mophine-3-glucuronide. Acetylmorphine carboxyesterase catalyses the hydrolysis of heroin to morphine in presence of $NADP^+$, while morphine dehydrogenase oxidizes morphine to morphinone.

Angiotensin converting enzyme inhibitors

Several enantioselective biosensors have been reported for the analysis of angiotensin converting enzyme inhibitors, which are drugs that are used in the treatment of hypertension. The enzyme L-aminoacid oxidase was immobilized on a carbon paste electrode and the drugs were applied by applying chronoamperometry.

Catecholamines

Epinephrine and dopamine are catecholamines and they have both been determined in pharmaceutical formulations using a biosensor based on carbon paste. The enzyme polyphenol oxidase catalyzes the oxidation of both of them to epinephrinequinone and dopaminequinone respectively. The enzymatic source of polyphenol oxidase was a crude extract of cara root.

Nicotine

Nicotine is found in many pharmaceutical formulations that are used in anti-tobacco treatment and the inhibiting action of nicotine on enzymes can be used to determine nicotine itself. Scientists have constructed a biosensor with butyrylcholinesterase and choline oxidase immobilized on a nylon membrane using butyrylcholine as substrate. In the reactions involved H_2O_2 is finally produced and detected amperometrically.

Carnitine

The determination of L- and D- enantiomers of carnitine has recently been reported using amperometric biosensors. L-amino acid oxidase and D-amino acid oxidase were used for the assay of L- and D-carnitine respectively, catalyzing convertion of the aminoacids into their ketoacids. The produced H_2O_2 was finally detected amperometrically, while horseradish peroxidase was also used in the construction of the biosensor in order to improve the reduction of H_2O_2.

L-methotrexate

L-methotrexate a drug showing antitumor activity has been determined with the help of seven amperometric biosensors and three more were used for D-methotrexate. Glutamate oxidase, L-amino oxidase and horsheradish peroxidase were used for the assay of L-mitoxanthrone and D-amino oxidase and horsheradish peroxidase for the assay of D-mitoxanthrone.

Rifampicin

Rifampicin is an antituberculosis agent and can be monitored with the help of a horseradish peroxidase-based biosensor. The enzyme was present in the electropolymerization of pyrrole on a Pt electrode. Rifampicin is reduced in the presence of a constant concentration of H_2O_2.

Benzydamine

Benzydamine is a compound having analgesic and anti-inflammatory activity. A flow-through biosensor was developed for the determination of this drug. The enzyme monoamino oxidase has been immobilized on a sol-gel film on carbon paste and platinum electrode. The produced H_2O_2 was finally electrochemically detected.

Creatinine

Creatinine is a final product of creatinine metabolism in mammals and is also used as an ergogenic aid. Creatine can be determined simultaneously with creatinine using amperometric biosensors. The biosensors are based on creatininase, creatiase and sarcosine oxidase enzymes, which are physically immobilized at a carbon paste electrode.

Daunomycin

Daunomycin is an anthracycline antibiotic and antitumor drug and its interaction with dsDNA has been studied. Daunomycin intercalates between the base pairs of DNA, causing a decrease in the daunomycin peak, plus a more positive shoulder appeared, while no changes occurred in the guanine oxidation peak.

Nitroimidazoles

Nitroimidazoles are drugs selectively toxic to anaerobic bacteria. The electrochemical reduction of metronidazole, tinidazole and nimorazole in the presence DNA was performed on a glassy carbon electrode.

Phenothiazine

A biosensor has been reported for the phenothiazine tranquilizers, applying potentiometric stripping analysis and immobilizing DNA at a carbon paste electrode surface.

Carboplatin

The anticancer drug carboplatin has been determined by previous studies in serum samples from women patients using a DNA-modified glassy carbon electrode with the help of DPV. Since carboplatin binds covalently to DNA, an indirect analytical method was developed for the determination of platinum compounds. After the conditioning of the electrode, oxidation curves of the electrodes were observed. Increasing the concentration of the drug in the solution, the oxidation current adenine decreased, while the guanine one decreased slightly.

Quinazoline

An electrochemical study of the interaction between arsenic trioxide and DNA the DPV oxidation signal of guanine obtained with the dsDNA-modified electrode before interaction with As_2O_3 was higher than the one obtained after interaction with the drug. The interaction of five non-electroactive quinazoline derivatives with DNA has been investigated on modified screen printed electrodes. The inactive quinazolines have been detected voltammetricaly and their interaction with dsDNA is based on competitive binding with the DNA electrochemical label $Co(phen)_3^+$.

Mitoxanthrone

Mitoxanthrone is an anthracycline antibiotic showing antitumor action and its voltammetric behavior has been studied at a DNA-biosensor the results showed that the interaction of the drug is not specific to guanine or to adenine bases, but has a preferential interaction with ssDNA in solution.

Mitomycin C

Mitomycin C another applied antitumor agent, has been studied on a hanging mercury drop electrode using stripping voltammetry.

Compared to the already established analytical techniques, electrochemical biosensors take advantage of very low concentrations of the analyte, offer simplicity, low cost and reliability, thus proving them to be an ideal analytical tool.

The electroactivity of DNA azo-bases has offered another very important analytical tool in the investigation of DNA drug interactions.

Electrochemical biosensors represent a challenging alternative in the chemical analysis as well as in the chemical synthesis offering analytical methods as well as impotant analytical tools complementary to other analytical techniques applied in the studies of drug interactions with important biomolecules.