Biosensors and Bioelectronics 108 (2018) 27–37

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

# Electrochemical sensors and biosensors for the analysis of antineoplastic drugs

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ARTICLE INFO

*Keywords:*
Electrochemical sensor
Biosensor
Chemotherapy
Anticancer
Antineoplastic

ABSTRACT

Cancer is a leading cause of death worldwide, often being treated with antineoplastic drugs that have high potential for toxicity to humans and the environment, even at very low concentrations. Therefore, monitoring these drugs is of utmost importance. Among the techniques used to detect substances at low concentrations, electrochemical sensors and biosensors have been noted for their practicality and low cost. This review brings, for the first time, a simplified outline of the main electrochemical sensors and biosensors developed for the analysis of antineoplastic drugs. The drugs analyzed and the methodology used for electrochemical sensing are described, as are the techniques used for drug quantification and the analytical performance of each sensor, highlighting the limit of detection (LOD), as well as the linear range of quantification (LR) for each system. Finally, we present a technological prospection on the development and use of electrochemical sensors and biosensors in the quantification of antineoplastic drugs. A search of international patent databases revealed no patents currently submitted under this topic, suggesting this is an area to be further explored. We also show that the use of these systems has been gaining prominence in recent years, and that the quantification of antineoplastic drugs using electrochemical techniques could bring great financial and health benefits.

## 1. Introduction

Cancer is considered to be a global epidemic. The main forms of treatment are surgery, radiotherapy, and chemotherapy (Silva et al., 2016). Chemotherapy involves the use of cytotoxic or cytostatic drugs to fight tumor cells. However, these drugs often have a high toxicity as well as mutagenic and carcinogenic potential, posing an occupational risk to those who are exposed to them, such as the patients themselves, the workers that prepare and administer them, health care professionals, and researchers (Martins and Della Rosa, 2004; Rizalar et al., 2012).

In addition, antineoplastic drugs, which are drugs used in cancer treatment, are often released into the environment in their complete form (without metabolization) and can cause environmental damage, even at low concentrations (Lutterbeck et al., 2015).

The monitoring of their environmental levels and exposure is therefore essential to minimize the risks associated with these drugs. Despite this, effective monitoring still faces major technical and analytical difficulties, and there is no appropriate standard regarding the type of sampling or the method of analysis that would allow an evaluation of the potential risks of exposure to antineoplastic drugs (Martins and Della Rosa, 2004).

In addition to environmental and occupational monitoring, the monitoring of plasma concentrations of antineoplastic drugs is of great importance. This is because ideal chemotherapy management depends on the personalized therapeutic schemes which are adapted according to the treatment response of each patient. The clinical monitoring of the plasmatic concentration of antineoplastic drugs is important for the optimization of therapy and management of side effects, because at doses that are too high, toxicity and side effects can occur, while at doses that are too low, the desired effects can be reduced or lost (Florea et al., 2015).

The techniques used for the analysis of antineoplastic drugs include: liquid chromatography coupled to mass spectrometry (LC-MS) or to fluorimetry, capillary electrophoresis coupled to UV detection, gas chromatography coupled to mass spectrometry (GC-MS), and others techniques that are sensitive, specific, and accurate, though in general, the equipment used is expensive and a trained technician is required for

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https://doi.org/10.1016/j.bios.2018.02.034
Received 4 November 2017; Received in revised form 2 February 2018; Accepted 12 February 2018
Available online 13 February 2018
0956-5663/ 

its operation (Connor and Smith, 2016). Often, samples require pre-treatment (Nussbaumer et al., 2011), which, along with being labor-intensive and time-consuming, can lead to the potential loss of the analyte (Dogan-Topal and Ozkan, 2011a; Connor and Smith, 2016).

The development of analytical methods for the detection and quantification of these drugs has gained significant interest. The fluorescence covalent microbead immunosorbent assay (FCMIA) and the lateral flow immunoassay (LFIA), are tests that have been demonstrated to be applicable to measuring low levels of surface contamination with antineoplastic drugs. However, in order to fully exploit these assays, some challenges must still be overcome, such as the need to develop antibodies and protein-drug conjugates for analyses, and the possibility of cross-reactivity between the analyzed drugs (Connor and Smith, 2016).

Electrochemical sensors and biosensors are interesting candidates for analyzing antineoplastic drugs, because of their high sensitivity, low cost, and the speed at which they are carried out (Florea et al., 2015). Biosensors are practical and economical tools, which play important roles in the analysis of specific compounds in biological assays, such as in plasma and blood analyses, and may still be miniaturized into small devices, applicable for domestic use or as medical aid devices (Bahadir and Sezgintürk, 2015).

The properties of electrochemical sensors are extremely promising for helping to diagnose cancer and monitor antineoplastic drug therapy. With investments and further research development, these devices may provide rapid analytical results for clinicians or for patients themselves (Dogan-Topal and Ozkan, 2011a).

In the future, these sensing systems may provide analytical results within a few minutes, as shown by the glycosometer currently available on the market, and are therefore extremely promising for improving the efficiency of cancer treatments, and reducing the risks from occupational exposure. Electrochemistry may also provide new insights into drug development and lead to a better understanding of the interactions between antineoplastic drugs and DNA (Dogan-Topal and Ozkan, 2011b).

This work aims to explain the current developments in electrochemical sensors and biosensors for the monitoring of antineoplastic drugs. We also present brief descriptions of the drugs analyzed, the principles of each methodology used, highlight the limit of detection found, as well as the linear range of quantification for various drugs.

## 2. Antineoplastics drugs that have been target of electroanalytical studies

Studies related to the determination of antineoplastic drugs using electrochemical sensors and biosensors have recently been gaining prominence. Some classes of antineoplastic drugs have been explored in electroanalytical studies. Table 1 summarizes the drugs that have been the targets of electroanalytical studies. The next sections provide more details about the research carried out on these drugs.

## 3. Electrochemical sensors for analysis of antineoplastic drugs

Chemical sensors are devices that allow information to be obtained with minimal manipulation of the system in situ, generating results that can be analyzed and correlated with other environmental parameters. Obtaining this analytical information depends on the capacity of the sensor to interact with an analyte. The sensor element is coupled to a transducer, which converts the analyte-sensor interaction into a measurable analytical signal (Lowinsohn and Bertotti, 2006). Examples include signals of electric current, capacitance, or potential, depending on the type of transducer used.

Fig. 1 shows the general scheme of operation of an electrochemical sensor. In this figure, the different ways of applying electric potential, as well as current response models obtained by different voltammetry techniques, are shown.

The active layer of the sensor, as shown in Fig. 1, may be composed of various types of materials, as outlined in Table 2. This active layer is designed to interact with the analyte of interest, and produces a measurable signal once bound. There are also electrodes of various types: gold (Hajian et al., 2015), platinum (Radhapyari et al., 2013), and carbon electrodes (Wei et al., 2014; Radhapyari and Khan, 2015), among others, can be used as transducers.

The use of electrochemical sensors to monitor the concentrations of molecules of biological relevance has been the object of increasing investigation in electroanalytical research (Lowinsohn and Bertotti, 2006). Erdem et al. (2011) showed considerable interest in the development of electrochemical sensors because of their important applications in medicine, bioengineering, clinical diagnosis, epidemic prevention and environmental protection.

The scientific literature already addresses the development and use of some electrochemical sensors for the determination of antineoplastic drugs. Table 2 summarizes some of the main electrochemical sensors found so far. These were developed using different materials and electrochemical techniques.

### 3.1. Electrochemical sensors that use nanoscale materials

Nanotechnology has been considered as a technology of general use, being common to almost all technological sectors. This is due to the fact that the interactions between different materials when they are in nanoscale ($10^{-9}$ m), are able to generate new properties (Bresnahan and Tajtenberg, 1995) where unique phenomena may occur, different from those observed at the macroscopic scale, thus giving rise to the possibility of new applications for these materials (Adams and Barbante, 2013).

Nanomaterials have often been used in the modification of bare electrodes in electrochemical analysis (Shojaei et al., 2016). Novel electrochemical sensors based on nanomaterials have gained special attention, due to their low cost and high sensitivity (Erdem et al., 2011). In the nanometer range, the surface area of materials greatly increase, resulting in new phenomena, such as improvement in electrical conductivity (Shojaei et al., 2016).

Some nanomaterials, such as carbon nanotubes (Zhu et al., 2013; Karadas and Ozkan, 2014), gold nanoparticles (Hajian et al., 2015), and silver nanoparticles (Ahmadi et al., 2015), have demonstrated potential for the development of sensing systems. Some of these materials have already shown promising results in the development of electrochemical sensors for the determination of antineoplastic drugs, such as methotrexate (Zhu et al., 2013), and flutamide (Ahmadi et al., 2015).

Methotrexate (MTX) is an analog of folic acid, which acts as an antimetabolite agent by tightly binding to the enzyme dihydrofolate reductase, inhibiting the conversion of dihydrofolate to tetrahydrofolate, which is necessary in the synthesis of DNA, RNA, and proteins, thus interfering in cell mitosis. For this reason, MTX is commonly used to treat cancers of the breast, head, neck, and bladder (Wei et al., 2014), as well as acute lymphoblastic leukemia and other diseases. It also has anti-inflammatory and immunosuppressive effects which are effectively exploited for the treatment of the rheumatoid arthritis, juvenile idiopathic arthritis, and other diseases (Zhu et al., 2013).

Zhu et al. (2013) developed an electrochemical sensor from the modification of a glass carbon electrode (GCE) with multiwalled carbon nanotubes, functionalized with quaternary amine (q-MWNTs) for the determination of MTX and folic acid. The use of q-MWNTs in the modification of the electrodes allowed significant acceleration of the electron transfer process on the surface of the modified electrode (q-MWNTs/ GCE), catalyzing the electrochemical oxidation of methotrexate and folic acid. However, because of the proximity in the oxidation potentials of these drugs, simultaneous determination could only be carried out by means of ion chromatography with electrochemical detection. Thus, the method was applied for the simultaneous

**Table 1**
Classes of antineoplastics drugs, according to the Anatomical Therapeutic Chemical Classification System (ATC), explored in electroanalytical studies. Available at http://www.whocc.no/atc_ddd_index/?code=L, accessed on Jan 09, 2018.

| Drug class | Antineoplastic drug | Reference |
|---|---|---|
| **Alkylating agents** | Cyclophosphamide | (Hassan et al., 1998); (Baj-Rossi et al., 2012) |
| | Ifosfamide | (Hassan et al., 1998); (Baj-Rossi et al., 2012) |
| | Lomustine | (Temerk et al., 2016) |
| **Antimetabolites** | 5-Fluorouracil | (Shojaei et al., 2016) |
| | Gemcitabine | (Florea et al., 2015); (Radhapyari and Khan, 2015) |
| | 6-Thioguanine | (Beitollahi et al., 2011); (Wang et al., 2006) |
| | Methotrexate | (Zhu et al., 2013); (Wei et al., 2014); (Wang et al., 2009); (Wang et al., 2012); (Guo et al., 2011) |
| | 6-Mercaptopurine | (Karimi-Maleh et al., 2015) |
| | Permetrexed | (Karadas and Ozkan, 2014) |
| **Cytotoxic antibiotics and related substances** | Daunorubicin | (Erdem et al., 2011); (Ribeiro et al., 2013); (Chandra et al., 2011); (Shamagsumova et al., 2015) |
| | Doxorubicin | (Guo et al., 2011); (Chandra et al., 2011); (Shamagsumova et al., 2015) |
| | Valrubicin | (Hajian et al., 2015) |
| | Idarubicin | (Chandra et al., 2011); (Shamagsumova et al., 2015) |
| | Mitoxantrona | (Chandra et al., 2011) |
| **Endocrine therapy** | Tamoxifen | (Yarman and Scheller, 2014); (Radhapyari et al., 2013) |
| | Flutamide | (Ahmadi et al., 2015); (Brahman et al., 2012) |
| | Fulvestrant | (Dogan-Topal and Ozkan, 2011b) |
| **Plant alkaloids and other natural products** | Taxol | (Tajik et al., 2015); (Mehdinia et al., 2008) |
| | Etoposide | (Baj-Rossi et al., 2012) |
| **Other antineoplastic agents** | Cisplatin | (Materon et al., 2014) |
| **Without ATC classification** | Tirapazamine | (Hu et al., 2009) |
| | Ftorafur | (Baj-Rossi et al., 2012) |
| | Leuprolide | (Dogan-Topal and Ozkan, 2011a) |

determination of methotrexate and folic acid, in plasma and human urine. This sensor presented sensitivity to the detection of methotrexate close to that observed in studies by Wei et al. (2014), Wang et al. (2012), Guo et al. (2011), and Wang et al. (2009), described in the following sections.

Beitollahi et al. (2011) described the preparation and characterization of 2,7-bis (ethylferrocenyl) fluorene-9-one (2,7-BF) modified carbon nanotube paste electrodes (CNPEs). These modified electrodes (2,7-BF/CNPE) showed efficient catalytic activity for the electro-oxidation of 6-thioguanine, folic acid, and uric acid individually and/or simultaneously, and have been used in the analysis of pharmaceutical samples and urine. The sensor demonstrated greater sensitivity in the determination of 6-thioguanine, compared with the biosensor developed in 2006 by Wang et al., discussed further in the section on DNA biosensors.

The antineoplastic drug 6-thioguanine is a purine analog used as a

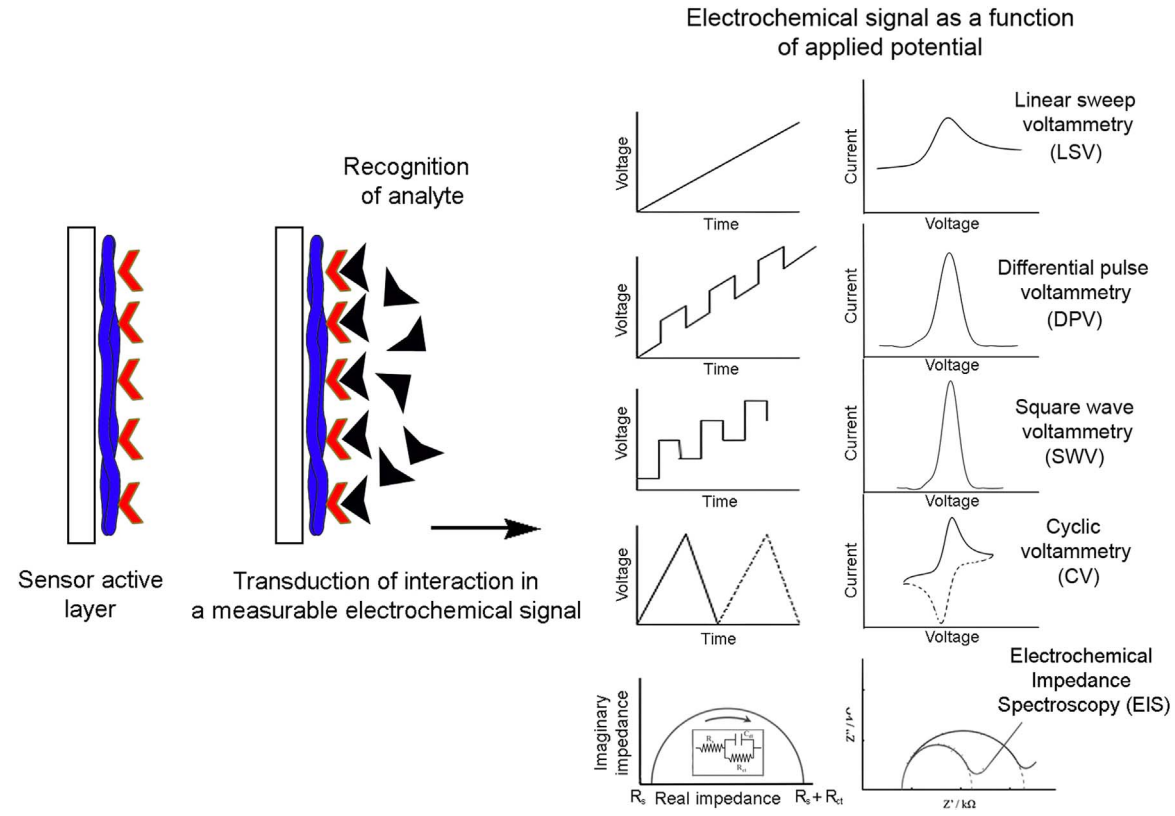


**Fig. 1.** Schematic representation of an electrochemical sensor, its interaction with the analyte, and the transduction of these interactions into measurable signals.

**Table 2**
Electrochemical sensors for the detection of antineoplastic drugs.

| Active layer | Drug | Electrochemical technique | Limit of detection (mol $L^{-1}$) | Linear range (mol $L^{-1}$) | Reference |
|---|---|---|---|---|---|
| ($ZnFe_2O_4$/MNPs/IL/CPE) $ZnFe_2O_4$ magnetic nanoparticles/1,3-dipropylimidazolium bromide ionic liquid modified Carbon Paste Electrode | 5-fluorouracil | (SWV) Square Wave Voltammetry | $7 \times 10^{-8}$ | 0.1–1400 $\times 10^{-6}$ | (Shojaei et al., 2016) |
| (MF/GPE) Mercury film coated graphite pencil electrode | Lomustine | (SWCASV) Square Wave Cathodic Adsorptive Stripping Voltammetry | $8.13 \times 10^{-8}$ | $1.92 \times 10^{-7}$ to $1.36 \times 10^{-5}$ | (Temerk et al., 2016) |
| (GMT-MMOF) Microporous metal organic framework for gemcitabine (p-aminothiophenol functionalized gold nanoparticles) | Gemcitabine | (LSV) Linear Sweep Voltammetry | $3 \times 10^{-15}$ | $3.8 \times 10^{-15}$ to $3.8 \times 10^{-8}$ | (Florea et al., 2015) |
| (PDR-PGE) Amino-terminated G4 PAMAM dendrimer modified disposable electrodes | Daunorubicin | (DPV) Differential Pulse Voltammetry | $1.28 \times 10^{-7}$ | – | (Erdem et al., 2011) |
| (q-MWNTs/GCE) Functionalized multi-walled carbon nanotubes modified glassy carbon electrode | Methotrexate | Ion chromatography with electrochemical detection | $0.4 \times 10^{-9}$ | $0.2 \times 10^{-7}$ to $0.4 \times 10^{-4}$ | (Zhu et al., 2013) |
| (2,7-BF/CNPE) 2,7-bis (ferrocenyl ethyl) fluoren-9-one modified carbon nanotube paste electrode | 6-thioguanine | DPV | $2.2 \times 10^{-8}$ | Two intervals: 0.06–10 $\times 10^{-6}$ and 1–16 $\times 10^{-5}$ | (Beitollahi et al., 2011) |
| (oo-PPY/MWCNT-COOH/GCE) Over-oxidized polypyrrole/multi-walled carbon nanotube composite on glassy carbon electrode | Pemetrexed | (ADSDPV) Adsorptive stripping with Differential Pulse Voltammetry | $3.28 \times 10^{-9}$ | $1 \times 10^{-8}$ to $1 \times 10^{-7}$ | (Karadas and Ozkan, 2014) |
| (CD-GNs/GCE) Cyclodextrin-graphene hybrid nanosheets modified glassy carbon electrode | (Doxorubicin) [Methotrexate] | DPV | ($0.1 \times 10^{-9}$) [$2 \times 10^{-8}$] | ($10 \times 10^{-9}$ to $0.2 \times 10^{-6}$) and [0.1–1 $\times 10^{-6}$] | (Guo et al., 2011) |
| A label-free sensor based on the direct ion transfer at the water/Dichlorohexane micro interface | Daunorubicin | DPV | $0.8 \times 10^{-6}$ | 1.2–8.2 $\times 10^{-5}$ | (Ribeiro et al., 2013) |
| (AuNPs/EN/MWCNTs/AuE) Gold nanoparticles/ ethylenediamine/ multi-wall carbon-nanotubes modified gold electrode | Valrubicin | CV | $1.8 \times 10^{-8}$ | 0.5–80 $\times 10^{-6}$ | (Hajian et al., 2015) |
| (PLL/GCE) Poly (L-lysine) modified glassy carbon electrode | Methotrexate | SWV | $1.7 \times 10^{-9}$ | $5 \times 10^{-9}$ to $0.2 \times 10^{-6}$ | (Wei et al., 2014) |
| (PVC-COOH) Poly (vinyl chloride) carboxylate membrane sensor | Cyclophosphamide and ifosfamide | Potentiometry | Not measured | $10^{-3}$ to $10^{-5}$ for both | (Hassan et al., 1998) |
| (AgNPs/GCE) Ag nanoparticles modified glassy carbon electrode | Flutamide | DPV | $9.33 \times 10^{-6}$ | 1–100 $\times 10^{-5}$ | (Ahmadi et al., 2015) |
| (TAM-MIP) Molecular imprinted sensor for Tamoxifen (o-phenylene-diamine-resorcinol) | Tamoxifen | CV | Not measured | 1–100 $\times 10^{-9}$ | (Yarman and Scheller, 2014) |
| (OMC/PGE) Ordered mesoporous carbon modified pyrolytic graphite electrode | Tirapazamine | DPV | $2 \times 10^{-11}$ | $5 \times 10^{-11}$ to $1.5 \times 10^{-5}$ | (Hu et al., 2009) |
| (Polymer-CPE) Polymer film modified carbon paste electrode | Flutamide | DPV | $1.8 \times 10^{-7}$ | 0.72–5.8 $\times 10^{-4}$ | (Brahman et al., 2012) |

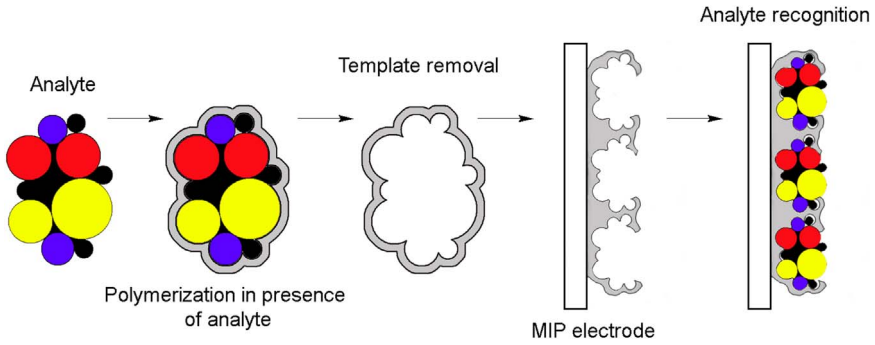


**Fig. 2.** Schematic representation of a molecularly imprinted polymer (MIP) electrode.

metabolic inhibitor with antitumor activity, for the treatment of leukemias and lymphomas (Wang et al., 2006). Uric acid is the final product of purine nucleoside catabolism, and is an important biomolecule present in physiological fluids (Beitollahi et al., 2011).

Ahmadi et al. (2015) modified glass carbon electrodes (GCE) with silver nanoparticles, using calixarenes as a template for the formation of nanoparticles, for the determination of flutamide. Flutamide is a non-steroidal, anti-androgen drug, often used in the treatment of prostate cancer, which has the ability to block the action of testosterone, a prostate cancer cell growth stimulant. Despite not being able to cure the cancer, it can keep it under control for long periods (Ahmadi et al., 2015). The calixarenes are macrocyclic compounds formed by units of phenolic compounds bound by methylene bridges, where hydrophobic cavities are formed. These cavities allow calixarenes to be used as templates for the synthesis of silver nanoparticles (AgNPs) on the surface of the GCE, controlling the size, quantity, and distribution of silver nanoparticles produced, and are later removed from the sensor (Ahmadi et al., 2015).

The formed film (AgNP/GCE) exhibited catalytic activity for the reduction of flutamide, which improved electron transfer activity. This modified electrode was used in the detection of flutamide in human urine and in medicinal tablets (Ahmadi et al., 2015). This sensor presented sensitivity to the detection of the flutamide close to that observed by the techniques developed by Brahman et al. (2012), described below, being slightly less sensitive, but displaying a wider linear interval of quantification.

Hajian et al. (2015) developed an electrochemical sensor for the determination of the antineoplastic drug valrubicin, where gold electrodes pretreated with multilayer carbon nanotubes were coated by the deposition of ethylenediamine films, with the addition of gold nanoparticles. Valrubicin is an antineoplastic drug that has been widely used in the therapy of bladder cancer, and is a second-generation anthracycline, derived from doxorubicin (Hajian et al., 2015). The sensor (AuNPs/EN/MWCNTS/AuE) possessed an increased surface area of the electrode and electron transfer rate, resulting in high catalytic capacity for the reduction of this drug in human urine and serum samples containing valrubicin.

Karadas and Ozkan (2014) developed an electrochemical sensor from the modification of GCE with carboxylic acid functionalized multiwalled carbon nanotubes (MWCNTS-COOH) and over-oxidized polypyrrole (oo-PPY), for the determination of the drug pemetrexed. This drug is an antifolate agent which exerts its action by interfering in folate-dependent metabolic processes essential for cell replication, therefore acting as an antineoplastic agent. It has proved to be effective against cancers of the lung, head and neck, colon, and breast.

With the use of oo-PPY, an improvement in the oxidation current of pemetrexed was obtained, due to an increase in the porosity of the film and consequently, in the active surface area of the electrode. These modified electrodes (oo-PPY/MWCNTS-COOH/GCE) were therefore promising for direct application to the analysis of the drug, even in its pharmaceutical form (for example in quality control), with no interference detected from excipients (Karadas and Ozkan, 2014).

In a study by Guo et al. (2011), glass carbon electrodes (GCEs) were modified with hybrid cyclodextrin-graphene nanosheets (CD-GNs), developing a sensor (CD-GNs/GCE) for the determination of doxorubicin and methotrexate. The electrochemical performance of the modified electrodes presented great advantages, resulting from their high electron transfer kinetics, generating a microenvironment favorable to the electrochemical reactions of these drugs. The sensor showed promise for the determination of both drugs at trace levels.

Shojaei et al. (2016) modified a carbon paste electrode (CPE) with $ZnFe_2O_4$ magnetic nanoparticles (ZnFe2O4/MNPs) and 1,3-propylimidazolium bromide ionic liquid (IL) for the determination of 5-fluorouracil, one of the most widely used drugs in the treatment of cancer, which acts as an antimetabolite, either by inhibiting essential biosynthetic processes or by its incorporation into nucleic acids.

The developed sensor (ZnFe2O4/MNPs/IL/CPE) showed a significant increase in the peak oxidation current of 5-fluorouracil, due to the increase in the surface area of the sensor resulting from the use of $ZnFe_2O_4$/MNPS and IL. These sensors were used in the detection of the drug by the method of standard additions, in pharmaceutical and biological samples (serum and urine) (Shojaei et al., 2016).

### 3.2. Molecularly imprinted polymer electrodes

The molecular imprinting technique used to produce sensors, consists of the polymerization of certain functional monomers in the presence of template molecules. This template is subsequently extracted from the resulting polymer matrix, generating a film filled with complementary cavities in the shape and size of the template molecule (Florea et al., 2015). The electrochemical signals observed from the contact between this film and a medium containing the analyte molecule can be used for sensing purposes. The advantage of this method is that the polymers used are generally low cost and have excellent specificity. Desorption of the template, shown in Fig. 2, is generally conducted using a solvent such as PBS at pH 7.2 (Florea et al., 2015) or mixtures of solvents such as water, methanol, and sodium hydroxide (Radhapyari et al., 2013).

Florea et al. (2015) developed an electrochemical sensor for the analysis of gemcitabine using the MIP technique. Gemcitabine is a antimetabolite drug, used to treat a large variety of tumors including pancreatic, breast, bladder, and non-small cell lung cancer (Radhapyari and Khan, 2015).

Here, using the cyclic voltammetry technique, the electropolymerization of gold nanoparticles functionalized with p-aminothiophenol on the surface of gold electrodes, was carried out in the presence of gemcitabine as a template molecule. Subsequently, the drug was removed, yielding a microporous metal organometallic framework (GMT-MMOF). Next, the voltammetric response of the redox ferrocyanide mediator was studied by linear scanning voltammetry against the microporous film, in the absence and presence of the drug (Florea et al., 2015).

When drug molecules contacted the microporous film, they were reincorporated, thus promoting changes in the linear scanning voltammograms, by facilitating the transfer of electrons at the surface of electrode, in proportion to the amount of analyte in the medium (Florea et al., 2015). The developed sensor was used for the detection of gemcitabine in serum samples and in drug formulations (Florea et al., 2015).

Tamoxifen is a non-steroidal anti-estrogen agent used in the prevention and treatment of breast cancer, which in view of its importance, has led to the development of several analytical methods for its quantification (Radhapyari et al., 2013).

Yarman and Scheller (2014) started from the electropolymerization of o-phenylene diamine resorcinol on the surface of a vitreous carbon electrode, using the cyclic voltammetry technique in the presence of a tamoxifen template molecule, to develop an MIP for this drug. Next, the voltammetric response of the redox ferrocyanide mediator was studied by cyclic voltammetry, in the absence and presence of tamoxifen. Using the formed sensor (TAM-MIP), a decrease in the peak current values was observed for the oxidation and reduction of the ferricyanide redox marker, observed by cyclic voltammetry, proportional to the concentration of tamoxifen in the electrolyte (a range of 1–100 nm). In this way, it was possible to perform indirect quantification from the observed relationship between the concentration of the drug in the electrolytic solution and the effect promoted in the voltammograms of the ferricyanide, where an increase in the drug concentration led to a reduction in the oxidation and reduction peaks of the redox pair. However, according to the authors, the decrease in current values observed in voltammograms may also be influenced by other factors acting on the MIP structure, and the development of methodologies for the direct detection of the target analyte may be a more reliable strategy.

This sensor presented a linear range for the detection of tamoxifen close to observations made by Radhapyari et al. (2013), and is described further in the section on DNA biosensors.

### 3.3. Electrochemical sensors with polymers as the active layer

Sensors with an active layer based on polymers can be prepared using various types of polymers and electrodes for electrochemical signal transduction. They are very common in the literature, with proposals for the detection of the majority of types of analytes, ranging from biological and pharmacological, to environmental samples (Farias et al., 2015). There are several techniques for the preparation of polymeric electrodes.

Depending on the technique employed, the polymer film produced at the surface of the electrode will have different characteristics, which influence the analytical response of the sensor. Fig. 3 illustrates some examples of the types of polymeric electrodes. The self-assembled-monolayer (SAM) technique involves preparing electrodes in which the polymers organize without an external influence, and exhibiting high molecular organization (Samanta and Amitabha, 2011).

SAMs are films of highly organized monomolecular structures (Ariga et al., 2006). However, the film prepared by this technique does not always cover the entire surface of the electrode, allowing non-specific interactions with the constituents of the sample. Blocking steps can be performed at these sites to minimize these interactions (Jesus et al., 2016).

Techniques such as spin-coating (Akmal et al., 2018; Sahu et al., 2009) and casting (Ambrosi et al., 2008) are able to produce thicker films that cover the entire electrode. However, the molecular organization of the films produced using this technique is not as consistent as that of the films produced using SAM and layer-by-layer (LbL) techniques (Avellaneda et al., 1998). In spin-coating, the film is produced on a rotating support and then transferred to the surface of the electrode (Lakiss et al., 2008). In casting, the polymer film is obtained by dripping the polymer solution onto the surface of the electrode, followed by drying at room temperature or by heating (Ambrosi et al., 2008). This technique is very common for modifying electrodes, especially when working with composite materials or blends, due to the technique's simplicity.

The LbL technique, like SAM, is a self-assembling technique, differentiated by films containing alternating layers of different materials, enabling the intercalation of layers of two or more polymers (Eiras et al., 2007). The advantage of this technique is the possibility of a synergistic effect between these polymers, which can improve selectivity as well as analytical signals during sensing of nanoparticles, nanotubes, clays, organo-metallic complexes, polymers, and a myriad of other materials (Therézio et al., 2011).

MIP electrodes are also examples of polymeric electrodes, prepared by specific methods, as described above. In addition to MIPs, there are studies that involve the electropolymerization of a compound onto the surface of an electrode, by applying electric potential even in the absence of a template, which have potential applications in sensing.

An example of a sensor using a polymer as the active layer for the detection of antineoplastic drugs can be found in the work of Hassan et al. (1998). The group developed a sensor based on carboxylated polyvinyl chloride (PVC-COOH), and from the interaction between molecules of the drugs cyclophosphamide and ifosfamide, and the carboxyl groups of the PVC-COOH sensor, finding that it was possible to quantify both drugs in pharmaceutical samples. Cyclophosphamide and its ifosfamide isomer are alkylating drugs which have been used clinically for the treatment of cancer for more than 30 years (Hassan et al., 1998).

Brahman et al. (2012) produced a modified carbon paste electrode (CPE) with glutamic acid polymer for the determination of flutamide, in electrolytic medium containing a surfactant, cetyltrimethylammonium bromide. Surfactants have been frequently used in electrochemical studies because of their ability to increase the electron transfer velocity between the electrode surface and the analyte, thereby improving detection limits. The addition of the polymer exerted a significant catalytic effect on the electrochemical reduction of flutamide.

The interference of several molecules which are commonly found in human fluids, were evaluated in order to determine the selectivity of the sensor. The use of the modified electrode did not show significant changes in the presence of these interferents and was therefore used for

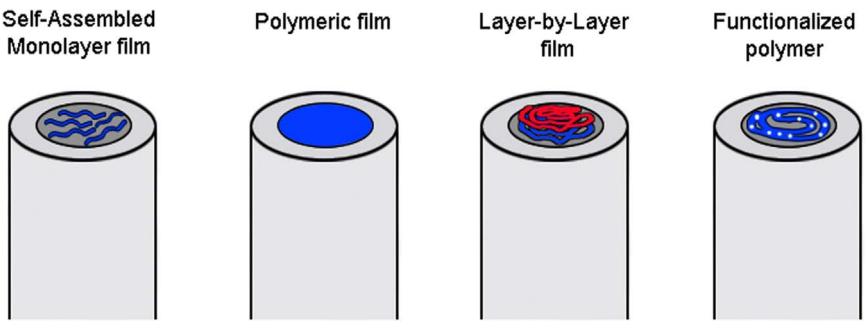


**Fig. 3.** Schematic representation of the types of polymeric electrodes.

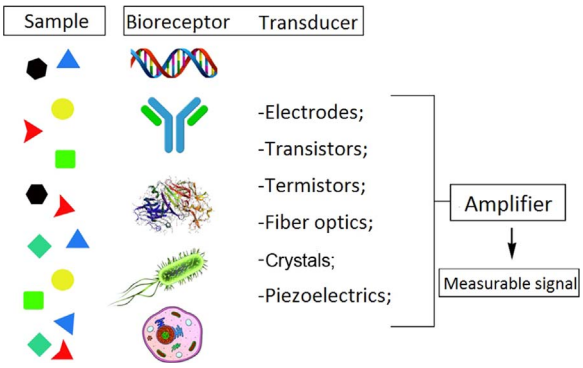


**Fig. 4.** Simplified scheme of biosensor operation.

the determination of flutamide in analytical samples and in its pharmaceutical form (Brahman et al., 2012).

In 2014, Wey and collaborators developed a simple and sensitive electrochemical sensor based on a poly L-lysine (PLL) modified glassy carbon electrode, for the detection of methotrexate in the presence of sodium dodecyl benzene sulfonate (SDBS) anionic surfactant in an electrolytic solution. This sensor (PLL/GCE) facilitated the drug oxidation process, while the SDBS surfactant helped to improve reproducibility by preventing the adsorption process of methotrexate oxidation products onto the electrode. The film exhibited excellent activity when detecting this drug in medical tablets.

Erdem et al. (2011) modified the surface of pyrolytic graphite electrodes (PGE) via the deposition of an amino-terminated PAMAM generation 4 dendrimer (PDR), and then used the PDR-PGE system as a sensor for the analysis of the drug, daunorubicin. The poly (amidoamine) dendrimers (PAMAM) are a family of dendrimers with extensive activity, recognized as a new class of synthetic nanostructures which allow precise control of the shape, size and placement of desirable functional groups for many scientific applications (Esfand and Tomalia, 2001).

Daunorubicin is an anthracycline with the ability to intercalate between DNA base pairs, and as such, possesses efficient antitumor activity (Ribeiro et al., 2013). The results of electrochemical tests indicated that the surface modification of the PGE improved the oxidation signal of the drug. It was hypothesized that daunorubicin could interact and accumulate by hydrogen bonding with the amine groups present in PAMAM, resulting in an increase in the magnitude of the drug oxidation signal (Erdem et al., 2011).

### 3.4. Other electrochemical sensors for the detection of antineoplastic drugs

Tirapazamine is an antitumor prodrug which is selectively activated by the low oxygen environment of some tumors, and is used in the treatment of cervical cancer, one of the most common cancers in women (Hu et al., 2009). Hu et al. (2009) modified PGE with ordered mesoporous carbon (OMC), producing a sensor to investigate the electrochemical behavior of tirapazamine. The modified electrode (OMC/PGE) showed a much higher electrochemical response for the drug when compared to the unmodified electrode. The authors attributed the reasons for this optimization to the increased surface area promoted by the OMC, and to the presence of functional groups containing oxygen on the surface of the electrode. The interference of several molecules such as uric acid, serotonin, catecholamines, and ascorbic acid commonly found in human fluids, were evaluated and did not affect the precise detection of the drug. The sensor was successfully used in the determination of tirapazamine in human serum.

Ribeiro et al. (2013) developed an electrochemical sensor for daunorubicin, based on the direct transfer of ions at the water/1,6-dichlorohexane interface. The voltammetric behavior of this drug was investigated, by observing the direct transfer of the $DNRH^+$ ion at the liquid/liquid micro interface. The determination of $DNRH^+$ in deproteinized human plasma was successful, but it was not possible to detect $DNRH^+$ in untreated plasma, due to its interaction with albumin, which impaired the amperometric test. However, this electrochemical sensing system was found to be less sensitive in the detection of daunorubicin, compared with the test developed by Erdem et al. (2011), as shown in Table 2.

Temerk et al. (2016) developed electrochemical sensors for the determination of lomustine, using the coating of a graphite pencil electrode (GPE) with mercury film (MF). Lomustine is an anticancer antineoplastic agent, widely used in the treatment of resistant brain tumors, relapses of Hodgkin's disease, lymphomas, and malignant melanomas, as well as other tumors (Temerk et al., 2016). In this study, an increase in the current value of the drug reduction process on the surface of the modified electrode was observed in comparison with the unmodified electrode. This provided evidence on the role of mercury as a facilitator of electron transfer between the surface of the sensor and the analyte, amplifying the electrochemical signal and enabling the accurate quantification of lomustine in human blood and urine samples.

## 4. Electrochemical biosensors for antineoplastic drugs

Electrochemical biosensors are distinguished from electrochemical sensors because they are systems composed of a recognition element of a biological nature such as enzymes, antibodies, drug receptors, or DNA, among others, immobilized in a transducer composed of an electroactive material, in this case an electrode, capable of converting an electrochemical signal (reactions involving electron transfer) into a measurable signal (Gil and De Melo, 2010). Fig. 4 shows the general scheme of biosensor operation.

Electrochemical biosensors have been employed in several areas and their great applicability is related to advantages such as their high sensitivity (detection of low concentrations of analyte), rapid responses, low cost, high specificity, and the number of commercially available enzymes for development of different types of enzymatic sensors, among others (Marques and Yamanaka, 2008). There are so far relatively few studies addressing the development and use of electrochemical biosensors for the determination of antineoplastic drugs. Table 3 summarizes some of the biosensors so far used, and the following text outlines the main aspects of the systems currently being developed.

### 4.1. Enzymatic biosensors

When the biological material used for the development of a biosensor is an enzyme, these are called enzymatic biosensors, with emphasis being given to those in which the analyte is monitores throught inhibition of the enzyme. This process is carried out by exposing the enzyme to a specific inhibitor for a certain period of time, and the percentage of inhibition of the enzyme is quantitatively related to the concentration of the inhibitory agent (Marques and Yamanaka, 2008).

An example of an enzymatic biosensor for the detection of antineoplastic drugs is described by Materon et al. (2014), where a biosensor was developed by modifying carbon paste electrodes (CPE) with the glutathione-S-transferase enzyme (GST). GST is an enzyme that protects cells from xenobiotic substances by catalyzing conjugation reactions with glutathione, thereby facilitating their excretion. This biosensor is based on the quantification of antineoplastic drugs through the competition between them and 1-chloro-2,4-dinitrobenzene (CDNB) at the catalytic site of the GST enzyme. The reaction of CDNB with glutathione, catalyzed by GST, generates an electrochemical signal that can be measured. However, this signal can be weakened by the addition of antineoplastic drugs, such as cisplatin, because of the competition between the drug and the CDNB substrate. The GST/CPE sensor was found to efficiently quantify this drug, however, it was not specific for a particular drug and could detect other substrates of this enzyme, such

**Table 3**
Electrochemical biosensors for the detection of antineoplastic drugs.

| Active layer | Drug | Electrochemical technique | Limit of detection (mol $L^{-1}$) | Linear range (mol $L^{-1}$) | Reference |
|---|---|---|---|---|---|
| (ds-DNA/PGE) Double-stranded DNA modified Pencil graphite electrode | Leuprolide | (ADSDVP) Differential Pulse Adsorptive Stripping Voltammetry | $4.9 \times 10^{-8}$ | $0.16–4.9 \times 10^{-6}$ | (Dogan-Topal and Ozkan, 2011a) |
| (ds-DNA/AET/Au) Composite film containing ds-DNA fabricated on 2-aminoethanethiol self-assembled monolayers film modified gold electrode | 6-Thioguanine | (1) Differential Pulse Stripping Voltammetry (2) SWV | (1) $2 \times 10^{-7}$ and (2) $1.2 \times 10^{-7}$ | (1) $1.1 \times 10^{-6}$ to $1.1 \times 10^{-5}$ and (2) $4 \times 10^{-7}$ to $2.2 \times 10^{-5}$ | (Wang et al., 2006) |
| (PANI/AuNP/HRP/ITO) Polyaniline-gold nanocomposite film modified horseradish peroxidase | Gemcitabine | DPV waveform | $1.1 \times 10^{-10}$ | $0.37–4.1 \times 10^{-9}$ | (Radhapyari and Khan, 2015) |
| (HRP/PANI/Pt) Horseradish peroxidase immobilized on polyaniline modified platinum electrode | Tamoxifen | CV | $1.9 \times 10^{-10}$ | $0.27–2.9 \times 10^{-8}$ | (Radhapyari et al., 2013) |
| (ds-DNA/GCE(ox)) ds-DNA Langmuir–Blodgett modified glassy carbon electrode | Methotrexate | SWV | $5 \times 10^{-9}$ | $2 \times 10^{-8}$ to $4 \times 10^{-6}$ | (Wang et al., 2009) |
| (ds-DNA/PGE) ds-DNA modified pencil graphite electrode | Fulvestrant | DPV | $6.7 \times 10^{-7}$ | $0.16–3.2 \times 10^{-5}$ | (Dogan-Topal and Ozkan, 2011b) |
| (ds-DNA/PGE) ds-DNA modified pencil graphite electrode | Taxol | DPV | $8 \times 10^{-8}$ | $2 \times 10^{-7}$ to $1 \times 10^{-5}$ | (Tajik et al., 2015) |
| (ds-DNA/SAM/Au) ds-DNA/ self-assembled monolayer modified Au electrode | Taxol | DPV | $1.2 \times 10^{-8}$ | $1.2 \times 10^{-7}$ to $1.5 \times 10^{-6}$ | (Mehdinia et al., 2008) |
| (*CYP450/CNTs/SPEs) Cytochrome P450 isoforms/ multi-walled carbon nanotubes modified screen-printed electrodes | Cyclophosphamide etoposide, ftorafur and ifosfamide | CV | $0.5–49 \times 10^{-7}$ for different drugs | – | (Baj-Rossi et al., 2012) |
| (GST/CPE) Glutathione-s-transferase modified carbon paste electrode | Various anticancer drugs | SWV | $8.8 \times 10^{-6}$ for cisplatin | $0.5–1.4 \times 10^{-4}$ for cisplatin | (Materon et al., 2014) |
| (ds-DNA/CL/AuNPs/PTTBA/SPE) ds-DNA and cardiolipin modified screen printed electrode | Anthracyclines: daunomycin, doxorubicin, idarubicin, and mitoxantrona | Chronoamperometry | $1.2–5.5 \times 10^{-15}$ for different drugs | $2–60 \times 10^{-12}$ | (Chandra et al., 2011) |
| (DNA/SWCNT/Nafion-modified/CGE) DNA-functionalized single-walled carbon nanotube and Nafion composite film modified glassy carbon electrode | Methotrexate | Square wave anodic striping voltammetry | $8 \times 10^{-9}$ | $2 \times 10^{-8}$ to $1.5 \times 10^{-6}$ | (Wang et al., 2012) |
| (PANI/DNA/GCE) Polyaniline–DNA modified glassy carbon electrode | Anthracyclines: daunomycin, doxorubicin and idarubicin | CV | $0.1–2 \times 10^{-10}$ for different drugs | $1 \times 10^{-8}$ to $1 \times 10^{-4}$ and $1 \times 10^{-10}$ to $5 \times 10^{-10}$ | (Shamagsumova et al., 2015) |
| (ds-DNA/PP/MWCNTS/PGE) ds-DNA immobilized on a polypyrrole and functional multiwalled carbon nanotubes modified pencil graphite electrode | 6-Mercaptopurine | DPV | $0.8 \times 10^{-7}$ | $0.2–100 \times 10^{-6}$ | (Karimi-Maleh et al., 2015) |

as the drugs gemcitabine, doxorubicin, or carboplatin, among others.

Radhapyari et al. (2013) developed an amperometric biosensor for the detection of tamoxifen. This biosensor was produced from the immobilization of the horse radish peroxidase (HRP) enzyme onto polyaniline (PANI) modified platinum (Pt) electrodes. This biosensor (HRP/PANI/Pt) was used to monitor the catalytic reduction of tamoxifen, and presented excellent properties such as high sensitivity and low detection limits for the drug.

Baj-Rossi et al. (2012) deposited carbon nanotubes on carbon paste electrodes, producing an electrochemical sensor that was used to quantify the drug etoposide. Subsequently, the carbon nanotubes were functionalized with three different cytochrome P450 isoforms (CYP1A2, CYP2B6, and CYP3A4), producing biosensors for the quantification of the drugs ftorafur, cyclophosphamide, and ifosfamide. In this study, the drugs were analyzed in phosphate buffered saline (PBS) and in human serum. The work was based on a biochemical reaction catalyzed by cytochrome P450, which oxidizes the target drug using an oxygen molecule and two electrons (provided by the current from the electrode), giving rise to an electrochemical signal that can be used for the quantification of these drugs. This system enabled greater sensitivity in the detection of cyclophosphamide and ifosfamide, compared to the system developed by Hassan et al. (1998).

Radhapyari and Khan (2015) developed a biosensor by immobilizing HRP onto nanocomposite films, based on polyaniline and gold nanoparticles (PANI/AuNP) on the surface of glass electrodes coated with indium tin oxide (ITO), for the direct determination of gemcitabine. The PANI/AuNP film used for the immobilization of HRP led to the production of a biosensor that was applied to the determination of this drug in pharmaceutical formulations, without the need for pretreatment of samples. This system was slightly less sensitive in the detection of gemcitabine, compared with the method developed by Florea et al. (2015) using MIP sensors.

### 4.2. Biosensors based on DNA

The construction of DNA electrochemical biosensors is based on the immobilization of nucleic acid films along an electrochemical transducer, where changes in DNA structure that occur during the intercalation of ligand molecules are detected, producing a measurable signal (Tajik et al., 2015). The signals are generated due to the oxidation of sites on the nitrogenous DNA bases. The electrochemical investigation into the interaction between nucleic acids and ligand molecules has therefore been explored because it allows the detection of changes that occur in the structure of DNA during its interaction with other molecules (Tajik et al., 2015).

Chandra et al. (2011) used gold nanoparticles (AuNPs) and terthiophene polymer with free carboxylic groups (PTTBA). They deposited films of these compounds onto screen-printed electrodes (SPEs), then immobilizing double-stranded DNA molecules (ds-DNA) and cardiolipin (CL) as elements in the production of biosensors. The biosensors (ds-DNA/CL/AuNPs/PTTBA/SPE) were used for the simultaneous detection of the antineoplastic drugs daunorubicin, doxorubicin, idarubicin, and mitoxantrone, previously separated in a microfluidic separation device (a system developed for the separation and detection of various biological analytes) (Shiddiky, Shim, 2007). These antineoplastic drugs are of the anthracycline class, which act by altering the fluidity and transport of ions from the cell membrane, promoting the formation of oxygen free radicals, and rupturing DNA filaments (Katzung et al., 2014). These drugs are used in the treatment of various types of cancer (Chandra et al., 2011). The proposed modification of the biosensor using ds-DNA and CL was based on known interactions between the studied drugs and the nitrogenous bases of DNA, alongside known interactions between the anthracyclines and the CL present on the surface of cancerous cells. Knowledge of these interactions was used to make a biosensor capable of interacting with the drugs of interest.

Mehdinia et al. (2008) prepared self-assembling monolayers (SAM) of the thiol derivative iodohexane, on gold electrodes (Au). These were then used to immobilize ds-DNA, thus producing a biosensor (ds-DNA/SAM/Au) where the interaction of ds-DNA with the drug Taxol was studied. Taxol is one of the most effective naturally occurring chemotherapeutic agents, and is commonly used in clinical practice for the treatment of ovarian and breast cancers (Tajik et al., 2015). In the study by Mehdinia et al. (2008), the observed decrease in the peak values of guanine oxidation current in ds-DNA was used to monitor interactions with the drug, thus allowing its quantification in human serum samples.

Wang et al. (2006) modified gold electrodes with self-contained aminoethanethiol monolayers (AET) and then deposited ds-DNA onto the surface of the electrode by electrostatic adsorption. This is a simple method for the immobilization of DNA onto electrodes, especially for ds-DNA. The biosensor (ds-DNA/AET/Au) was developed for the quantification of the drug 6-thioguanine, using two different electrochemical probes $[Fe(CN)6]^{3-/4-}$ e $Co(bpy)3^{2+/3+}$; however, because of the ds-DNA characteristics and the complexity of factors related to the ideal electrochemical response, it was not possible to completely elucidate the mechanism of interaction between ds-DNA and the drug.

Wang et al. (2009) developed a highly sensitive electrochemical biosensor for the detection of methotrexate. A glass carbon electrode was progressively polished and cleaned, giving rise to an electrode which was named (GCE-ox), and ds-DNA was then immobilized on its surface using the Langmuir-Blodgett (LB) technique.

Electrochemical tests for drug detection using the ds-DNA/GCE-ox biosensor showed well-defined oxidation processes, whose currents were approximately 100 times higher than for the unmodified GCE electrode and 5 times higher than for GCE-ox, also unmodified. The increase in current was attributed to the interaction between the drug and DNA, which form a complex by high affinity intercalation. The authors suggest that oxygen-containing groups, formed on the surface of the GCE-ox electrode because of the treatment process used, allowed a better interaction between the drug and the electrode, via hydrogen bonding, improving the electron transfer rate. The proposed method was applied for the determination of methotrexate in its pharmaceutical form and in human urine (Wang et al., 2009).

Leuprolide is a gonadotropin-releasing hormone (GNRH) agonist, which in continuous therapeutic doses, causes a constant and repetitive stimulation of its receptors in the pituitary gland. This stimulation promotes a decrease in the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The positive effects of this have been observed in the treatment of hormone-responsive cancers such as breast, prostate, and uterine fibroids (Dogan-Topal and Ozkan, 2011a).

Dogan-Topal and Ozkan (2011a) modified the surface of a pencil graphite electrode (PGE) with ds-DNA, and developed a biosensor for the detection of leuprolide. The results obtained for the oxidation of this drug using the biosensor (ds-DNA/PGE), showed a linear dependence between the analytical signals and the concentrations of the analyte in the electrolytic medium, related to the decrease in the guanine signal after the interaction with the drug. This can be explained by the damage to the oxidizable groups of the guanine bases, due to the adsorption of the drug. The proposed method could be applied to the determination of leuprolide in its pure form and its pharmaceutical dosage form, without any interference.

Fulvestrant is a novel type of estrogen receptor antagonist, capable of forming a complex with these receptors, preventing their dimerization and rendering them transcriptionally inactive. This drug therefore inhibits the growth of estrogen-responsive tumors and is used in the treatment of postmenopausal women with advanced breast cancer, even in addition to tamoxifen therapy (Dogan-Topal and Ozkan, 2011b).

Dogan-Topal and Ozkan (2011b) developed an electrochemical biosensor (ds-DNA/PGE) for the determination of fulvestrant, using immobilized ds-DNA on PGE. The results obtained in differential pulse voltammograms indicated that the oxidation signal of the guanine bases decreased with increasing concentration of this drug in the electrolytic

**Table 4**
Number of patents deposited in databases involving electrochemical sensors and biosensors, and their correlation with the quantification of antineoplastic drugs.

| KEYWORDS | INPI | EPO | USPTO | WIPO | FPO |
|---|---|---|---|---|---|
| Sensor/sensor | 3045 | > 10,000 | > 10,000 | > 10,000 | > 10,000 |
| Biosensor/biossensor | 63 | 8129 | > 10,000 | > 10,000 | > 10,000 |
| Electrochemical sensor/Sensor eletroquímico | 38 | 1932 | 3841 | 3887 | 7053 |
| Electrochemical biosensor/Biossensor eletroquímico | 8 | 428 | 595 | 692 | 2645 |
| Antineoplastic drug/Antineoplásico | 6 | 58 | 657 | 578 | 685 |
| Chemotherapy drug/Quimioterápico | 22 | 37 | 1398 | 342 | 1168 |
| Electrochemical sensor and chemotherapy drug/ Sensor eletroquímico e quimioterápico | 0 | 0 | 13 | 0 | 9 |
| Electrochemical sensor and antineoplastic drug/ Sensor eletroquímico e antineoplásico | 0 | 0 | 0 | 0 | 0 |
| Electrochemical biosensor and chemotherapy drug/ Biossensor eletroquímico e quimioterápico | 0 | 0 | 0 | 0 | 0 |
| Electrochemical biosensor and antineoplastic drug/ Biossensor eletroquímico e antineoplásico | 0 | 0 | 0 | 0 | 0 |

Patent Title Search; INPI: Instituto Nacional da Propriedade Industrial; EPO: European Patent Office; WIPO: World Intellectual Property Organization; USPTO: United States Patent and Trademark Office; (FPO) Free Patents Online.

medium. This could be explained by the detection of oxidizable groups in the nitrogenous bases, such as guanine. For the method developed, it was not necessary to perform pre-treatment of the samples, and it was successfully used for the determination of fulvestrant in its medicinal pharmaceutical form.

Subsequently, Tajik et al. (2015) developed an electrochemical biosensor (ds-DNA/PGE) for the quantification of Taxol. In electrochemical tests, it was observed that an increase in Taxol concentration promoted a corresponding decrease in the oxidation signal from guanine and adenine bases present in DNA, possibly due to the intercalation of the drug into DNA. The biosensor was used for determination of the drug in its injectable dosage form, as well as in urine and human serum. However, the sensor demonstrated a sensitivity and linear range for Taxol determination close to those observed using methods developed by Mehdinia et al. (2008).

Shamagsumova et al. (2015) produced biosensors from the electropolymerization of aniline on a GCE surface, using the cyclic voltammetry technique in the presence of DNA and oxalic acid, as a doping agent for the determination of anthracyclines (doxorubicin, daunorubicin, and idarubicin). In this study, the voltammetric response of the redox ferrocyanide mediator was studied, in the absence and presence of anthracyclines. The addition of anthracyclines to the electrolytic medium resulted in decreased currents being observed, and an increased resistance to electron transfer between the ferrocyanide mediator used as the electrochemical probe and the electrode surface. This was probably due to the ability of the anthracycline molecules to penetrate the surface layer of the PANI and intercalate into the DNA, making it difficult to transfer electrons between the redox probe and the electrode (Shamagsumova et al., 2015).

The determination of anthracyclines was possible even in the presence of sulfanilamides, as well as some molecules commonly found in the blood which could potentially interfere with the assays. This sensor was also used in the determination of doxorubicin in a pharmaceutical preparation (Shamagsumova et al., 2015), though it was less sensitive in the detection of anthracyclines than that observed for the sensor developed by Chandra et al. (2011).

Karimi-Maleh et al. (2015) developed a biosensor for the detection of 6-mercaptopurine, using DNA immobilized on PGE modified with polypyrrole (PP) and functionalized multi-walled carbon nanotubes (dsDNA/PP/MWCNTS/PGE). The drug 6-mercaptopurine acts as an anti-metabolite, inhibiting the biosynthesis of adenine nucleotides, essential for cell replication, and has been widely used in the treatment of leukemias (Karimi-Maleh et al., 2015). This biosensor was able to detect the drug at very low concentrations, and it was observed that the addition of the drug to the electrolytic medium promoted a considerable decrease in the oxidation processes of guanine and adenine in the DNA present in the biosensor, proportionally to the amount of drug present in the sample, at a given concentration range. The biosensor was also applied for the detection of 6-mercaptopurine in pharmaceutical samples and in human urine by the standard addition method (Karimi-Maleh et al., 2015).

Wang et al. (2012) developed a biosensor for methotrexate detection, using DNA-functionalized single-walled carbon nanotubes and Nafion composite film, for GCE modification. The DNA/SWCNT/Nafion-modified/GCE was able to detect this drug, demonstrating a high sensitivity and low overpotentials, which can be attributed to the synergic action promoted by DNA-functionalized SWCNT and Nafion.

With an excellent film-forming ability, the Nafion could increase the immobilization stability of DNA/SWCNT on the GCE surface. In addition, it could also attract the positively-charged methotrexate (in acidic media) to the electrode surface, affecting its accumulation. The biosensor was also applied to assay methotrexate in medicinal tablets and spiked human blood serum samples (Wang et al., 2012).

## 5. Technological prospecting

Based on the number and variety of different electrochemical sensors and biosensors already described in the literature, it is clear that this subject has been gaining prominence, especially in recent years. It is a promising scientific area which could bring great advantages to human health and the environment. However, to achieve these objectives, many aspects must still be improved, and the defense of industrial property rights related to such sensors are of paramount importance.

A technological prospection was carried out in order to report on the patents encompassing the use of electrochemical sensors and biosensors in the analysis of antineoplastic drugs. The search was carried out in October 2017 at the headquarters of the Instituto Nacional de Propriedade Industrial (INPI), European Patent Office (EPO), World Intellectual Property Organization (WIPO), United States Patent and Trademark Office (USPTO), and Free Patents Online (FPO). This research was carried out on patent titles, and when the exact terms were used individually, many patents were located. However, when search terms were combined, relating electrochemical sensors and biosensors to antineoplastic drugs, no patents were found in the INPI, EPO, WIPO, and FPO databases, as presented in Table 4. This shows that the technology of electrochemical sensors and biosensors in the detection of antineoplastic drugs, although growing, is still little explored when considering patents.

In the USPTO database, some patents were found by combining the terms "electrochemical sensor" and "chemotherapy drug", but none of them were specifically related to the development of electrochemical sensors for the analysis of antineoplastic drugs. Among the patents found, some reported the production of implantable devices for dynamic monitoring of physiological and biological properties of tumors, whereas others related to the development of wireless implantable sensors for the detection of local radiation received in radiotherapeutic treatments. Further patents related to sensors for the detection of molecules used in the treatment of cancer, but they were associated with the use of radiotherapy and not chemotherapy.

Thus, it is observed that the protection of reserved rights in the

commercial exploration of electrochemical sensors and biosensors for antineoplastic drugs is still an unexplored area. Given the different possibilities arising from the practical use of these tools, it is a promising area to be explored.

## 6. Conclusion

This work provides for the first time a simplified and thorough evaluation of the main electrochemical sensors and biosensors developed so far for the analysis of antineoplastic drugs. Different electrochemical sensors and biosensors have been found to be promising for the analysis of these drugs, and based on the large number of antineoplastic agents currently available on the market, and the potential demonstrated by these sensors in the quantification of such drugs, new studies should be carried out for the development of new sensing technologies.

However, according to the information collected in the databases of the INPI, EPO, WIPO, USPTO, and FPO, to date no patent deposits have been registered, indicating that these sensors are still a promising field to be explored.

Thus, the improvement of electrochemical sensors and biosensors in order to ensure their practical and safe use for the detection of antineoplastic agents, especially those that are harmful, is of great value for the safety of professionals working with these drugs, patients, and the environment, which we know to be at risk of contamination.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## 7. Future perspectives

The research in sensing systems for antineoplastic drugs can bring great advantages in different fields, since they may be used in the quality control of pharmaceutical products, in the monitoring of environmental contamination, in the control of occupational exposure, and even for the optimization of the therapies in patients. However, some difficulties must still be overcome for the practical and safe use of these tools. Many of the materials used in the production of these (bio) sensors are still expensive or scarce, while some sensors do not fulfill all the necessary requirements for an analysis methodology, needing validation and analyses in real samples, and in concentrations within ranges of clinical importance. The reserved rights in the commercial exploration of electrochemical sensors and biosensors for the antineoplastic drugs still are an unexplored area.

The development of sensors with financially accessible and abundant materials, which have potential for use in electrochemical sensors, should be encouraged. The validation of analytical methodologies, and how best to guide the regulatory agencies of each country, also represent a crucial point to be assessed.

## Acknowledgements

The authors thank Coordination Support in Higher Education (CAPES), the National Council for Scientific and Technological Development (CNPq), and the Foundation of Support to Research of Piauí (FAPEPI). The authors also thank The Federal University of Piauí (UFPI) and Federal Institute of Piauí (IFPI).

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