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Nanosensor Prototype for Antidepressant Drugs Based on Cytochrome P450 Enzyme

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Abstract

Cytochrome P450 (CYPs) monooxygenases are well-known drug-metabolizing enzymes involved in the metabolism of xenobiotic molecules in animals and plants. Particularly active in drug metabolism are the mammalian liver CYP species, which exhibit broad and overlapping substrate specificity for one another. About 30% of currently available medications are metabolized by the CYP2D6 isoform, including b-adrenergic blockers, antihypertensive medications, and antidepressants like paroxetine and fluoxetine. CYP2C19 is involved in the metabolism of other medications like amitriptyline and citalopram (CIT). The idea of using these enzymes to make electrochemical nanosensors to monitor drug levels in blood samples appears to be receiving a lot of attention. This biosensor represents a device that can be used in various fields of clinical diagnostics, including the monitoring of drug therapies and vital parameters. It is important to underline that the choice of antidepressant may be guided by the past response to a specific drug, the side effect profile, the presence of comorbidities and potential interactions with other drugs. Starting from this evidence, it can be stated that sensors for antidepressants can be useful in clinical and forensic contexts to monitor adherence to therapy, plasma concentration of drugs, the onset of adverse effects or toxicity and the presence of abuse or overdose of drugs. In our work drug concentration in the samples can be determined using the electrochemical signal produced by the interactions of the drugs with CYP. The two P450 enzyme isoforms that are employed to electrochemically identify the interaction between an antidepressant drug and a cytochrome are sensitive enough to guarantee the precision and specificity of the nano-biodevice.

Keywords

Cytochrome P450, Nano-biodevice, Drug detection, Electrochemical sensor

Introduction

Antidepressant detection has emerged as a key research area with the global incidence of depression rising to the point where it will rank second in the International Burden of Disease ranking [1, 2]. The main enzymes involved in drug metabolism are CYPs, which make up about 75% of the body's total metabolism [3, 4]. Many substances are bioactivated by CYPs to create their active compounds, and most drugs are deactivated by CYPs [5]. Since it depends on a variety of variables, including the patient's condition and the presence of other medications during the same therapy (many pharmacological treatments for serious diseases frequently rely on cocktails of various medications), drug metabolism

is difficult to predict. For these reasons, it is necessary to understand drug concentrations caused by the real and effective patient's metabolism at the time of the pharmacological cure in order to optimize an ongoing drug therapy individually. Recent years have seen significant research into developing workable electrochemical biosensors based on CYP for the detection of drugs and other chemical compounds stimulated by the growing demand for the development of personalized therapy. Each CYP isoform has a unique inhibitor and substrate. The key enzymes involved in the metabolism of many medications, including citalopram and pyridoxine, are represented by the CYP2C19 and CYP2D6 isoforms, which is why they are so crucial in the field of psychopharmacology chemistry. It was thought interesting to investigate the possibility of controlling these drugs since the monitoring of these drugs (in particular, the true dose-effect relationship), as previously discussed, is fundamental in clinical diagnostics.

The US Food and Drug Administration approved the bicyclic phthalate medication citalopram, also known as 1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile), for the treatment of depression in 1998 [6]. Additionally, it is recommended for other central nervous system disorders like anxiety, obsessive compulsion disorders, phobias (including social phobia and agoraphobia), borderline personality disorders, bipolar disorders, and situations where serotonin reuptake inhibition is desired [7]. The CIT is used frequently because it is more widely accepted and causes fewer discontinuations. It is already known that CYP2C19 polymorphisms are crucial for the N-demethylation of citalopram to produce its primary metabolite, desmethylcitalopram (DCIT) [8]. Individual pharmacokinetic properties vary greatly between and within species because of CYP2C19's polymorphism. The activity of CYP2C19 and its polymorphism may be reflected in the relative proportion of CIT and DCIT. Therefore, monitoring compliance or overdose [9], optimizing the therapeutic effect, and investigating the gene-dose effect [10] all benefit from the measurement of CIT and DCIT.

Even though it only makes up about 2% of the total CYP protein in the liver, cytochrome CYP2D6 is primarily a hepatic enzyme that has been shown to significantly contribute to the metabolism and elimination of about 25% of prescribed drugs [11]. One of the most crucial enzymes in the body's xenobiotic metabolism is CYP2D6, which also breaks down several endogenous compounds like hydroxytryptamine and neurosteroids [12]. In addition, CYP2D6 is in charge of the metabolism of neuroleptic and antidepressant drugs [13].

The effectiveness and quantity of CYP2D6 enzyme produced varies greatly between individuals. Genetic polymorphism suggests that clear population differences in enzyme expression or activity result from variations in DNA sequence, which can affect drug metabolism and CYP2D6 substrate clearance. People are categorized as either extensive (normal) or poor metabolizers. Poor metabolizers, who not only lack functional CYP2D6 but also have impaired metabolism of more than 70 drugs, do not produce functional enzymes and are unable to metabolize drugs via this pathway. Contrarily, because these drugs are metabolized by CYP2D6, some people (ultrarapid metabolizers) can get rid of these drugs quickly. Drug efficacy may be reduced if it is metabolized too quickly, and toxicity may develop if it is metabolized too slowly [14]. As a result, the dosage of the medication may need to be changed to account for how quickly CYP2D6 metabolizes it [15-17].

Paroxetine is a nonlinear kinetic, selective serotonin reuptake inhibitor that is both a CYP2D6 substrate and inhibitor [18-21]. Major depression, obsessive compulsive disorder, panic disorder, social anxiety, post-traumatic stress disorder, and generalized anxiety disorder are all conditions that paroxetine is used to treat in adult outpatients [22, 23]. With a half maximal inhibitory concentration (IC50) ranging from 150 nM to 2.0 M depending on the substrate, paroxetine is metabolized by CYP2D6 via demethylenation of the methylenedioxy group, producing a catechol metabolite and formic acid [24]. As a result, paroxetine has been classified as a competitive, reversible inhibitor of CYP2D6.

The literature has reported some drug concentration assays, including those combined with Ultraviolet [25], photodiode array [26], fluorescence [27], and mass spectrometry [28] detection, all of these techniques require multi-step extractions, significant amounts of organic solvent, and time-consuming sample preparation processes that can be overcome by the use of a biosensor that allows for easy and rapid testing.

It is possible to get around all these restrictions, at least in theory, by using electrochemical biosensors based on CYP for drug detection [29]. These enzymatic amperometric biosensors use the measurement of the current generated at the electrode surface because of the redox reaction of the enzyme when a substrate is present in the sample as their detection mechanism. Due to their capacity for recognition, CYPs have been widely utilized as components for biosensor construction [30-34]. CYP nano-biosensors are therefore a promising technology that can deliver quick measurements for drug and metabolite concentrations with good selectivity, accuracy, sensitivity, and affordable hardware.

The creation of a biosensor that can measure a drug mixture is still a challenge despite numerous attempts to measure drug concentration using CYP-based systems [35-37]. Immobilizing CYP on the electrode surface in a way that optimizes the electron transfer from the enzyme's active site and the orientation of the protein relative to the electrode is one of the key problems. The immobilization of cytochromes in nanofilms has previously been the subject of in-depth study [38-41]. The main objectives of this work are to demonstrate that it is possible to use two isoforms of the CYP enzyme (2C19 and 2D6) to obtain the measurement of the concentration of antidepressant drugs; furthermore the cyclic voltammetry results establish that it is possible to measure the concentrations of paroxetine and citalopram within the range of therapeutic use.

In the present work, we show the capability of a P450 enzyme based electrochemical biosensor useful for determining the concentration of antidepressant drugs.

Materials and Method

Chemicals and reagents

Potassium phosphate buffer, human recombinant cytochrome P450 (CYP2D6 and CYP2C19) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Paroxetine solution was purchased by Italfarmaco (Milano, Italy) and CIT was purchased by Innova Pharma (Milano, Italy). Electrolyte (Potassium chloride, KCl) solution 3 M/L silver chloride (AgCl) saturated was purchased by Mettler Toledo S.p.A. (Novate Milanese MI, Italy).

Electrode modification and functionalization

Electrochemical electrodes, provided by BVT Technologies (Brno, Czech Republic; http://www.bvt.cz), were AC1.W5.R1 (graphite working electrode), and AC1.WS.R1 (gold and platinum working electrode). The planar sensors were produced on a corundum ceramic base with applied metal/graphite working electrode, Ag/AgCl reference electrode and platinum counter electrodes. Silver conducting paths connected the electrodes to the external control circuit; they were covered by a dielectric protection layer at the end of the sensor. Both gold and graphite working electrodes were utilized. The dimensions of these central working electrodes were 1.00 ±0.05 mm and 2.00 ± 0.05 mm, respectively. The diameter of the external counter electrode was 6.00 ± 0.05 mm.

Development of potentiostat electronics

A classic book [41], specifically Chapter 15, provided a summary of the fundamental knowledge of the electronics needed to control a three-electrode cell in cyclic voltammetry experiments. A further signal amplifier A4 and a potential control amplifier A1 are used in the fundamental circuit for cyclic voltammetry and amperometric experiments, respectively, to prevent resolution loss during the analog-to-digital conversion to the 12-digit DAQ card input.

A current follower, whose output is proportional to the current, is fed by a buffer amplifier A_2 that was added to the reference electrode R. The time-dependent ramp signal from the DAQ card and the amplifier A_1 were separated by an additional buffer A_0. The working electrode W is kept at the virtual ground with the aid of the current follower, which is necessary for the system to function properly. A booster amplifier can bepositioned between the amplifier A_1 and the counter electrode C, as suggested in several papers in order to extend the output voltage range. This amplifier is not present in our circuit because currents are expected to be very low in all practical situations. The amplifier A_1 is the most important component of the circuit because operational amplifiers have two major drawbacks that have a significant impact on all low-frequency experiments: input offset voltage and temperature dependence. The Texas Instruments Incorporated (Dallas, Texas, USA) chopper-stabilized high-precision TLC2652A amplifier was chosen because it enables extremely high precision in DC measurements by continuously resetting input offset voltage variations in temperature, time, common-mode voltage, and power supply voltage.It achieves an average input offset voltage of 500 nV and an input offset voltage temperature coefficient of 3 nV/°C. This amplifier needs to be changed when conducting experiments with higher currents. The system was tested by substituting the electrochemical cell with dummy resistors and using the solid-state planar electrochemical cell described above with different copper, iron, and ferricyanide solutions.

Control software and data acquisition

The experiment is run using graphical code developed for the LabVIEW software version 8.0 by National Instruments, which drives a 6062E card (National Instruments Corporation, Assago (MI), Italy). The version 8.0 fits the DAQ card with NI drivers' version 9.9.0. The device uses two 12-bit D/A converters and a 12-bit A/D converter, and it can be mounted on a portable computer's Type II PC card using the device's PCMCIA bus connector on the DAQ card-6062E. For simpler connections between the 6062E card and the electronic circuit, National Instruments' SCB-68 shielded board with 68 screw terminals was used (Assago (MI), Italy). The two-stage current-to-voltage amplifier in figure 1 was used in the LabVIEW graphical program to generate the voltage ramps on an output channel and collect the resulting voltage response (related to the current in the electrochemical cell) using one input channel. The software enables to customize the voltage ramp's starting and ending voltages, switching voltage, speed, and direction.

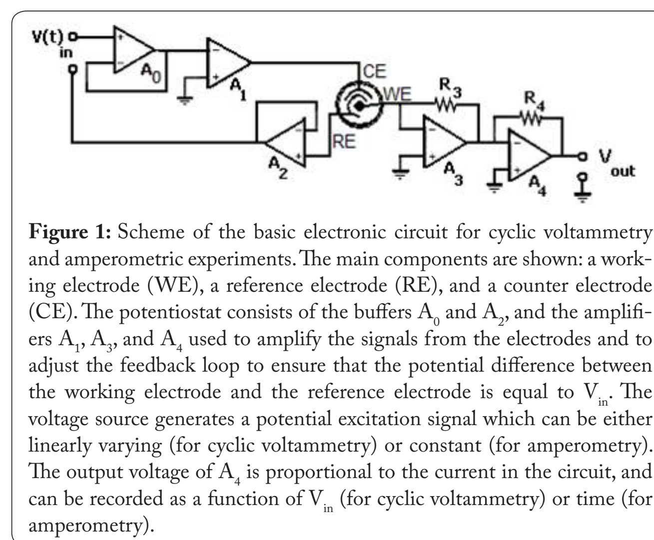

Figure 1: Scheme of the basic electronic circuit for cyclic voltammetry and amperometric experiments. The main components are shown: a working electrode (WE), a reference electrode (RE), and a counter electrode (CE). The potentiostat consists of the buffers A_0 and A_2, and the amplifiers A_1, A_3, and A_4 used to amplify the signals from the electrodes and to adjust the feedback loop to ensure that the potential difference between the working electrode and the reference electrode is equal to V_{in}. The voltage source generates a potential excitation signal which can be either linearly varying (for cyclic voltammetry) or constant (for amperometry). The output voltage of A_4 is proportional to the current in the circuit, and can be recorded as a function of V_{in} (for cyclic voltammetry) or time (for amperometry).

Results

The apparatus was tested by inserting two impedances between the collector-reference and reference-working positions both connected to the working one. Since changing the collector-reference resistance from 10 kΩ to 35 kΩ resulted in a voltage change of less than 1%, the independence of that decision was examined. High resistances of 218 kΩ (blue), 690 kΩ (red), and 4.7 MΩ (black) were used to test the apparatus's response to low currents. As a result of the cyclic voltage sweep, results reported in figure 2 demonstrate that the apparatus can detect currents of the order of 50 nA within the instrumental resolution and a voltage resolution of 3 mV.

Gold-platinum and graphite electrodes were both used for enzyme deposition. Gold-platinum electrodes produced better results, demonstrating the need for a computational

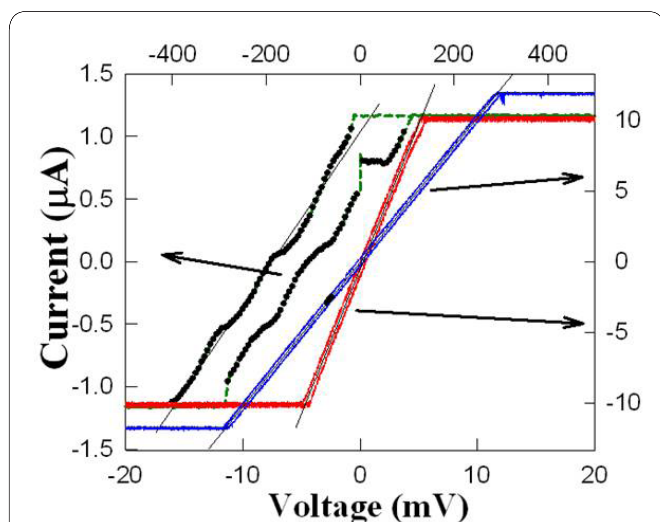

Figure 2: A test of the device's performance under low current conditions was conducted using high resistances of 218 kΩ (blue), 690 kΩ (red) (both referred to the right and upper scales, see arrows), and 4.7 MΩ (referred to the left and bottom scales, see arrow). The blue and red lines show the linear relationship between current and voltage, as indicated by the right and upper scales. This suggests that the device behaves as expected under these conditions, with a direct proportionality between current and voltage. The line corresponding to a resistance of 4.7 MΩ, referred to on the left and bottom scales, shows a different behavior that can be attributed to the higher resistance leading to a very low current through the device, at the edge to non-linear effects.

process to subtract a continuous contribution from the semiconducting graphite contact in graphite electrodes. A fully differential approach with two differential action circuits was created based on the scheme shown in figure 1.

The cyclic voltammetry analyses were conducted throughout the nanosensor hardware described above, and the enzymes CYP2C19 and CYP2D6 were immobilized by drop-casting on the electrode active surface. The overall reliability of the apparatus for the electrochemical study of CYP was tested in comparisons with the commercial EG&G PARC 263A potentiostat/galvanostat in the same experimental conditions to check the reliability to study the interaction between P450 enzyme on clozapine [33] and cholesterol [34].

The measurements with the functionalized CYP2D6 electrode are shown in figure 3. According to published data [33, 34] its characteristic peaks were obtained at +200 mV and -450 mV. In the buffer phosphate environment, the CYP enzyme exhibits cyclic voltammetry behavior that is superimposed by such peaks. The observed currents at scanning potential variation were significantly reduced after the addition of the paroxetine solution at 100 ng/ml concentration. The finding that peak intensities decrease while their voltage positions remain constant in both figure 3 and figure 4 is crucial.

Figure 4 reports the results of the cyclic voltammetry of CYP2C19 immobilized on the screen-printed electrode in potassium phosphate buffer with and without 100 ng/ml concentrations of the antidepressant citalopram. When this molecule is dissolved in a buffer, the electric current changes very slightly, as shown in figure 4, but they are still detectable within the apparatus's sensitivity limits. These findings suggest

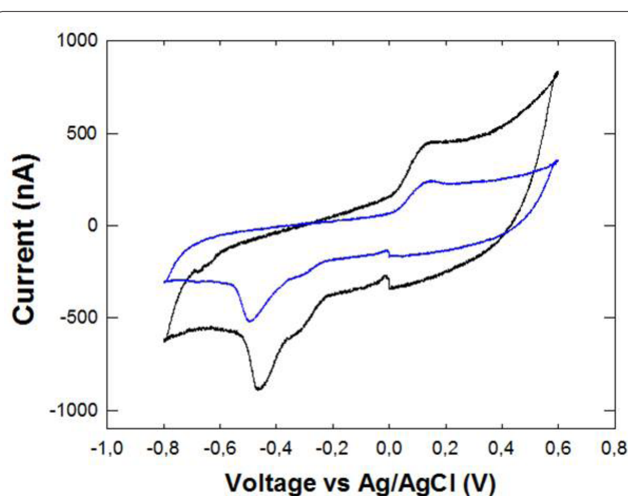

Figure 3: Cyclic voltammetry of an Ag/AgCl electrode functionalized by CYP2D6 deposition to detect paroxetine. The analysis was done in 10 mM potassium phosphate buffer with a pH of 7.4. Both curves were obtained at 50 mV/s scan rate, demonstrating the electrochemical behavior of the system under these specific conditions. The black curve starts at around (-1.0, 1000) and ends at around (0.6, -500), indicating a positive oxidation slope. The curve represents the baseline behavior of the functionalized electrode in the absence of paroxetine. The blue curve illustrates the interaction between CYP and a 100 ng/ml paroxetine solution, while the black curve represents the baseline behavior of the functionalized electrode. It starts at around (-0.8, -1000) and ends at around (0.8, 500), with a lower slope with respect to the baseline curve. The different behavior of the two curves in the figure effectively demonstrates the interaction between CYP2D6 and paroxetine.

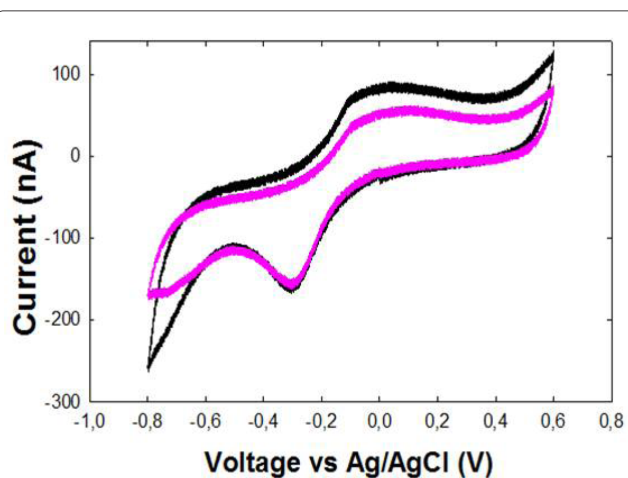

Figure 4: Cyclic voltammetry of an Ag/AgCl electrode functionalized by CYP2C19 deposition in a solid state to detect CIT. The analysis was done in 10 mM potassium phosphate buffer with a pH of 7.4. The black curve represents the baseline behavior of the functionalized electrode in the absence of the drug. This curve shows how the current changes with voltage when only the enzyme (CYP2C19) is present. It starts at (-1, -300) and ends at (0.8, 100), indicating a positive slope. This suggests that as the voltage increases, the current also increases, which is typically indicative of an oxidation process occurring at the electrode. The pink curve represents the behavior of the electrode in the presence of a 100 ng/ml CIT solution. It starts at (-1, -200) and ends at (0.8, 0) with respect to Ag/AgCl. The deviation of the pink curve from the black curve indicates that CIT is interacting with CYP2C19 and affecting its electrochemical behavior. The specific shape of the pink curve provides insights into the redox behavior of CIT and how it gets oxidized or reduced in the presence of CYP2C19.

that the concentration of paroxetine and CIT within the range of therapeutic use can be measured. As a result, the apparatus's sensitivity is adequate for routine measurements with clinical relevance. It is necessary to miniaturize the instrumentation in accordance with accepted standard practices in order to convert the current prototype into a tool usable in a clinical if not domestic, environment.

Discussion

The results of the present study demonstrate the feasibility and reliability of a CYP enzymes based nanosensor for clinical application through antidepressant concentration monitoring the apparatus was able to detect low currents and voltages with high resolution and accuracy, as well as to distinguish the characteristic peaks of different CYP enzymes immobilized on the electrode surface. The apparatus was also able to measure the interaction between CYP enzymes and antidepressant drugs, such as paroxetine and CIT, at clinically relevant concentrations. These findings suggest that the nanosensor can be used as a tool for drug metabolism studies and drug-drug interaction screening. The main advantages of the nanosensor are its low cost, simplicity, and portability. Moreover, the nanosensor can be miniaturized and integrated with other components of the biosensors, including wireless communication devices, to create a compact and versatile device for point-of-care or home-based applications. The results showed that gold-platinum electrodes produced better results than graphite electrodes, indicating that the choice of electrode material and fabrication method is crucial for the performance of the apparatus. Furthermore, the results showed that changing the collector-reference resistance had a negligible effect on the voltage output, suggesting that this parameter can be further tuned to improve the signal-to-noise ratio and the sensitivity of the apparatus. The future directions of this study can include the improvement of the design and functionality of nanosensor, testing its applicability for other CYP enzymes and drugs, and validation of results with clinical samples. The nanosensor prototype can be improved by using differential circuits, feedback loops, or digital signal processing techniques to enhance its stability and accuracy. The results in figure 3 and figure 4 show that both paroxetine and CIT can be detected by our nanosensor at clinically relevant concentrations by means of cyclic voltammetry profiles.

Conclusion

A nano-biosensor prototype for the detection of antidepressants was developed and tested in the current work. Experiments were conducted to confirm the viability of using two CYP isoforms, such as 2C19 and 2D6, for the biochemical measurements of antidepressants drugs present in blood or saliva.

The present study demonstrates that proposed biosensor, can be a useful and economical device for the determination of drug concentrations based on the electrochemical characteristics of CYP enzymes and their interactions with drugs. This device can potentially facilitate drug development and personalized medicine by providing rapid and accurate information on drug metabolism and drug-drug interactions.

In fact, the tendency for more frequent monitoring of biochemical values in antidepressant-treated subjects raises the case for the requirement of multiple measurements of various clinical parameters at once. There are currently no novel tools on the market that can adequately meet these requirements because modern technology uses research instrumentation that is inappropriate for large-scale screening [42]. The technology created in the present work offers fast and effective clinical analytical system that would enable multiple detections by utilizing the CYP propriety. The nano-biosensor can be used to monitor the drug levels and the drug metabolism of patients undergoing antidepressants therapy, such as paroxetine and CIT. This can help to limit the drug dose, avoid adverse effects, and improve the effectiveness of the treatment. Moreover, nanosensor can be used in clinical settings to check for potential drug-drug interactions between various medications. By enhancing the therapeutic result and optimizing the drug combination, adverse or unwanted interactions can be avoided. In order to personalize drug therapy, choose the most effective drug, and lower the risk of toxicity or resistance, it is possible to detect the drug metabolism and response of specific patients. This approach can be used to assess the new drugs' safety, effectiveness, and bioavailability and to compare them to currently available medications.

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None.

Conflict of Interest

The authors declare no conflicts of interest.

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