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# Detection Methods and Tools of Administered Anti-Epileptic Drugs - A Review

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## Abstract

Anti-Epileptic Drugs (AEDs) are prescribed for extended periods of time for patients suffering from disabling seizures. This manuscript reviews main methods and tools available for the detection of AEDs for patients, assessing the advantages and drawbacks of each technique. On one hand, chromatography-based sensors are highly selective and sensitive; however, they are often time-consuming and require specialized technicians and bulky equipment. Optical-based and micro cantilever sensors are sensitive and easy to manipulate; nevertheless, they generally lack selectivity and also require specialized technicians. On the other hand, electrochemical-based sensors, although not as sensitive as chromatography-based ones, are easiest to operate and possess the potential to be selective, miniature, and implantable. After a thorough analysis of the scientific literature, it was found that electrochemical biosensors are most likely the best for Therapeutic Drug Monitoring (TDM) since they are simple, do not require much labor and expertise, are inexpensive, have a quick time of analysis, only require a small sample and can be miniaturized for Point of Care (POC) applications. More- over, electrochemical sensors may be modified through immobilizing Molecular Imprinted Polymers (MIP) at the Working Electrode's (WE's) surface. All these factors make electro- chemical techniques most suitable for TDM and remove a lot of the hassle associated with other methods.

**Keywords:** Anti-Epileptic Drug (AED); Biosensor; Chromatography; Electrochemical; Epilepsy; Therapeutic Drug Monitoring (TDM)

## Introduction

The history of biosensors dates back to as early as 1906 when Cremer demonstrated that the concentration of an acid in a liquid is proportional to the electric potential that arises between parts of the fluid located on opposite sides of a glass membrane [1]. However, a first complete biosensor was only developed in 1956 by Leland Clark Jr for oxygen detection [2,3]. After these initial contributions, much attention has been allocated to the research and development of a large variety of biosensors. A biosensor is an analytical device which often contains immobilized biological material which specifically interact with an analyte and produce a physical, chemical or electrical signal proportional to the amount of analyte present in the solution [2]. Among commonly immobilized material are enzymes, antibodies, nucleic acid chains, and hormones. An analyte is defined as a component of interest generally measured by its chemical or physical properties [4-6].

Biosensors are nowadays ubiquitous in biomedical diagnosis as well as a wide range of other areas such as point-of-care monitoring of disease treatment or progression [4], environ- mental monitoring [4], food quality control [5], drug discovery [6], forensics and personalized treatment [2,4]. One of their main applications is the detection of biomolecules that are either indicators of a disease or targets of a drug [2]. In particular, electrochemical biosensors can be used as clinical tools to detect protein cancer biomarkers [7-9]. Currently, glucose biosensors are the most widely used biosensor accounting for 85% of home-used biosensors worldwide as diabetes mellitus treatment involves precise control of blood-glucose levels [10,11].

(Figure 1) illustrates the general layout of a biosensor. The elements that interact, recognize or detect an analyte are referred to in this text as biosensing elements, and components that generate or help generate a signal related to a certain physical or chemical property of an analyte are referred to as transducing elements.

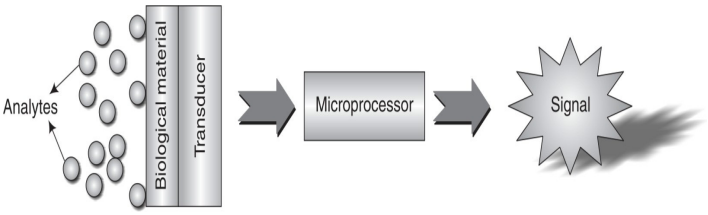


**Figure 1:** The general layout of a biosensor [12].

Biosensors essentially involve the quantitative analysis of various substances by converting their biological properties into measurable signals. (Figure 2) illustrates the process of generating a signal from an analyte. The performance of a biosensor is mostly dependent on the specificity and sensitivity of the biological reaction, which is highly determined by the sensor's biosensing and transducing elements [5,13]. This review presents the latest biosensing and transducing elements applied to the detection of AEDs. Biosensors were categorized according to their main blocks: transducing element and biosensing element. Sections were assigned to the different categories with a focus on methods deployed, results and efficacy.

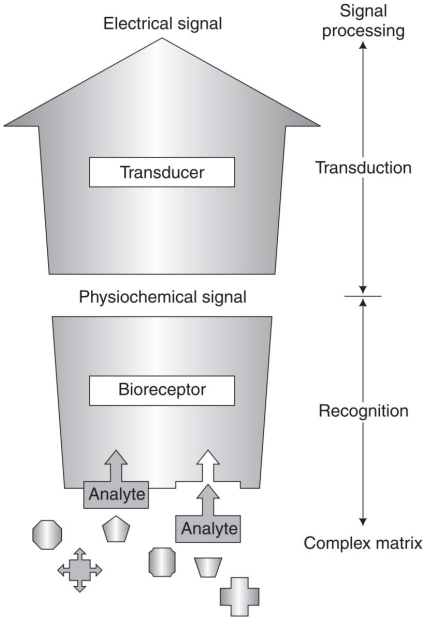


**Figure 2:** Transducer signaling process in a biosensor [12].

## Biosensor Characteristics

When analyzing biosensors, there are numerous characteristics that allows us to assess their performance including selectivity, reproducibility, stability, sensitivity, and linearity. Selectivity is the ability of a bioreceptor to detect a specific analyte in a sample containing contaminants. The best example of selectivity is depicted by the interaction of an antigen with the antibody. Reproducibility, on the other hand, is the ability of the biosensor to generate identical responses for a duplicated experimental set-up. Furthermore, reproducibility is characterized by the precision and accuracy of the transducer and electronics in a biosensor [5,13]. Reproducible signals provide high reliability and robustness to the biosensor's inference. Stability, the most crucial feature of a biosensor, is the degree of susceptibility to ambient disturbances in and around the biosensing system.

These disturbances can cause a drift in the output signals of a biosensor under measurement causing an error in the measured concentration and can affect the precision and accuracy of the biosensor. Sensitivity is the biosensor's signal strength which along with the sensor's stability affect the minimum amount of analyte that can be detected by a biosensor, referred to as its Limit of Detection (LOD). Linearity is the attribute that shows the accuracy of the measured response (for a set of measurements with different concentrations of analyte) to a straight line [2]. The linearity of the biosensor can be associated with the resolution of the biosensor and the range of analyte concentrations under test. Finally, the resolution of the biosensor is defined as the smallest change in the concentration of an analyte that is required to bring a change in the response of the biosensor [2].

In addition to all above characteristics, biosensor miniaturization has proved to be beneficial for various reasons. For instance, reducing the size of the biosensor to the micro- or nanoscale can result in a better signal-to-noise ratio as well as the possibility of using smaller sample volumes, which means lower assay costs. Moreover, when going towards nanoscale dimensions, the surface-to-volume ratio of the sensing active area increases, and the sizes of the detecting electrodes and that of the target biomarker become comparable. This reduces non-specific binding and increases binding efficiency towards the target molecule. As a result, the bioreceptor becomes an active transducer for the sensing system and it becomes possible to perform single-molecule detection [2,14]. Miniaturization also allows for easier integration of these sensors in point-of-care monitoring allowing them to be implantable.

## Anti-epileptic Drugs and Therapeutic Drug Monitoring

AEDs are the first line of treatment offered to epileptic patients. These drugs are gener- ally taken by mouth once, twice or three times a day depending on their pharmacokinetic properties. Post absorption, AED levels reach a point where the patient is most protected against seizures, and where risks of dose-related

side effects are highest. Levels will then gradually decrease until reaching a trough where the patient has the least seizure protection. Anew, levels will rise with the intake of the next dose. While low serum concentrations may have no therapeutic effect, high serum concentration may cause side effects [15].

Common dose-related side effects of AEDs include dizziness, blurred vision, dysarthria, ataxia, somnolence, and psychomotor slowing. Currently, more than fifteen AEDs are avail- able on the market. The most commonly used include Carbamazepine (CBZ), Valproic Acid (VPA), Levetiracetam (LEV), Phenytoin (PHT) and Lamotrigine (LTG) [16]. Therapeutic Drug Monitoring (TDM) of concentrations of drugs in body fluids, usually plasma, can be used during treatment. This information is used to individualize dosage so that drug concentrations can be maintained within a target range. In the routine management of epileptic patients, physicians will frequently order punctate blood levels of AEDs for various reasons such as a) to ensure that the patient has reached a sufficiently protective dosage, especially in the context of elderly patients, liver or renal disease, pregnancy, and polypharmacy (due to the possibility of drug interactions); b) assessing adherence or com- pliance; and c) detect or avoid therapeutic overshoot of dosing and development of overt clinical toxicity.

The current practice of ordering punctate AEDs levels has several limitations: a) there are numerous conditions which may affect drug levels, some of which are unpredictable and others for which the neurologist may be unaware of before it is too late (e.g. poor compliance, new onset co-morbid condition, pregnancy, prescription by another physician of a drug which can interact with AEDs such as oral contraceptives pills, grapefruit consummation which may elevate for example carbamazepine levels etc.); b) results from AED testing will vary according to the time of measurement (before the AED was taken versus several hours after the dose was taken); c) because patients need to go to a health care facility and wait in line for blood collection, obtaining these AED levels can be time consuming and sometimes logistically difficult, especially for elderly patients and those who require frequent drug levels.

A sensor capable of continuously measuring AED levels in the bloodstream or tissue *in vivo* would give clinicians a valuable window into patients' health and their response to therapeutics. This device could allow for example: a) a better understanding of (intra- or inter-individual) drug level variations whether it can be over 24h, days, weeks or years, at various ages, during certain concomitant physiological (e.g. pregnancy, sleep) or pathological conditions (surgery, alcohol intoxication etc.); b) a comprehensive assessment of compliance by patients; c) a thorough causality assessment between drug levels and side effects or seizure protection; d) a better assessment of the impact of switching from brand-name to generic AEDs. Unfortunately, continuous, real-time measurements are currently only possible for a handful of targets, such as glucose, lactose, and oxygen and the few existing platforms for continuous measurement are not generalizable for the monitoring of other analytes, such as small-molecule therapeutics.

## The Transducing Element

A biosensor is a sensing device that compromises a biological component-referred to as a sensing element in this text - and a transducer that transforms biochemical activity into a measurable signal proportional to the quantity of analyte present. In this section, the transducing element of the biosensor will be discussed and categorized into chromatography- based, optical-based, and electrochemical-based sensors.

### Chromatography Based Methods

Chromatography-based methods are one of the most adopted methods for TDM of AEDs because of their high accuracy, sensitivity and selectivity [17-22]. Drawbacks include that they require expensive bulky instruments and a long time for sample pretreatment. The four main types of chromatography techniques are liquid chromatography, gas chromatography, thin-layer chromatography and paper chromatography. In liquid chromatography, the liquid solvent containing the sample mixture travels by gravity through a column containing solid adsorbent material. Contingent upon the mixture's interaction with the adsorbent material, different flow rates separate the components as they flow out of the column. An improved version of liquid chromatography is High-Performance Liquid Chromatography (HPLC) [23-25] where the solvent is pressurized by a pump through the column reducing the time of separation. In gas chromatography, helium is used to move a gaseous mixture through a column of absorbent material. Gas chromatography is applied in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition for testing the purity of a substance or separating the different components of a mixture [26].

Both thin-layer chromatography and paper chromatography use an absorbent material on flat glass or plastic plates as stationary phases; however, in contrast to paper in paper chromatography, silica or alumina is used in thin-layer chromatography. All mentioned chromatography techniques are often coupled with mass spectroscopy to enhance detection after separation [27,28]. The latter is an optical-based detection technique which relies on quantifying particles through measuring their mass-to-charge ratio after ionization. Due to their precision, accuracy and high selectivity, HPLC and gas chromatography are the most widely used techniques for therapeutic drug monitoring. Shah et al. [29], implemented a HPLC technique by taking a sample of dried blood spots from patients for the simultaneous determination of AEDs

LEV, LTG, Phenobarbital (PHB), CBZ and carbamazepine 10,11 epoxide (CBZE). Dried blood spots sampling provides multiple advantages over conventional venous sampling such as: a) only a small volume of blood is required, beneficial for amassing samples from newborns, youth and seniors; b) it can be performed by non-professionals; c) the dried blood spots samples do not need to be processed and prepared; e) most importantly, once dried, many analytes including antibodies are stabilized on filter paper [30]. However due to the use of minimal sample volumes, there is a high risk of false negatives with dried blood spots sampling [31]. Prior to analysis, whole blood aliquots were prepared from the dried blood spots samples by adding 75% buffer (25 mM phosphate buffer pH 6.2), 15% acetonitrile and 10% methanol.

Afterwards, 10 ml of analyte diluted in methanol at concentrations corresponding to recorded therapeutic ranges were added to 0.95 mL of the prepared blood aliquots. HPLC analysis using an XBridgeT M $C_{18}$ column (150 mm x 4.6 mm, 3.5 µm; Waters, UK) combined with ultraviolet detection was then carried out for a total run time of 28 min. The method was then validated by evaluating selectivity, linearity, limit of detection, accuracy and recovery. It was found that the mobile phase consisting of the above-mentioned mixture enabled the best chromatographic conditions to achieve good resolution of all analytes, including LEV which is highly polar and requires a mobile phase with little organic strength. Ultraviolet detection using a wavelength of 205 nm was then carried out to monitor the absorbance of the analyzed AEDs. Ultraviolet detection was used adjacent to liquid chromatography mass spectrometry since all the AEDs of interest are active at relatively high concentrations (µg/ml, rather than ng/ml). Apart from good selectivity, linearity, and accuracy within 15% at all quality control concentrations, acceptable Limit of Quantification (LOQ) and Limit of Detection (LOD) values were achieved as provided in (Table 1). Finally, it was found that extraction of AEDs from the tested samples using methanol: acetonitrile (3:1, v/v) gave the best recovery enabling the extended use of blood samples.

| AED | LOD (µg/ml) | LOQ (µg/ml) |
|---|---|---|
| LEV | 0.38 | 1.15 |
| LTG | 0.223 | 0.676 |
| PHB | 0.318 | 0.963 |
| CBZE | 0.3 | 0.908 |
| CBZ | 0.258 | 0.78 |

**Table 1:** Calculated LOD and LOQ for AEDs in dried blood spots samples [29].

De Almeida, et al. [32] on the other hand, used liquid chromatography coupled with mass spectrometry for the determination of AEDs bromazepam, lorazepam, CBZ, and diazepam. Specifically, samples were pre-concentrated with the aid of $C_{18}$ Premium 300 mg/3 ml cartridges pre-conditioned with 10 ml methanol and 10 ml water (pH 2.0), and the analytical determinations were carried out using a liquid chromatograph equipped with a binary pump, a degasser, a column oven and an automatic injector. Furthermore, the operational conditions were the following: analytical column = Zorbax SB $C_{18}$ 5 µm, 4.6 x 150 mm; mobile phases = water and methanol, both containing 1.0% v/v formic acid. The gradient program adopted began with 10% mobile phase methanol, then the mobile phase was increased linearly up to 80% for 5.5 min. Afterwards, it was kept at 80 % until 8 min was reached, then increased again to 90% until 11 min, finally increased to 100% from 13 min to 15 min before dropping it back to initial conditions (Table 2).

| Time (min) | Flow rate (ml/ min) | Injection volume (µl) | Methanol % |
|---|---|---|---|
| Initially | 0.7 | 25 | 10 |
| 0 - 5.5 | 0.7 | 25 | 10 - 80 |
| 5.5 - 8 | 0.7 | 25 | 80 |
| 8 - 11 | 0.7 | 25 | 80 - 90 |
| 11 - 13 | 0.7 | 25 | 90 |
| 13 - 15 | 0.7 | 25 | 90 - 100 |
| Final | 0.7 | 25 | 10 |

**Table 2:** HPLC gradient program [32].

After separation, the compounds were quantified using a mass spectrometer equipped with an electrospray ionization source. The spectrometer was optimized by infusing the working solution of each analyte and determining both the ionization mode and precursor ion. Furthermore, the ionization conditions were found by injecting a standard solution of each analyte at a rate of 100 µg/l. The mass spectrometer parameters used are presented in (Table 3). De Almeida et al. were able to achieve satisfactory precision and exactitude with intra-day precision values between 3.6 % and 5.8 % and inter-day precision values between 5.1 % and 9.5 %. Finally, the LOD values were found to be between 4.9 and 6.1 ng/l and the LOQ between 30 and 50 ng/l.

| Drug | Precursor Ion (m/z) | Product Ion (m/z) | DP. | CE. (%) | CCEP. |
|---|---|---|---|---|---|
| Bromazepam | 316 | 214 | 106 | 39 | 12 |
| | | 156 | 106 | 45 | 10 |
| CBZ | 237 | 194 | 130 | 53 | 10 |
| | | 179 | 130 | 53 | 10 |
| Clonazepam | 316 | 270 | 101 | 35 | 14 |
| | | 181 | 101 | 67 | 8 |
| Diazepam | 285 | 193 | 56 | 53 | 10 |
| | | 154 | 130 | 53 | 10 |
| Lorazepam | 321 | 275 | 101 | 53 | 10 |
| | | 302 | 106 | 47 | 10 |
| DP: Declustering Potential; CE: Collision energy; CCEP: Collision Cell Exit Potential | | | | | |

**Table 3:** Mass spectrometer parameters used [32].

Hashem, et al. [33] implemented an HPLC method that is simple, rapid, accurate, and stable for the quantification of PHB and PHT in various forms: powder forms, dosage form and in urine samples. Prior to analysis, stock solutions of PHB (1 mg/ml) and PHT (1 mg/ml) were dissolved in methanol and acetonitrile respectively. Furthermore, apart from control samples, others were loaded with either HCL, NaOH or $H_2O_2$ or exposed to ultraviolet radiation in order to perform forced degradation studies. The different samples containing PHB were separated using an analytical column with an isocratic binary mobile phase of MeOH/$H_2O$ (38.0/62.0, v/v) at a flow rate of 3 ml/min at a temperature of 40°C and detection was achieved at 214 nm. Whereas the PHT containing samples were separated using an analytical column with an isocratic binary mobile phase of ACN/$H_2O$ (25.0/75.0, v/v) at a flow rate of 1 ml/min at 40°C and detected at 220 nm. Phenobarbital showed a strong degradation with NaOH, and a weak degradation with HCL, $H_2O_2$, and upon exposure to ultraviolet radiation. Phenytoin, on the other hand, showed weak a degradation with all NaOH, HCL, $H_2O_2$, and upon exposure to ultraviolet radiation. In order to study the detection limit of the HPLC method developed, 13 concentrations of PHB and PHT solutions ranging from 0.061-100 µg/ml were prepared. The graph of the peak area versus concentration provided linearity in the range of 1 - 20 µg/ml for PHB and 1 - 50 µg/ml for PHT. The limits of quantification were found to be 0.250 µg/ml for PHB and 0.500 µg/ml for PHT. Both drugs were further analyzed, PHB in suppository, and PHT in capsules and spiked urine. Results are provided in (Table 4).

| | PHB | | PHT | | |
|---|---|---|---|---|---|
| | Recovery | St. Addition (suppository) | Recovery | St. Addition (suppository) | Spiked urine |
| Average | 100.78 | 99.3 | 96.91 | 96.05 | 96.69 |
| RSD | 2.62 | 2.94 | 1.06 | 1.67 | 0.68 |
| SD | 2.6 | 2.96 | 1.09 | 1.74 | 0.7 |

**Table 4:** Determination of PHB and PHT in Dosage forms or in Urine [33].

Shah, et al. [34] developed an accurate, simple, rapid, precise and linear technique using reverse phase HPLC for simultaneous estimation of PHT and PHB. The term reverse phase describes the chromatography mode that is opposite of a normal phase, namely the use of a polar mobile phase and a non-polar hydrophobic stationary phase. Shah et al. tested different chromatographic conditions for better separation and resolution. A mobile phase of methanol: phosphate buffer (pH 5) adjusted with 0.1 M NaOH (50:50), a flow rate of 1.0 ml/min and a run time of 9 min were found to be fit for the analysis. Furthermore, ultraviolet detection was at 215 nm for both drugs. The mobile phase was also used as the solvent to prepare the drug solutions. The linearity of the test solutions for the assay using 5 different concentrations of each drug was found to be within the concentration range of 10 - 30

µg/ml and 3 - 9 µg/ml for PHT sodium and PHB respectively. The retention time for PHT was 3.97 min and 6.90 min for PHB. The LOD for PHT was 1.44 µg/ml and 4.36 µg/ml; the LOD for PHB was 0.4 and 1.35 µg/ml. The proposed reverse phase HPLC method used methanol and phosphate buffer which are both easily available and achieved a recovery rate of 98 % to 100 % for each drug which made it simple, easy to perform and economical.

## Optical Based Methods

Optical biosensors employed in AED detection offer great advantages because they enable direct, real-time and label-free detection of many biological and chemical substances [35,36]. Their advantages also include high specificity, sensitivity, smallness and cost-effectiveness. Optical-based detection is performed by exploiting the interaction of an optical field with a biorecognition element [37]. Spectrophotometry is a simple optical technique that relies on measuring the absorbance of a medium to light. It requires a photometer to measure the intensity of light through an analyte containing medium by studying the wavelength. This simple technique has been employed in AED detection systems [38-44] to detect and characterize analytes according to their absorbance spectra. Revanasiddappa, et al. [45] used a UV-Vis Spectrophotometer to study the determination and degradation of oxycar- bamazepine, a keto analog of carbamazepine, in HCl, NaOH, $H_2O_2$, thermal and UV radiation. They concluded that the absorbance spectra of oxycarbamazepine solution prepared in methanol:acetonitrile (50:50, v/v) showed a direct correlation with the amount of analyte present at a wavelength of 255 nm. The LOD and LOQ measured by Revanasiddappa were 0.0550 µg/ml and 0.1667µg/ml respectively. However, despite good sensitivity, Revanasid- dappa's method does not claim to be selective.

On the other hand, Rezaei, et al. [46] used partial least-squares regression combined with UV-spectrophotometry in order to achieve selective detection of both CBZ and PHT. Partial least-squares regression is a linear statistical method that measures the correlation of two variables and has been utilized in spectrophotometric-multicomponent analysis of various drugs in biological and pharmaceutical samples [47-53]. Authors found a correlation with the drugs and their respective absorbance spectra and reported recovery percentages of CBZ and PHT to be 98.4 and 98.2 respectively. However, the authors do not yet propose this method as an alternative to HPLC for in plasma detection which is a complex medium. In addition to spectrophotometry, surface plasmon resonance, the most common optical technique, relies on a phenomenon that occurs on the surface of metals (or other conducting materials) at the interface of two media (usually glass and liquid) when it is illuminated by polarized light at a specific angle.

This generates surface plasmons and consequently a reduction of the intensity of reflected light at a specific angle known as the resonance angle. This effect is proportionate to the mass on the surface. As a result, a sensogram can be obtained by measuring the shift of reflectivity, angle or wavelengths against time. (Figure 3) demonstrates the basic schematics of a surface plasmon resonance setup as well as the sensogram measurements. Multiple and various interacting molecules or biosensing elements could be immobilized depending on the analyte of interest, which makes surface plasmon resonance a universal technique suitable for various biosensing applications. For example, to measure a ligand-analyte interaction, one interacting molecule must be immobilized on the sensor surface. However, small molecules, such as most therapeutic drugs, once captured on the surface, may not affect the refractive index significantly, which makes their direct detection and quantification difficult. Furthermore, nonspecific binding is an issue that occurs in most universal detectors and is defined as the adsorption or binding on the sensor's surface of molecules that are not related to the analysis [54].

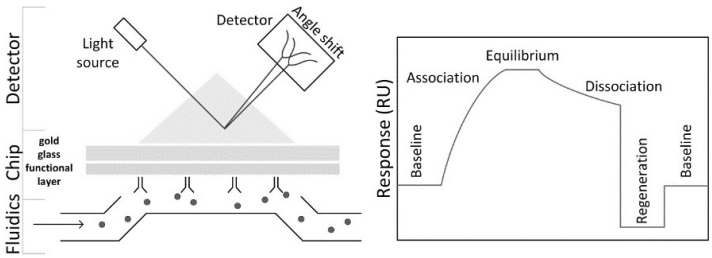


**Figure 3:** The principle of a surface plasmon resonance instrument (left) and a typical surface plasmon resonance sensorgram showing the steps of an analytical cycle (right).

Fu, et al. [55], developed a prototype biosensor that takes advantage of surface plasmon resonance for the detection of PHT in saliva. Saliva is not often used as a sample for TDM; however, it is an ideal sample for patients since its collection is non-invasive and painless, and samples can be obtained more frequently than would be practical with blood [56]. Nevertheless, analytes, especially PHT, are often present in saliva at concentrations that correlate well with their free levels in the blood. Phenytoin was tested in both phosphates buffered saline and human saliva. Saliva was preconditioned by a 0.2-µm-pore conventional polymeric filter and a microfluidic diffusion-based separations device, the flat H-filter [57,58]. This combination of filters removes 98 % of the glycoprotein/mucin content and 92 % of the protein content, while retaining 27 % of the small-molecule analytes [59].

To act as a source, a near infrared light-emitting diode was used along with stationary wide-field image imaging optics. A compact liquid crystal polarizer enabled electronic switching of the source between a transverse magnetic mode, where the magnetic field is perpendicular to the wave-guide axis, and a

transverse electric mode, where the electric field is perpendicular to the wave-guide axis. Additionally, the concept of folded optics, to miniaturize the optical module whilst still achieving a long optical path producing a strong and focused beam.

Apart from that, Fu, et al. [55] utilized a 1/3" CCD with 640 x 480 pixels' image detector with a fast readout to enable improvement of image statistics through averaging and a low background noise to operate at high light levels. The CCD, integrated into a camera with a powerful digital signal processor was able to acquire and sum images at 30 frames per second. Fu, et al. [55] then used a custom-coded software to control both data acquisition functions such as light-emitting diode translation, polarization switching and image acquisition, and fluid motion such as valve and pump actuation. Finally, the data analysis function was created using MATLAB and implemented on a tablet-style paper chromatography enabling large processing power and a high-resolution display.

In order to produce a change in the refractive index, the unknown amount of PHT was mixed with a known amount of anti-PHT which was then introduced to the PHT-surface immobilized detection zone. Only the free unbound anti-PHT binds to the immobilized PHT causing a change in the refractive index quantifiable by the SPR imaging system. The SPR signals provided in (Figure 4) indicate that both the rate of binding and the total coverage of anti-PHT are inversely correlated with the concentration of PHT. Furthermore, in order to detect different AEDs, antibodies for the AED of interest could replace that of PHT and by a small-range translation of the source the instrument response can be optimized.

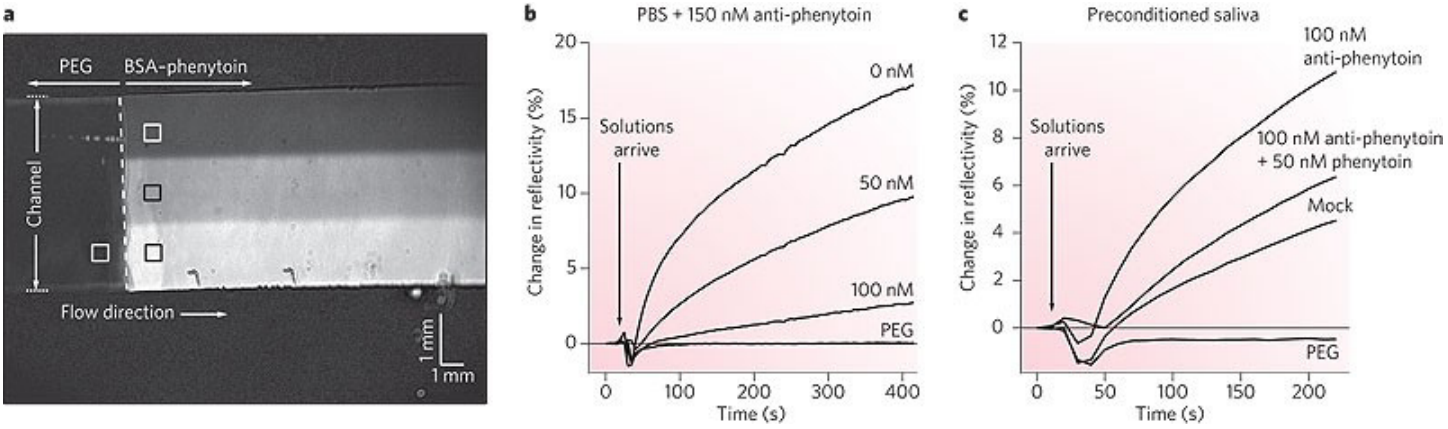


**Figure 4:** Parallel indirect immunoassays for PHT conducted using multiple flows. (A) The SPR difference image shows the outcome of anti-PHT binding to the surface from samples containing 0, 50, or 100 nM PHT in phosphate buffer premixed with 150 nM anti-PHT after 5 min (B) Assay results for PHT spiked into PBS (C) Assay results for PHT spiked into preconditioned saliva [59].

## Electrochemical Methods

Electrochemical methods are a class in analytical chemistry which rely on either current or voltage to detect and measure the analyte. Located in an electrochemical cell, the analyte is subjected to an excitation signal followed by measuring the response signal representing the concentration of the analyte. Such methods are mainly potentiometry, voltammetry, and impedance spectroscopy. Due to ease-of-application, sensitivity, low-cost, minimal need for labor and least damage to analyte, electrochemical methods are highly reliable. Biosensing elements could be immobilized on the surface of the working electrode to improve upon its selectivity. However, the working electrode could also be modified with different materials to amplify the strength of the signal which will improve the signal-to- noise ratio [60,61]. Several factors may affect electrochemical evaluation, such as the electric conductivity of the electrode, solvent type, scan rate, and distance between electrodes. Therefore, it is important to control such factors in order to achieve reproducible results.

Raoof, et al. [62] developed a highly sensitive voltammetric sensor for the determination of PHB in the presence of acetaminophen. Cyclic voltammetry and differential pulse voltammetry were conducted using a multiwalled carbon nanotube paste electrode as the working electrode, a platinum wire as the counter electrode and an Ag|AgCl|KCl (3 M) electrode as the reference electrode. Multiwalled carbon nanotubes are allotropes of carbon with cylin- drical nanostructures possessing large-surface area, high stability at nanoscale, and high thermal, electrical and mechanical conductivity. They were able to attain detection limits for AC and PHB of 0.17 and 0.1 M, respectively.

On the other hand, chemical doping at the electrode's surface has proved to be effective for chemo-biosensing applications [63-65]. Lavanya, et al. [66], developed an electrochemical sensor

for the determination of CBZ levels. They modified the working electrode by Fe- $SnO_2$ doped with $Fe^{3+}$. The added active elements stabilize the $SnO_2$ surface and promote a decrease in grain size which enhances higher catalytic activity and sensor response than that of pure $SnO_2$. The behaviour of CBZ at the Working Electrode (WE) was investigated using CV and Square Wave Voltammetry (SWV). Despite achieving oxidation peaks at 0.78 V for a bare screen-printed electrode which is much lower than literature values ~1.15 V [14,62,66,67], modifying the electrode resulted in a significant increase in anodic peak currents of CBZ (Ipa = 14.8 µA) (Figure 5). This increase in anodic current is due to the large effective electrode surface area and higher electron conductivity of the Fe doped $SnO_2$ NPs. To conclude, their fabricated sensor displayed a good electro-oxidation response towards the detection of CBZ at a lower oxidation potential of 0.8 V in phosphate buffer solution at pH 7.0with a wide linear range of 0.5-100 µM and a low detection limit of 0.5-100 µM.

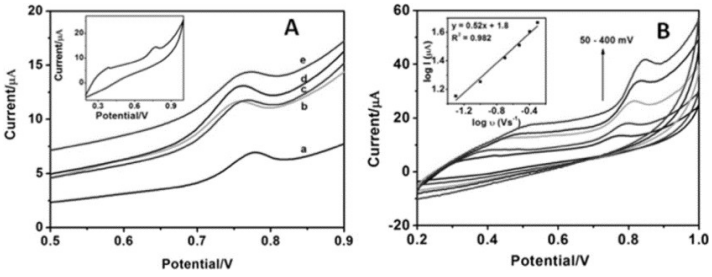


**Figure 5:** A) Anodic scan of CVs of 50µM CBZ at a) bare SPCE, b) $Sno_2$; B) CVs obtained at different scan rates [66].

On the other hand, Balasubramanian, et al. [68] developed a CBZ detection biosensor based on both graphene oxide and Graphitic Carbon Nitride Composite (GO/g-C3N4). Despite its specific electronic structure, low toxicity and stability, g-$C_3N_4$ was found to be limited due to its low electrical conductivity [69,70]. Therefore, graphene oxide was added along with g-$C_3N_4$ to the electrodes surface. This improves electrocatalytic activity along with the detection limit, sensitivity and linear range. Cyclic Voltammetry was used in order to prove the superior electro-catalytic activity of (GO/gC3N4). Performance of the GO/g C3N4 modified glassy carbon electrode was compared g-$C_3N_4$ / GCE, in a $N_2$ -purged 0.05 M phosphate buffer solution. The working electrode had excellent electro-catalytic activity with an overpotential of only 0.1V. and attained the best rate of the electron transfer as can be seen in (Figure 6). Furthermore, an amperometric assay calculated the limit of detection of CBZ on the modified electrode along with the sensitivity to be 10.5 nM and 1.727 µA $\mu M^{-1}cm^{-2}$ respectively.

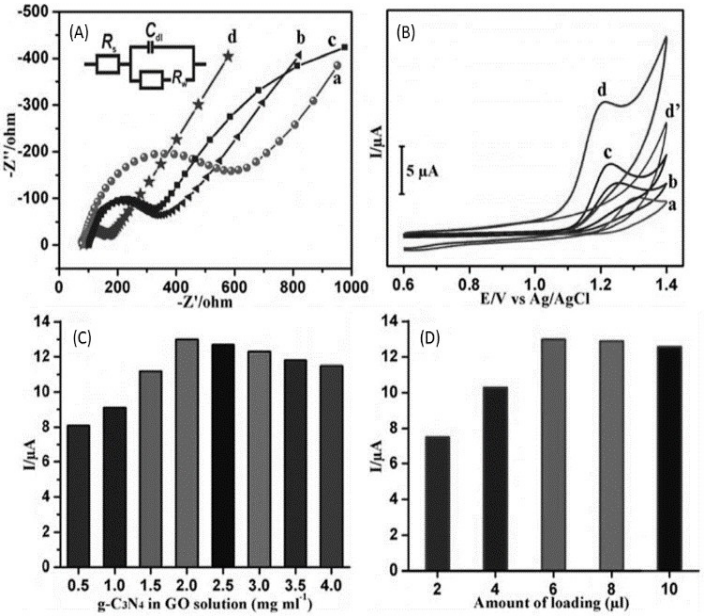


**Figure 6:** A) EIS spectrum of bare GCE (a), g-C3N4/GCE (b), GO/GCE (c), and GO/g-C3N4/GCE (d). (B) CVs of bare GCE (a), g-C3N4/GCE (b), GO/GCE (c), and GO/g-C3N4/GCE (d) in 0.05 M PBS (pH 7) containing 20 µM CBZ and GO/g-C3N4/GCE (d') absence of 20 µM CBZ at scan rate of 50 $mVs^{-1}$. (C) Anodic current response of 20 µM CBZ on various amount ofg-C3N4 loaded GO. (D) The effect of loading amount of GO/g-C3N4 composite on GCE [68].

Lin, et al. [67], developed a biosensor to find serum levels of CBZ in rabbits. This was accomplished using DPV with GCE as the working electrode. Initially, dropping mercury electrodes were to be used; however, mercury is not only toxic to the environment but can also enter the human body by inhalation or ingestion, potentially resulting in acute mercury intoxication, which can manifest as chills, chest pain, dyspnea, and pulmonary infiltration, or chronic mercury intoxication, which can cause tremors, social withdrawal, irritability, perspiration, rash, and paresthesia [71]. Furthermore, pre-treatment of the test samples with acetonitrile was crucial to maintain a good performance of the DPV method which would be affected by the presence of N, S, or O the common elements in serum. Blood samples from rabbits that were fed CBZ were obtained and added to a 3-electrode setup containing a glassy-carbon electrode as the working electrode, a platinum wire as auxiliary electrode, and an Ag/AgCl, KCl as reference electrode. Different concentrations of CBZ were tested: 4, 8, 12 µg/ml. The detection limit was 0.14 µg/ml for the DPV technique. For comparison reasons Lin also performed the fluorescence polarization immunoassay method which resulted in a detection

limit of 0.2 µg/ml. The correlation between the CBZ concentrations from DPV compared with those by fluorescence polarization immunoassay was good (RSQ = 0.998). Lin concluded that the electrochemical sensor had a superior detection limit, precision and accuracy compared to fluorescence polarization immunoassay.

In a work similar to Lin, Wang, et al. [72], also developed a biosensor for the determination CBZ levels in serum using DPV and a bare GCE as the working electrode. Furthermore, the performance of the sensor was also compared with fluorescence polarization immunoassay technique. The tested samples were prepared in 7 different concentrations (0, 2, 4, 8, 12, 20 and 23.6 µg/ml) by dissolving CBZ in 0.1 M TBAP/ acetonitrile (Figure 7). The DPV parameters were the following: pulse amplitude: 50 mV; pulse width: 0.05 (sec); sample width: 0.0167(sec); pulse period: 0.2 (sec); scan rate: 20 mV/sec. The detection limit was 1 µg/ml for the DPV technique however was 0.5 µg/ml for the fluorescence polarization immunoassay technique. Despite the fact that fluorescence polarization immunoassay performance exceeded DPV in Wang's work, the performance of the DPV technique was within the FDA guidelines for bioanalytical methods, which ensures the clinical applicability of the DPV technique.

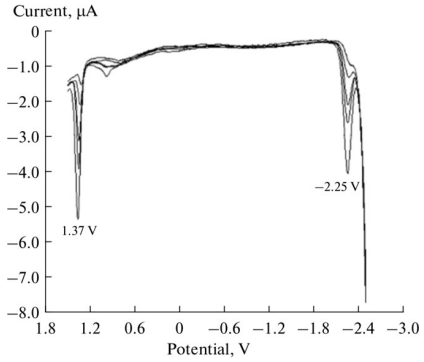


**Figure 7:** Typical DPV potential of CBZ in serum developed at +1.37 V and -2.25 V [72].

Finally Pan et al. [73] confirmed the reliability of DPV for measuring CBZ levels in human serum. The latter used a GCE with the following setup parameters: DPV pulse amplitude, 50 mV; pulse width: 0.05 s; sample width: 0.0167 (s); pulse period: 0.1 s; and scan rate: 20 mV/s. Again, the correlation between the results obtained by the DPV technique and the fluorescence polarization immunoassay technique were very good with RSQ = 0.998 (Figure 8).

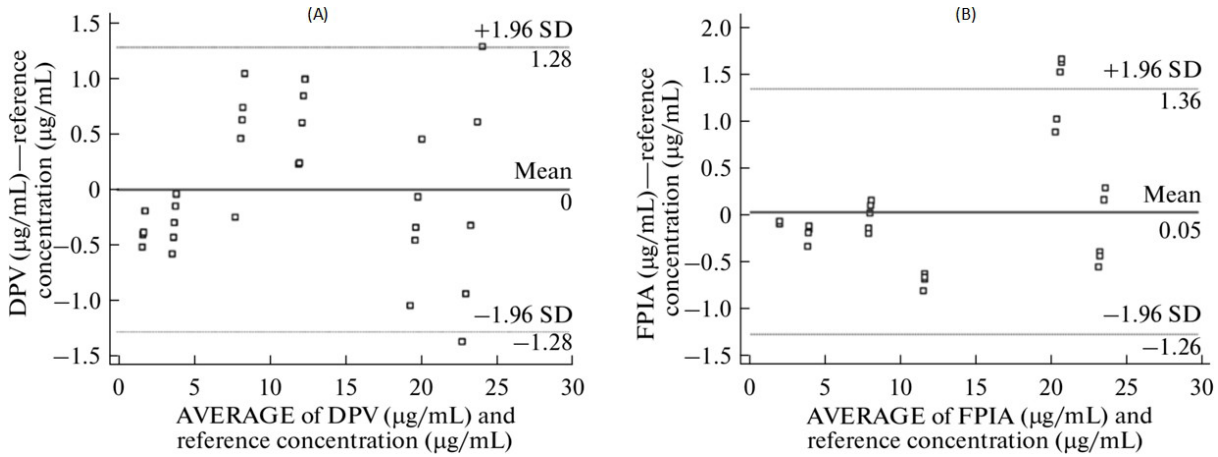


**Figure 8:** (A) Bland-Altman plots of the DPV results and reference value concentrations. The solid line represents the mean difference, and the dashed line represents 1.96 SD; (B) Bland-Altman plots of the FPIA results and reference value concentrations. The solid line represents the mean difference, and the dashed line represents 1.96 SD [72].

## The Biosensing Element

In the context of biosensors, a receptor is used to aid in analyte detection; it is often im- mobilized on the transducer's surface to specifically bind to the target analyte or a molecule related that target. Generally, bioreceptors include a vast category of molecules including enzymes, polymers, nucleic acids, proteins, and aptamers; however, given the paper is limited to at AED bioreceptors, the below sections will only cover bioreceptors utilized for AED detection. According to the reviewed papers, AED bioreceptors utilized in recent research centralize around antibodies [74-80] or molecular imprinted polymers (MIPs) [81-88].

### Antibody-Based Bioreceptors

The concept of antibodies generated from the field of immunoassays which relies on the selective binding and high affinity of the antigen to the antibody. Not only have antibodies

been incorporated on large-scale AED sensing systems, but their use has migrated towards nano-scale systems such as micro-electrodes, microcantilevers and miniature microfluidic systems. In his research, Huang [89] added PHT-antibodies to his piezoresistive micro- cantilever beam for PHT detection. The antibodies were incorporated into a microfluidic channel where analytes can enter and bind to the receptors. The use of a microfluid channel enhanced detection by filtering out unwanted particles, thus enabling the PHT particles to solely enter and bind to the antibodies. The binding yielded a deflection and thus an associated resistance change due to the molecular recognition. This change in resistance was then measured to interpret variations in PHT levels. The sensor had a linear detection response ranging from 10 to 80 µg/ml with a signal resolution of 0.005 Ω and a sensitivity of 2.94 x 10-6 µg/ml.

In a similar research, Huang [90] utilized VPA selective antibodies which allowed the detection of a different drug, VPA. The VPA sensor had a calculated LOD of 45 µg/ml, and a measured drug-antibody binding affinity of around 90 ± 21 µg/ml. Both biosensors were label-free and were compared to Fluorescence polarization immunoassay (FPIA) measure- ments of PHT/VPA in bovine and were shown to yield comparable results in the relevant clinical concentrations. Huang's research demonstrates the versatility of piezoresistive mi- crocantilevers where all that is required is a change of the bioreceptor used. (Figure 9) demonstrates the side-view of the biosensor and the various layers that were used in its construction.

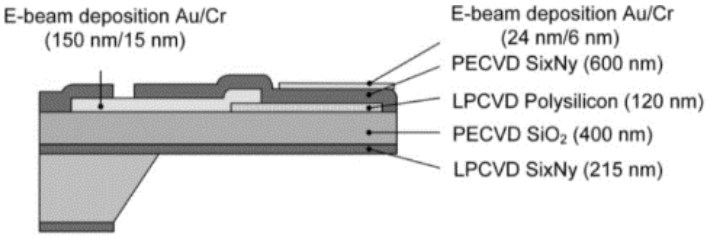


**Figure 9:** Fabrication process of a piezoresistive microcantilever sensor [90].

In an antibody based detection sensor, Yang, et al. [74] reported the detection of CBZ, PHT, and VPA in serum utilizing a Cloned Enzyme Donor Immunoassay (CEDIA). To per- form concurrent detection, they fabricated a multi-well ImmunoChip by xurography. The ImmunoChip surpasses conventional analytical devices by requiring small volume of samples, and being smaller in size due to automated manufacturing. CEDIA is an immunoassay technique were the reaction requires the addition of two separately inactive antibody-fragments. Once added together, these inactive fragments form an active antibody ready to bind to the target analyte. This procedure does not need any separation steps since fragments are initially inactive. To proceed with detection, Yang mixed AED spiked serum samples with the first CEDIA reagent. The second CEDIA reagent was then immediately added to the solution. Once mixed, the mixture was transferred to the multi-well ImmunoChip containing freshly Beta-Glo reagent solution. Once exposed to a bright luminescent signal, graphs of relative light intensity versus AED concentrations were generated. Although the LOD and LOQ was not specified, Yang provided evident graphs that relative light intensity varied over the range of AED concentration. Furthermore, the highest calculated coefficients of variation intra-assay and inter-assay, were reported to be 6.9% and 9.3% for CBZ, 5.3% and 9.6% for PHT, and 5.6% and 9.2% for VPA.

## MIP-Based Bioreceptors

As a cheap alternative to natural antibodies, MIPs have been widely researched [91-93]. MIPs are chemically synthesized molecules designed to mimic the behavior of antibodies. They typically involve a monomer, a porogen, a cross-linker, an initiator, and the template of interest. Once the MIP is polymerized across the template, the template is removed to leave a gap which resembles its structure. This gap is similar to the target template and has an affinity to bind to a molecule with similar structure. MIPs have proven to be robust, slightly affected by pH variations, reproducible, simple to fabricate, and most importantly cheap. It is however important to determine the initiating reagents proportions and conditions to synthesize functional MIPs.

Since MIPs are robust, it is relevant to explore their usage in electrochemical based systems which highly rely on the immobilization of biosensing molecules on the electrode's surface. Commercially available electrodes are often expensive and lack selectivity, hence, it is necessary to fabricate application specific electrodes. These application specific electrodes differentiate from the aforementioned by the ease of fabrication, reproducibility and strong affinity towards the analyte. To achieve these characteristics, MIP are added to the electrode's setup.

Gholivand et al. developed an electrochemical MIP-based biosensor for the selective detection of LTG [94]. MIPs were fabricated using a non-covalent molecular imprinting approach was used. After polymerizing, LTG was extracted from the polymers by soxhlet extraction; tetrahydrofuran was used and the removal of the template. The LTG-free MIP was then immobilized on the Carbon Paste (CP) working electrode and cyclic voltammetry was performed. In order to confirm the ability of MIPs to bind to LTG it was compared to an electrode immobilized with Non-Imprinted Polymers (NIP) along with a bare CP electrode. NIPs have been prepared similarly to MIP without the addition of a template. Cyclic voltammetry graphs in (Figure 10) show that MIP-CP had the highest current response, validating the selective detection of LTG.

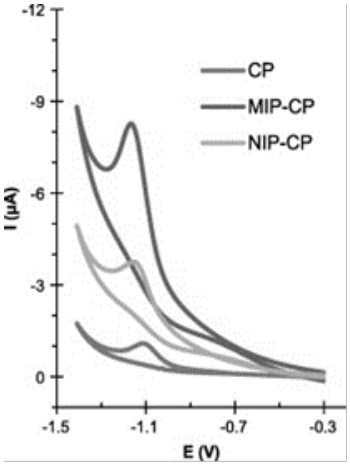


**Figure 10:** Cyclic voltammograms of different electrodes immersed in the 1.0 x 10-6 M LTG solutions after 7 min preconcentration. Determination conditions: acetate buffer pH = 5.5 and scan rate 100 mV/s [94].

The selectivity of the designed MIP-based CP electrode through DPV was further evaluated by inserting the electrodes into aqueous solutions of LTG and LTG similar compounds.

(Figure 11(B)) shows a distinct response to LTG when compared to LTG-similar compounds, unlike (Figure 11(A)) and (Figure 11(C)) were no significant LTG-related response could be concluded. Furthermore, the sensor's response was linear throughout LTG concentrations of 0.8-25 nM and 25-400 nM with a calculated limit of detection of 0.21 nM.

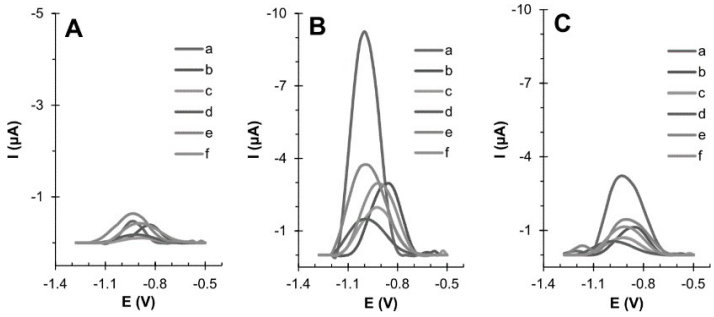


**Figure 11(A-C):** The DPV response of sensors based on a **(A)** CPE, **(B)** MIP-CPE and **(C)** NIP-CPE immersed in solutions containing LTG and LTG similar compounds [94].

Furthermore, MIPs have replaced antibodies and other expensive biosensing elements in chromatography based methods. Hoshina, et al. [95] developed a MIP-based biosensor for the successive determination of four different AEDs. Rather than utilizing an electrochemical approach, they combined MIPs as biosensing elements with a chromatography-based detection method. In order to study the effect of different monomers on functionality of the imprinted polymers, polymerization was tested with MAA, 2-VP and 4-VP as monomers. It was found that the 4-VP based polymer exhibited the best selectivity when compared to other monomers. Drug induced samples were pre-treated prior to chromatographic analysis by passing through a MIP packed column, then into the analytical column. 2 mM ammonium acetate-acetonitrile (60:40, v/v) was then administered at a flow rate of 0.2 mL/min. The proposed method was both accurate and reproducible and graphs of peak area ratio versus AED concentrations were linear with a correlation of >0.999. Furthermore, limits of detection and limit of quantification were as follows: PHB 15 and 5.0 ng/L, amobarbital 8.0 and 2.0 ng/L, and PHT 2.0 and 0.50 ng/L.

## Discussion and Conclusion

AEDs are given as a treatment to control seizures; therefore, detecting optimal dosage on purely clinical grounds can be difficult. Furthermore, continuous AED monitoring rises as a necessary technique to optimize patients' outcome and providing them with a safe course of treatment. TDM is currently performed in centralized laboratories equipped with bulky instruments, such as immunoassay analyzers and mass spectrometry, which can be used by trained personnel only. Particularly mass spectrometry, coupled with high-performance liquid chromatography (HPLC-MS), is routinely used in core facilities. The financial costs related to instrumental operation and maintenance, and the time required for the preparation and analysis of samples, for processing the results, affects the application of TDM in medical practices. Therefore, a new generation of analytical tools, capable of providing rapid, sensitive and reliable diagnosis, is necessary to respond to the timely need of drug control aiming at effectively treating epileptic patients.

With all the various assets brought forward by detection techniques, it is often hard to specify an optimal solution. Vastly developing transducing techniques along with biosensing elements are spiraling towards sensitive, accurate, label-free, cheap and easy to operate biosensors. Because AEDs have a narrow therapeutic range, are often administered as a form of poly-treatment, and are susceptible to high toxicity reactions, AED detection biosensors must be sensitive but also highly selective in order to eliminate or decrease the effect of unwanted particles. Hence, it is necessary to combine the advantages offered by both transducing and biosensing elements to produce the desired outcome. (Table 5) sums up the common biosensors employed for AEDs detection or monitoring.

The main detection techniques regarding PHT and PHB determination have focused on techniques such as: a piezoresistive cantilever combined with the immobilization of biomolec- ular receptors operating as biosensing elements; electrochemical DPV using a multi-walled carbon nanotube modified Pt electrode; and HPLC combined with UV spectroscopy. For LEV, the main detection technique is HPLC-ES-MS/MS coupled with immobilized

protein for selective detection. Furthermore, VPA main detection techniques are mostly piezoresistive microcantilever combined with antibody immobilization. Finally, most CBZ detection sensors are electrochemical based combined with chemically modified working electrodes. It is justifiable to say that no specific biosensor has yet been employed to detect multiple AEDs without the need for bulky equipment, sample pre-conditioning, or specialized clinicians. By examining the existing techniques provided in (Table 5), it is possible to choose one detection technique that may be integrated into a miniature, label-free, economical, easy to operate, and accurate biosensor. Although not the most sensitive or selective, electrochemical transducing techniques such as CV, DPV, SWV, and EIS are the simplest to operate, enable label-free detection and fast response times. Combined with MIPs surface immobilized electrodes, electrochemical biosensors could become highly selective and suitable for multi AED detection. Furthermore, using appropriate dopants or by adding graphene or carbon nanotubes, electrodes could also become highly conductive, thereby increasing the sensitivity of the sensor.

We described in this paper the most recent AED detection systems by highlighting their respective biosensing and transducing elements. When designing an AED detection biosensor, it is important to opt for the optimal combination of biosensing and transducing elements. The latter mainly affect signal quality and accuracy of the biosensor whereas biosensing elements achieve selectivity towards the analyte of interest. All aforementioned transducing elements present good signal quality and high accuracy. Different elements offer varying degrees of ease-of-operation, damage to analyte, response time, and ability to be rendered implantable. Electrochemical methods turn out to be least destructive, easiest to operate and suitable to be integrated on a miniature implantable chip. However electro- chemical biosensors often lag selectivity if not coupled by a biosensing element. Microfluidic channels, antibodies, and MIPs all compensate through enhancing selectivity, particularly MIPs prove to be convenient, cheap and highly reliable. A MIP immobilized electrochemical biosensor may be a good alternative to bulky centralized equipment enhancing AED monitoring and providing point-of-care treatment.

| Ref. | Tran. | Sens. | Drug | LOD | Sel. | L.R. | Com. |
|---|---|---|---|---|---|---|---|
| [54] | RP-HPLC | - | PHT | 1.44 µg/mL | High | PHT:10-30 mg/L pheno-barbitone 3-9 µg/mL | High |
| [55] | SPR | Antibodies | PHT | 50 nM | High | - | High |
| [63] | CV and SWV | NPs | CBZ | 0.34 µM | Low | 0.5-100 µM | moderate |
| [67] | DPV | MWCNT/ Pt-NPs | PHB | 0.1 µM | Low | 0.4-60 µM | Low |
| [72] | DPV | - | CBZ | 1 µg/mL | Low | - | Low |
| [72] | FPIA | - | CBZ | 0.5 µg/mL | High | - | High |
| [89] | PM | Antibodies | PHT | 9.5 µg/mL | High | 10-80 µg/mL | High |
| [90] | PM | Antibodies | VPA | 45 µg/mL | High | 50-500 µg/mL | High |
| [94] | DPV | MIP | LTG | 0.21 nM | High | 0.8-25 and 25-400 nM | moderate |
| [96] | CV | GO-g-C3N4 | CBZ | 10.5 nM | Low | 0.092-266 µM | Low |
| [97] | HPLC | - | LEV | 0.380 µg/mL | High | - | High |
| | | | LTG | 0.223 µg/mL | | | |
| | | | PHB | 0.318 µg/mL | | | |
| | | | CBZ | 0.258 µg/mL | | | |
| [98] | HPLC-ES-MS/ MS | Protein | LTG, LEV, Primidone | 50 µg/mL | High | - | High |
| [99] | DPV | - | CBZ | 0.14 µg/mL | Low | - | Low |
| PM: Piezoresistive Microcantilever IRTD: Iron doped tin dioxide. | | | | | | | |

**Table 5:** Various drug transducing and sensing systems along with their LOD, selectivity, linear range, and complexity.

## Acknowledgments

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