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DESENVOLVIMENTO DE UM SENSOR ELECTROQUÍMICO BASEADO EM PAPEL DE CARBONO PARA DETERMINAÇÃO DE KETOPROFENO

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DEVELOPMENT OF A CARBON PAPER BASED ELECTROCHEMICAL SENSOR TO DETERMINE KETOPROFEN

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obtain the master´s degree in Bioresources

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Abstract

Among contaminants of emerging concern (CECs), pharmaceuticals are of paramount concern. Because these products are widely used, CECs keep being released into the environment where they can accumulate over time. Innumerous studies performed throughout the world have confirmed the detection of CECs in different aquatic matrices such as rivers, seas, lakes and marine animals. However, these analyses are usually performed with chromatographic methods using bulky and expensive equipment, only operated by specialized technicians. Therefore, the development of electrochemical sensors is a potential alternative to these conventional methods, and it can be seen as an opportunity for the development of sustainable analytical practices.

Non-steroidal anti-inflammatory drugs (NSAIDs) are some of the most consumed pharmaceuticals worldwide. A literature review showed that several studies confirmed the presence of NSAIDs in the environment. Until now, electrochemical sensors for NSAIDs such as diclofenac, acetylsalicylic acid and acetaminophen have been the most widely reported. However, they have been developed in a lesser extent for the propionic acid derivatives NSAIDs, such as ketoprofen, which is one of the most prescribed drugs in Portugal.

The aim of the present work is the development of a simple and efficient sensor to be applied in the monitoring of ketoprofen. The developed sensor is a paper-based carbon substrate, which is connected in one end to a copper mesh and used directly as working electrode. The electrochemical performance of the sensor was characterized by cyclic voltammetry in a standard solution of the indicator $Fe(CN)_6^{3/4-}$ while quantification of ketoprofen was performed at the optimum conditions (pH 5.0, pulse amplitude of 70 mV, pulse time of 5 ms, step potential of 17.5 mV and interval time of 0.117 s) by differential pulse voltammetry. The developed sensor presented a linear range of 0.125 to 8.60 µM, a sensitivity of 24.02 µA/(µM.cm^2), and a limit of detection of 0.233 µM. In the reproducibility and repeatability assays, it was possible to obtain relative standard deviation values lower than 8.2% and 6.1%, respectively. The proposed electrochemical sensor was successfully tested for ketoprofen monitoring in WWTP water samples with recoveries ranging from 86% to 99%. The carbon paper-based electrode developed during this work and used in the presented electrochemical sensor can also be integrated into a miniaturized and portable device, that allows *in-situ* analysis of environmental samples.

Keywords: Contaminants of Emerging Concern; Ketoprofen; Electrochemical Sensor; Toray Carbon Paper

Resumo

Os compostos farmacêuticos são um grupo de contaminantes emergentes que causam uma enorme preocupação. Devido à elevada utilização destes compostos, estes são libertados para o meio ambiente, onde se podem acumular ao longo do tempo. Diversos estudos realizados em todo o mundo têm vindo a detetar contaminantes emergentes em diversas matrizes aquáticas, tais como rios, mares, lagos e animais marinhos. No entanto, geralmente, estas análises são realizadas através de métodos cromatográficos utilizando equipamentos volumosos e caros, que só podem ser operados por técnicos especializados. Como tal, o desenvolvimento de sensores eletroquímicos é uma potencial alternativa ao uso destes métodos convencionais e surge como uma oportunidade para o desenvolvimento de práticas analíticas mais sustentáveis.

Os medicamentos anti-inflamatórios não esteroides (NSAIDs) são dos mais consumidos mundialmente. Uma revisão da literatura permitiu encontrar vários estudos que confirmam a presença destes contaminantes no ambiente. Atualmente, existem sensores descritos na literatura para alguns NSAIDs, nomeadamente o diclofenac, o acido acetilsalicílico e o acetaminofeno. No entanto, não têm sido desenvolvidos sensores para NSAIDs derivados do ácido propiónico, como é o caso do ketoprofeno, que é um dos medicamentos mais prescritos em Portugal.

O objetivo deste trabalho é desenvolver um sensor simples, eficaz e que possa ser aplicado na monitorização de ketoprofeno. O sensor desenvolvido é um substrato à base de papel de carbono, no qual um dos lados do substrato de papel de carbono foi ligado a uma liga de cobre e foi diretamente usado como elétrodo de trabalho. A resposta eletroquímica do sensor foi caracterizada através de voltametria cíclica com uma solução padrão de $Fe(CN)_6^{3/4-}$, enquanto a quantificação do ketoprofeno foi realizada às condições ótimas (pH 5, amplitude de pulso de 70 mV, amplitude de tempo de 5 ms, incremento de potencial de etapa de 17.5 mV e intervalo tempo de 0.117 s) utilizando-se voltametria de pulso diferencial. O sensor desenvolvido apresentou uma gama linear de 0.125 a 8.60 µM de ketoprofeno, uma sensibilidade de 24.02 $\mu A/(\mu M.cm^2)$ e um limite de deteção de 0.233 µM. Nos ensaios de reprodutibilidade e repetibilidade obtiveram-se valores de desvio padrão relativos menores do que 8.2% e 6.1%, respetivamente. O sensor eletroquímico proposto é capaz de monitorizar ketoprofeno em matrizes aquosas com recuperações entre 86% a 99%. O elétrodo de papel de carbono desenvolvido durante este trabalho e utilizado neste sensor eletroquímico pode também ser integrado num dispositivo miniaturizado e portátil de forma a permitir análises *in-situ* amostras ambientais.

Palavras-chave: Contaminantes emergentes; Ketoprofeno; Sensor Eletroquímico; Papel de Carbono *Toray*

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List of Abbreviations

ACV	Affinity Cyclic Voltammetry
ADSPV	Affinity Differential Stripping Pulse Voltammetry
AgNPs	Silver Nanoparticles
AuNPs	Gold Nanoparticles
BDDE	Boron Doped Diamond Electrode
BRB	Britton-Robinson Buffer
BSA	Bovine Serum Albumin
CB	Carbon Black
CE	Counter Electrode
CECs	Contaminants of Emerging Concern
CNTs	Carbon Nanotubes
CP	Carbon Paper
CPE	Carbon Paste Electrode
CV	Cyclic Voltammetry
DBPs	Disinfection By-Products
DPAdSV	Differential Pulse Adsorptive Stripping Voltammetry
DPV	Differential Pulse Voltammetry
DSP	Dithiobis Succinimidyl Propionate
EIS	Electrochemical Impedance Spectroscopy
GC	Gas Chromatography
GCE	Glassy Carbon Electrode
HMDE	Hanging Mercury Drop Electrode
HPLC	High-Performance Liquid Chromatography
IA	Ion Associates
ISE	Ion Selective Electrode
LC	Liquid Chromatography
LOD	Limit of Detection

LOQ	Limit of Quantification
LSV	Linear Sweep Voltammetry
MWCNT	Multi-Walled Carbon Nantubes
NPDES	National Pollution Discharge Elimination System
NPV	Normal Pulse Voltammetry
NSAIDs	Nonsteroidal Anti-inflammatory Drugs
PAuE	Polycrystalline Gold Electrode
PCPs	Personal Care Products
PGE	Pencil Graphite Electrode
PME	Potentiometric Membrane Electrode
PyrGE	Pyrolytic Graphite Electrode
RE	Reference Electrode
rGO	Reduced Graphene Oxide
RSD	Relative Standard Deviation
SMDE	Static Mercury Drop Electrode
SPAuE	Screen-Printed Gold Electrode
SPCE	Screen-Printed Carbon Electrode
SPEs	Screen-Printed Electrodes
SWAdSV	Square Wave Adsorptive Stripping Voltammetry
SWCNTs	Single-Walled Carbon Nanotubes
SWV	Square-Wave Voltammetry
TCP	Toray Carbon Paper
TCPE	Toray Carbon Paper Electrode
USA	United States of America
WE	Working Electrode
WWTP	Wastewater Treatment Plant

1. Introduction

1.1. Contaminants of Emerging Concern

Contaminants of Emerging Concern (CECs) are chemical compounds that are commonly present in water and only recently started being recognized as significant water pollutants since there are no maximum levels defined for these substances in European Union legislation, or if maximum levels have been set for some substances, they require revision [1]. These contaminants can be natural or synthetically occurring substances that are not routinely monitored in the environment.[2]. CECs are known for having undesirable effects on humans and in the ecosystems and are typically in concentrations that range ng/L (or lower) to µg/L. Natural attenuation and most conventional water treatment processes are not capable of removing these pollutants from wastewater [2]. Furthermore, with the advancements in the chemical industry, there are more and more potentially harmful compounds being released to the environment. There are multiple groups of CECs, such as pharmaceuticals, personal care products (PCPs), illicit drugs, steroids, hormones, biocides, pesticides, and more [2][3]. However, there are still doubts regarding the list of compounds that should be monitored [2].

1.1.1. Pharmaceutical

The use of pharmaceuticals in a large-scale increased, as did their presence in surface water, groundwater, wastewater and stormwater runoffs in urban areas [2]. It is known that these contaminants occur widely in the environment of industrialized countries as a result of the significant volume of pharmaceuticals that are used for the treatment of diseases, injuries or illnesses [4].

Painkillers, antibiotics, antidiabetics, beta-blockers, contraceptives, lipid regulators, antidepressants and impotence drugs are amongst the 3000 substances that are estimated to be used as pharmaceutical ingredients. It becomes necessary to stress that only a small part of these has been investigated in environmental studies [2].

Pharmaceuticals are a significant group of CECs since they can have both unpredictable and unknown side effects on different organisms, especially after a long-term exposure to low concentration [5].

Researchers have proposed that certain emerging contaminants are a serious threat to human health because they may enhance the antibiotic resistance of disease-causing microorganisms. There are two kinds of antimicrobial agents: disinfectants and antibiotics. The first is used outside of a living body and is found in many soaps and cleaning supplies, the latter are compounds capable of destroying or inhibiting the growth of bacteria [5].

Antibiotics can be structurally categorized into sulfonamides, fluoroquinolones, nitroimidazoles, penicillin, cephalosporins, tetracyclines and macrolides. All antibiotics have the tendency to persist or to just partially degrade during treatment, this way contributing to their load in receiving waters which can be then used for recreational and drinking purposes [3].

Nonsteroidal anti-inflammatory drugs (NSAIDs) is another category of pharmaceuticals that has a significant impact when found in large concentrations in the environment, even though they present a relatively low toxicity to humans [6]. NSAIDs are a large group of compounds that include salicylates, propionic acid derivatives, indole derivatives, phenylacetic acid derivatives, pyrazoles, fenamates and others [7]. These pharmaceuticals act by inhibiting cyclooxygenases enzymes, which will cease prostaglandin synthesis and lead to inflammation relieve [8]. Figure 1.1 shows the chemical structure of some widely used and monitored NSAIDs. Most NSAIDs are well absorbed in the gastrointestinal tract and are metabolized in the liver and excreted in the urine [8]. In fact, the excretion of humans and animals is the most important source of NSAIDs residues. It was reported that around 15-20% of ibuprofen and diclofenac consumed is excreted by urine unchanged [6].

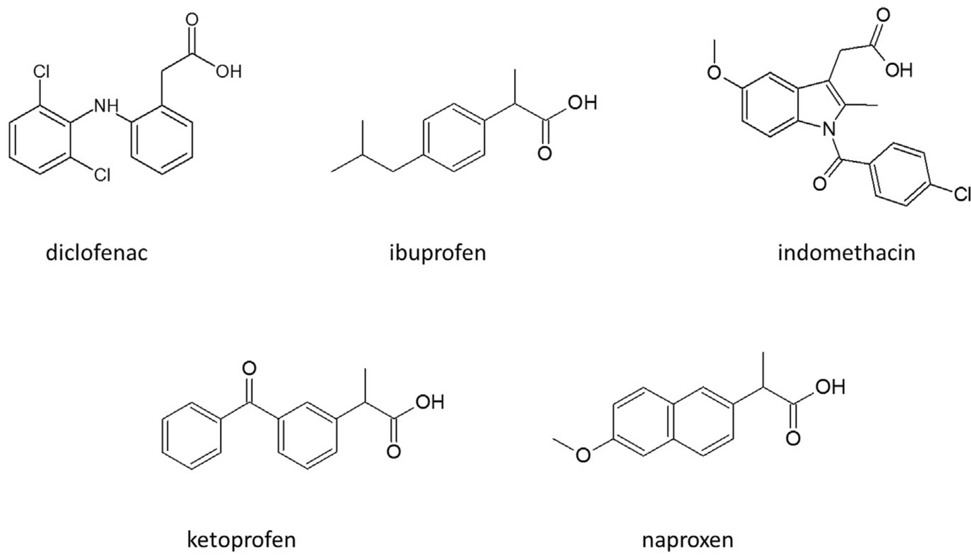

Figure 1.1 – Structure of some of the most used NSAIDs. Source: [6].

Ketoprofen is part of the propionic acid derivatives group, having analgesic and antipyretic effects and works by reversibly inhibiting cyclooxygenase 1 and 2 enzymes. This allows for a decreasing production of pro-inflammatory prostaglandin precursors [9]. This drug is used in the treatment of muscular-skeletal disorders, such as rheumatoid arthritis, osteoarthritis and symptoms caused by traumatic lesions [10].

A study conducted in Italy in 2008 stated that ketoprofen was one of the drugs with the most spontaneous reports due to adverse events of which around 30% were considered serious. Still in Italy, in 2012, ketoprofen ranked 6th for adverse drug reaction incidence, being involved in 560 adverse drug reactions, of which 31% were serious. This increase in reports of this drug reflects the incremented use of this NSAID, even after multiple warnings of toxicity [9].

Ketoprofen use may cause distinct adverse reaction, such as [9]:

- cardiovascular reactions;
- headache, drowsiness, sweating, hives;
- dermatological reactions;
- blood edemas;

- liver malfunction;
- gastrointestinal reactions (vomiting, diarrhea, ulcers, stomach bleeding);
- respiratory problems.

1.1.2. Personal Care Products

These chemicals include fragrances, sunscreens, insect repellents and antifungal agents and can be found very commonly in urban environments. Like pharmaceutical compounds, the presence of personal care products (PCPs) in urban and groundwater runoffs has also increased recently and they also tend to persist during water treatment [2]. PCPs are quickly released into aquatic environments because they are designed for external usage, and since there is no metabolic reaction, they maintain their chemical properties [3] [2]. Esters of p-hydroxybenzoic acid or parabens are a class of chemicals widely used as preservatives in cosmetics and pharmaceuticals. These include bisphenol A and other esters such as methyparaben, ethylparaben and propylparaben which have been used for decades [4].

1.1.3. Illicit drugs

There is a worldwide concern regarding the use of illicit drugs due to their impacts on human health and wellbeing of the society. Illegal drugs are those whose non-medical use is prohibited by law and belong to the classes of opiates, cocaine, cannabinoids, amphetamines, and their metabolites [3].

The chemicals associated with illegal laboratories can be building blocks for the syntheses of illicit drugs, by-products resulting from the syntheses of the drug, or even the illicit drug. These chemicals are often not only illegally buried in the soil or public waste managing facilities, but there are also cases where they are disposed into sinks or toilets, after which they enter the sewerage system [3].

Research on illicit drugs has shown that the removal of these compounds by wastewater treatment was generally greater than 50% [3].

1.1.4. Steroids and hormones

The primary source of artificial steroids entering the environment are wastewater treatment plants. When natural and synthetic steroids are released from the human body, they do so in an inactive form. However, they can undergo chemical or enzymatic dissociation in bacterial sludge and reform as active steroids being this way present in the sewage as free and active steroids. This activity in wastewater treatment plants effluents results in adverse effects on the ecosystem [3].

Likewise, hormones are known to have adverse effects, specifically on human and wildlife endocrine systems. The primary origin of steroidal hormones in the aquatic environment is human and animal excretion. Eventually, engineered and natural hormones along with their metabolites, end up reaching wastewater treatment plants. Like antibiotics, hormones are mainly introduced to the aquatic environment through wastewater [4].

Studies on the occurrence of hormones in the aquatic environment have confirmed the presence of these compounds in surface waters. Hormones such as 17β-estradiol and 17α-ethinylestradiol have been found in river waters of some countries, namely South Africa, USA and Brazil [4].

Studies conducted to determine endocrine compounds in municipal wastewaters, surface and drinking waters found these compounds to be present in all three types of water in Germany. In contrast, in Japan they were present in municipal wastewaters and surface waters, and in the United Kingdom they were only found in municipal wastewaters [11].

1.1.5. Biocides and pesticides

Pesticides are chemicals used for agricultural purposes and biocides are chemicals or microorganisms used in urban environments to exert a controlling effect on any harmful organism. Pesticides can be classified according to their use: fungicides, herbicides, bactericides and insecticides and represent the main source of pesticide contamination through surface runoffs from agricultural areas and wastewater in urban areas [4].

The most used herbicide in the United States of America (USA), Atrazine, was detected in surface waters in 2015. In 2018, it was reported that the pesticide metolachlor is the most frequently detected pesticide in surface water from wetlands of the USA [4].

Biocides are commonly used in roof sealing membranes or grass maintenance and weed control [2].

During rain events, biocides and pesticides are assimilated in surface waters and groundwaters via runoffs. Therefore, the biggest concern regarding these CECs is the development of bacterial resistance after their release into the environment and the effects on the biodegradation of plant materials, which may cause the disruption of the primary food chain in aquatic ecosystems [2].

1.2. CECs in the environment

Since CECs are commonly found in many of everyday used products, they keep being released into the environment where they can accumulate over time. Many CECs have chemical properties that allow them to remain unchanged and resist natural environmental degradation processes that would break them down. Even the compounds that undergo transformation and degradation sometimes form other chemicals that can be more problematic than the original chemical [5].

One of the many pathways of CEC exposure relates to discharges of municipal wastewater effluents. Conventional Wastewater Treatment Plants (WWTPs) remove organics and pathogens and can also convert the mass of the sewage into common gases and water. However, these facilities are not designated to remove all CECs. Although essential conventional wastewater treatment is not effective in removing some CECs, well-operated wastewater treatment plants can reduce the concentration of many CECs substantially. Even after advanced wastewater treatment, some CECs may persist and be discharged into the environment when the effluent is released to surface water or recharged into groundwater [5].

The CECs removed from the effluent can still remain in the treated sewage sludge produced as a result of the wastewater treatment process. Treated sewage sludge (biosolids) is usually applied on agricultural land as a soil conditioner or fertilizer, which can be a pathway for surface and groundwater contamination, even though CEC breakdown can be facilitated by biosolid composting before application [5].

Other sources of CECs include industrial wastewater effluents, untreated wastewater from manufacturing facilities, landfill leachate, effluents from poultry farms, agricultural runoffs containing pesticides, and animal feeding facilities where antibiotics are used by veterinarians. Figure 1.2 shows the multiple pathways through which CECs can enter the environment [5].

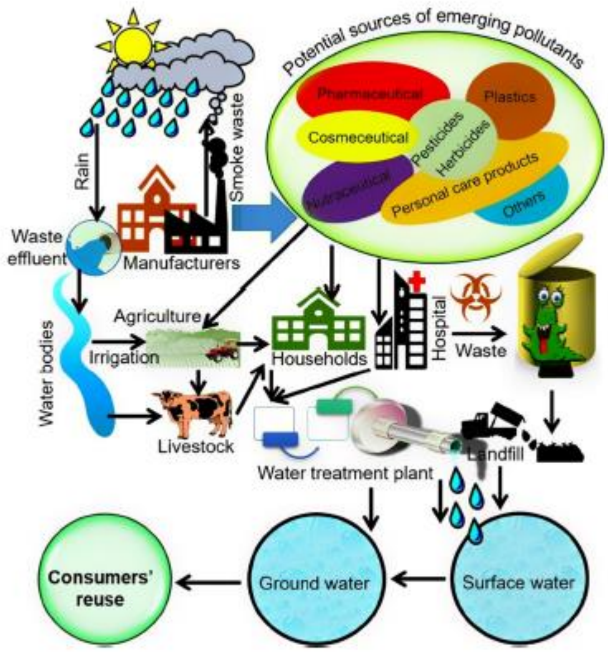

Figure 1.2 – Potential pathways through which CECs can enter ground and surface water. Source: [11].

Each own geographical region has its specific topography, climate, land-use, and ultimately, also has a particular type of contaminants. The production of CECs and their impact on the environment predominantly depends on their use and disposal. These contaminants can be produced through an on-filled route such as wastewater treatment plants, soil, waste from medical centers, poorly treated raw materials, animal and livestock industries [12].

Wastewater treatment plants are the primary source of pharmaceutical CECs as they are designed to remove the readily biodegradable compounds present in wastewater. Many pharmaceutical contaminants enter the environment through wastewater treatment plants [12].

Antibiotics are a particular class of pharmaceutical compounds since they enter the environment through human and animal urine and feces. Ten commonly used antibiotics (amoxicillin, clindamycin, sulfamethazine, minocycline, chlortetracycline, chloramphenicol, ciprofloxacin, tylosin, trimethoprim and vancomycin) are commonly found in influent and effluent wastewater. The concentration in which they are detected in samples of wastewater depends on the usage pattern, consumption, weather conditions and environmental persistence [12].

Antimicrobial and antifungal agents are commonly used in households in the form of soaps, shampoos, shower gels, aerosol sprays, dermal creams, and toothpaste. After usage, these compounds are discarded to the domestic sewage system and subsequently enter the aquatic environment [12].

Organic UV filters (4-methylbenzylidence camphor, oxybenzone, and octocrylene) are compounds used in cosmetics, skin lotions, hair sprays, lipsticks, beauty creams, shampoos and sunscreen products. The concentration of organic UV filters depends on the seasonality. Different studies showed that in the hot season, between July and September, the maximum concentration of these compounds is recorded [12].

Pharmaceutical-based CECs like anti-inflammatory drugs, carbamazepine, β-blocker, antiepileptic and antidepressants are also present in the environment [12]. About 90% of pharmaceutically active compounds enter the domestic wastewater through excretion [13].

The mobility of the different CECs depends on their original phase in which they exist in the environment. Many organic pollutants present in the liquid state and organic contaminants immiscible in aqueous media persist for a long time in the environment as compared to the miscible ones. Their fate and transportation are also affected by other physicochemical properties such as evaporation, solubility, boiling point, melting temperature, chemical structure, the reactivity of inorganic contaminants to for complexes, volatility and adsorption potential [12].

1.2.1. Removal

The performance of a wastewater treatment plant in removing specific chemicals can be influenced by many factors, such as chemical properties, level of treatment applied, retention time, pH, temperature, and others [5]. Recent studies have shown that conventional treatment plant operations can be optimized in order to reduce the concentration of CECs present in the effluent. However, the persistence of CECs has been observed often in wastewater treatment effluents, sewage sludge and land-applied biosolids in Europe and in the USA [5]. There are also studies describing the relationship between wastewater treatment performance levels and CEC bioaccumulation levels in aquatic organisms since aquatic organisms living downstream of wastewater treatment plants are exposed to effluents containing CECs [5].

The use of advanced treatments in wastewater plants, as defined by EPA´s National Pollution Discharge Elimination System (NPDES), can improve the removal rate of CECs from wastes. This was demonstrated in bioaccumulation studies for pharmaceuticals and PCPs performed in different river zones located downstream from wastewater treatment facilities. [5].

Techniques like advanced oxidation processes, granular activated carbon, nanofiltration and reverse osmosis have been shown to provide high levels of removal for most tested chemicals. However, some of them persist, being therefore detected at trace levels [5].

Some disinfection technologies such as oxidation processes, which include ozonation, ultra-violet treatment and combined treatments between hydrogen peroxide and ozone or ultraviolet radiation can convert chemicals into by-products with more toxicity, which are designated as Disinfection By-Products (DBPs) [5].

Using a combination of advanced technologies is possible to remove all contaminants of concern to below the current detection limits, but it would result in a high energy consuming process. Therefore, without knowing the long-term health impacts of these CECs, it is uncertain if the expense would provide any significant improvement in human and environmental health [5].

Besides wastewater treatment plants, CECs may also be removed or attenuated in the environment by natural phenomena such as exposure to sunlight, biodegradation and adsorption. However, the efficiency regarding these processes is highly influenced by the properties of the contaminant and the environment. Natural attenuation is very hard to assess since it is a site-specific process [5].

1.2.2. Biodegradation

Biodegradation is often considered as one of the most effective and eco-friendlier ways to remove CECs. Caffeine, acetaminophen, estradiol and ibuprofen are easily biodegradable because of their high biodegradation constant. On the other hand, contaminants like tetracycline, carbamazepine and iopamidol have a low biodegradation constant and are harder to degrade [12].

The biodegradation of contaminants is influenced by many biotic and abiotic factors, among them are redox potential, molecular features, microbial diversity, temperature, physicochemical properties, pH, temperature, toxicity of CECs, etc. [12].

Biotransformation of CECs performed by microorganisms may encounter come obstacles since some antibiotics and antimicrobial agents are extremely resistant towards the growth of the microorganism.

Thus, it becomes mandatory for the existence of ammonium and carbonate salts to maintain the optimal conditions for enzymes and induce their biotransformation of CECs. There are two main groups of bacteria involved in biotransformation of CECs: heterotrophic bacteria and autotrophic oxidizers. The latter can be ammonium oxidizing bacteria and ammonium oxidizing archaea. Ammonium oxidizing bacteria play an important role in the degradation of many CECs, such as trimethoprim, acetaminophen, ibuprofen and bisphenol, with the rate of biodegradation depending on the concentration of ammonium substrates [12].

1.2.3. Effects

Environmental and health concerns are mostly due to the lack of laws about a concentration limit of CECs in wastewater, drinking water, air, soil and sludge. Since these contaminants easily enter the food chains through the sewage of sludge used in agricultural applications, they have direct contact with humans and animals. Although they occur at trace concentrations, they may still cause serious health problems with long term effects. Thus partial/incomplete biodegradability of various CECs along with their derivatives can cause a severe impact in ecological life [12].

To investigate the toxic effects of CECs in the environment, it becomes vital to monitor the eco-toxicity levels of CECs in the aquatic environment and the air. Using the small planktonic crustacean *Daphnia magna* to assay the environmental eco-toxicity, CECs having a concentration between 10 and 100 mg/L are categorized as harmful, from 1 to 10 mg/L as toxic and <1 mg/L as very toxic to aquatic life [12].

The primary health concerns in humans and animals regarding PCPs are the disruption of developmental functions, hypothalamic-pituitary thyroid axis, and reproductive functions [5] [12].

1.3. Electrochemical sensors and electrochemical techniques

Sensors are devices that detect and register a physical, chemical, or biological change and convert it into a measurable signal [14]. The fact that they allow real-time and *in situ* analysis without needing a technician or a specialist for their operations makes sensors advantageous over other traditional analytical techniques encouraging, therefore, the use of these devices. Sensors are very promising due to characteristics such as [15]:

- Sensitivity – allows detection of low concentrations of target analyte;
- Selectivity – ability to differentiate between the target analyte and other compounds present in the sample;
- Analysis time – they usually have quick responses to the target analyte and in real-time;
- Reproducibility – analysis is reliable and present the same result when reproduced by different people;
- Precision and accuracy – in a series of analysis, the results are very similar to each other and do not differ much from the actual value;
- Miniaturization potential – sensors are portable and easy to install at the location where the analysis is performed, allowing real-time and *in situ* analysis.

Most sensors use electrochemical detection for the transducer due to the low cost, ease of use, portability and simplicity of construction. These are designated as electrochemical sensors and are widely used due to their reliability, wide detection ranges, rapid response and, usually, because no sample treatment is required [14, 16].

Sensors are composed of a recognition element which enables the selective response to a specific analyte, minimizing interferences from other sample components. If the recognition element is a biological system, the device is called a biosensor. However, there is not an ideal detection technique that works for all recognition systems. The choice of the transductor must be made considering features such as [16]:

- Compatibility with the immobilized biological material;
- Specificity for the target analyte, being that the transducer must be capable of detecting small variations that occur during the biological reaction;
- Response frequency;
- Compatibility to the environment where it is operated;
- Possibility of signal amplification;
- Mechanical and electrical toughness.

According to the transduction system, sensors can be classified as electrochemical, optic and piezoelectric. Electrochemical sensors can be characterized according to the principle of the transduction signal. The transducer is the component that detects the signal originated from a reaction or a stimulus and converts that signal into an electric measurable signal.

The transducer is the component that detects the signal originated from a reaction or a stimulus and converts that signal into an electric measurable signal. In this specific case, electrochemical sensors make use of an electrode as the transducer. So, chemical energy (analyte) is converted through redox reactions at the surface of the electrode, and then the generated electrical signal is relayed to the amplifier and the computer system for signal display.

Electrochemical sensors are characteristic for their simplicity, sensitivity, reliability, wide detection ranges, rapid response and because, usually, no sample treatment is required [16].

When the target analyte contacts an electrochemical cell, these biosensors are capable of providing information such as mass transfer velocity and chemical reaction equilibrium velocities and constant, which allow the characterization and description of the system based on the electrical properties of the analyte solution [16].

Electrochemical sensors are the most common and the most studied mainly because electrochemical cells are specific for an oxidation state and their instrumentation is relatively inexpensive.

Electrochemical reactions can be followed by generating a detectable current (voltammetric/amperometric), potential, charge gathering (potentiometric) or even by inducing a significant variation in the conductivity of the reaction medium (conductometric) [17].

1.3.1. Amperometric/Voltammetric sensors

Amperometric sensors measure continuously the current resulting from the reduction or oxidation reactions of electroactive species in the electrodes. The analytical technique used by these systems is called amperometry, which consists of tracing variations in the current through time while potential is kept constant between working and reference electrodes in the electrochemical cell [17].

In this electrochemical detection technique, the current is proportional to the concentration of the analyte in the sample [14]. Amperometric sensors are extremely selective, due to the potential resulting from the reduction and oxidation reactions being a distinctive property of the target analyte [17].

Amperometry presents a high simplicity of operation and a low limit of detection (LOD), which makes it very appropriate to be applied in catalytic (enzymatic) or in affinity sensors (immunosensors and genosensors), but also sensors. Furthermore, the potential during amperometric detection is fixed, which results in a negligible charging current that subsequently minimizes the background signal that affects the LOD [14].

However, if the current is measured during controlled variations of the potential, this is referred as voltammetry. In voltammetry, the information regarding the analyte is obtained through varying a potential and then measuring the resulting current, making it an amperometric technique [18].

Because there are many ways to vary the potential, there are also several forms of voltammetry such as cyclic, linear sweep, differential staircase, normal pulse, reverse pulse, differential pulse, etc. Among these techniques, cyclic voltammetry is one of the most used and it is very useful to learn information about the redox potential and electrochemical reaction rates of analyte solutions [18].

In cyclic voltammetry (CV) the measurements are plotted as current *vs.* voltage. The voltage is measured between the working electrode and the reference electrode, while the current is measured between the working electrode and the counter electrode. The resulting plot is a voltammogram (Figure 1.3) [18].

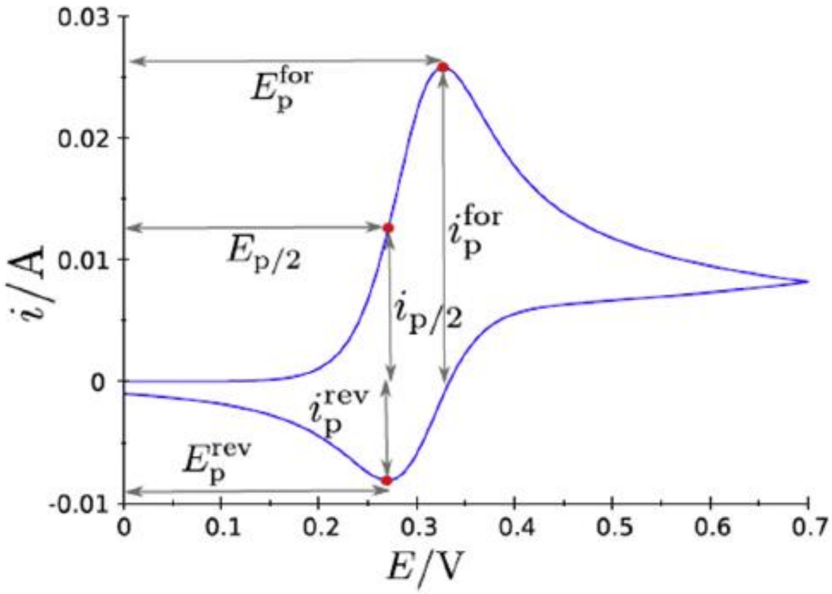

Figure 1.3 – Example of a cyclic voltammogram. Source: [32].

Voltage is swept, at a fixed rate, from a given value (V_1) to a predetermined value (V_2). When it reaches V_2, the scan is reversed, and the voltage is swept back to V_1. The scan rate, which is given by $(V_2 - V_1)/(t_2 - t_1)$, is a critical and decisive parameter, so that varying it will lead to different results [17]. The shape of the voltammogram depends not only on the scan rate, but also on the catalyst concentration and on the electrode surface, which is different after each adsorption step [18].

In addition to CV, voltammetric techniques such as Differential Pulse Voltammetry (DPV), Normal Pulse Voltammetry (NPV) and Square Wave Voltammetry (SWV) have been reported as some of the most useful electroanalytical techniques. These procedures alter the potential of a sample through pulsing from one potential to another, instead of scanning through varying potentials. [19].

The principle of pulse techniques resides in the difference in the decay rates of the faradaic and charging currents. Since the charging current decays much faster than the faradaic current, at the conclusion of each pulse, the capacitive current is negligible compared to the faradaic current. This allows for a lower detection limit and a higher sensitivity, making it very adequate for analytical purposes. SWV can be applied for the analysis of irreversible reactions, catalytic reactions and reactions with slow electron transfer [19].

One of the most popular pulse voltammetric techniques is DPV. In DPV, the potential perturbation, which consists of small pulses, is superimposed upon a staircase waveform, which is represented in the Figure 1.4 [20].

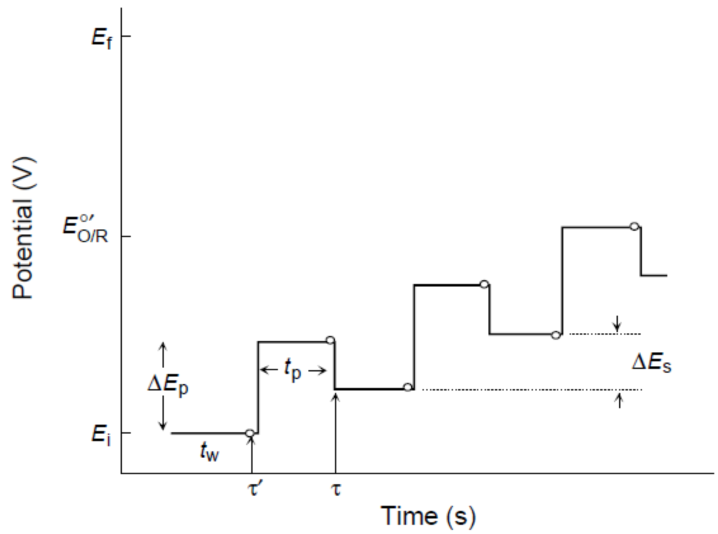

Figure 1.4 – DPV experimental parameters and waveform. Source: [35].

Magnitude or pulse amplitude, ΔE_P, and each pulse time, t_P, are preceded by a uniform waiting time, t_W. At the end of each pulse time two measurements are made. The first measurement (i_1) is made at t¢ and the second one (i_2) is made at the end of the pulse, at t.

When the pulse is completed, the potential does not return to Ei, instead it rises progressively by a step represented by ΔE_S. The output of the experiment reflects the name of the method, which is the difference between i_2 and i_1. The experiment results in a peaked output shown in Figure 1.5 [20].

DPV studies can provide improved selectivity in the observation of different redox processes compared to those of Linear Sweep Voltammetry (LSV) and CV [21]. The enhanced sensitivity and detection limited of DPV is due to a reduction in the charging current [20].

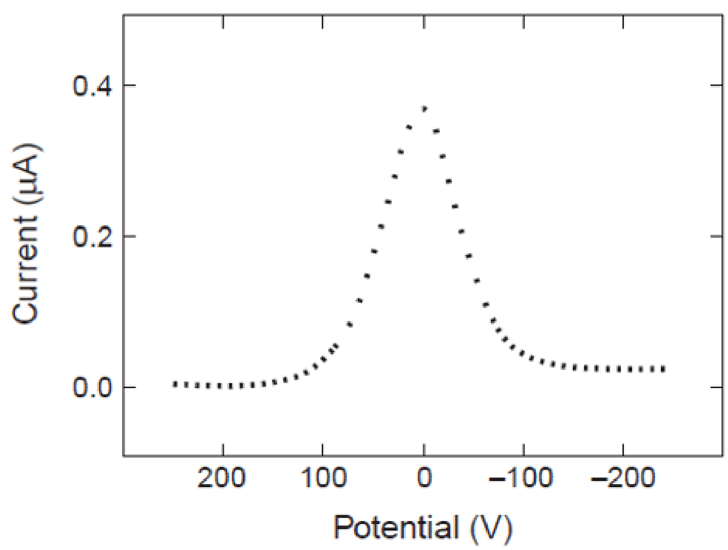

Figure 1.5 – Illustration of a differential pulse voltammogram. Source: [35].

The peaked output has many advantages regarding quantification and speciation. In DPV, sloping baselines tend to be more flatten and the height of the resulting peak varies with the pulse amplitude, ΔE_P [20].

1.3.2. Potentiometric sensors

Potentiometric sensors measure the potential of an electrochemical cell while drawing negligible current, so that the indicator electrode does not consume or produce species in a thermodynamic equilibrium with the analyte [14]. The potential of the working electrode is measured against that of a reference electrode [22]. Ion selective electrodes for K^+, Ca^{2+}, Na^+ and Cl^- are examples of working electrodes in this type of electrochemical technique [14].

In potentiometric devices, the potential measured is associated to the number of electroactive species in electrochemical cell. Direct potentiometry directly measures analyte concentration with the application of Nernst equation. Potentiometric devices currently have detection limits between 10^{-8} and 10^{-11} mol/L [17][18].

The main advantages of potentiometry are the selectivity of the signal produced for the target analyte, the ability to study a large range of compounds and a good detection limit since potentiometric sensors do not chemically affect the sample [22][17].

1.3.3. Conductimetric/Impedimetric sensors

Conductimetric devices monitor changes in the electrical conductivity of the sample solution, as the composition of the medium changes due to the occurring of the chemical reaction [14].

These devices also evaluate the ability of an analyte to conduct an electrical current between electrodes, for instance in electrolyte solutions [18].

Conductometry is a technique very used in environmental monitoring and clinical analysis, and has been reported to be applied coupled with enzymes and antibodies to create biosensors [14].

Conductimetric devices can also be considered as a subset of impedimetric devices. The most used impedimetric technique is Electrochemical Impedance Spectroscopy (EIS) [18]. In this method, the impedance of a system is determined by varying the excitation frequency of the applied potential over a range of frequencies [23].

This procedure allows the analysis of complex electrical resistance of a system and is sensitive to surface phenomena and changes of bulk properties [23].

EIS has been widely used to study corrosion mechanisms, characterization of charge transport across membranes and membrane/solution interfaces, and for the optimization of batteries [23]. In the field of biosensors, it has been applied in the detection of binding events on the transducer surface and in the monitoring of biosensor fabrication processes through characterization of surface modifications that result from immobilization of biomolecules on the transducer [23].

The main differences between the signal measured with the discussed electrochemical techniques is shown in Figure 1.6.

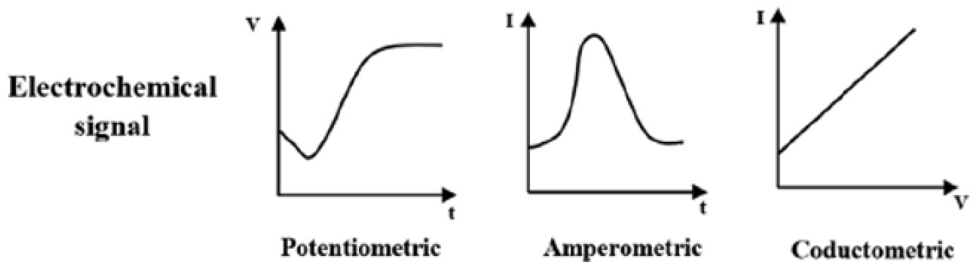

Figure 1.6 – Electrochemical signals of different electrochemical techniques. Source: [15].

1.4. Sensors application in the environment

As CECs occur at trace concentrations, the difficulty in their detection encourages the development of analytical methods that are highly sensitive and selective. The main analytical techniques for CEC monitoring are based on Gas Chromatography (GC) and Liquid Chromatography (LC) coupled to Mass Spectrometry (MS) [4].

However, these techniques present some disadvantages. They are time consuming since it is required one or more sample preparation steps and subsequently *in situ* analysis cannot be performed. Furthermore, GC, LC and High-Performance Liquid chromatography (HPLC) techniques must be performed by a technician capable of operating chromatography equipment.

In this context, electroanalysis are very promising since sensors possess important characteristics that make them very adequate to environmental monitoring, such as portability, low cost in construction and analysis, minimal sample treatment and selectivity, rapid analysis and low costs associated [24].

Therefore, interest in electrochemical sensing applications is increasing, mostly in areas like clinic diagnosis, food and beverage industries, agriculture and environmental monitoring [16].

There have been reported cases of biosensors application in environmental monitoring, mostly in the detection of heavy metals in soil samples and herbicides and pesticides in water samples [16].

1.4.1. Electrochemical sensors in pharmaceutical analysis

Regarding oxidizable drugs, the best choices are voltammetric/amperometric sensing systems. When biosensors are equated for the detection of these drugs, the best biological recognition element is usually enzymes such as peroxidases, laccases, tyrosinase, superoxide dismutase, among other oxidases [24].

Concerning drugs containing hydrolysable and labile groups and when proton H^+ liberation occurs, the most frequently used biosensors are potentiometric biosensors or ion-selective electrodes [24].

In 2010, it was reported that methamphetamine detection in human urine has also been performed using conductimetric sensors [14].

Since 2016, the use of electrochemical sensors for determining pharmaceuticals have increased. Thus, biosensors have become a promising tool for pharmaceutical analysis. Reports show that many biosensors have been applied for the determination of countless drugs such as: salicylates, acetaminophen, fluoxetine, theophylline, opiates, angiotensin converting enzyme inhibitors (ACEI), catecholamines, nicotine, carnitine, L-methotrexate, rifampicin, benzydamine, creatinine, daunomycin, nitroimidazoles, phenothiazine, carboplatin, quinazoline, mitoxanthrone and mitomycin C [25].

Amperometric DNA biosensors were reported to be used in the determination of salicylate hydroxylase. Salicylates are the main metabolite from the hydrolysis of acetylsalicylic acid. The sensors are designed by immobilizing salicylate hydroxylase, which catalyzes the hydroxylation of salicylate to catechol in the presence of NADH and oxygen. The product, catechol, is detected amperometrically. The use of the salicylate hydroxylase biosensor allowed the quantification of salicylate in the range of 7.27×10^{-6} mol/L to 4.35×10^{-3} mol/L [25].

Although many strategies are already implemented for paracetamol analysis, amperometric biosensors based on direct enzyme immobilization on the transducer surface are the most used because, in the presence of hydrogen peroxide, the horserdish peroxidise enzyme catalyzes the oxidation of paracetamol into N-acetyl-p-benzoquinoneimine [24].

Later, in 2016, a biosensor was developed to detect and quantify acetaminophen, an electrochemical biosensor was built using the enzyme aryl acylamidase. Acetaminophen is an analgesic and antipyretic drug that is used alternatively to acetyl-salicylic acid. The enzyme hydrolysis the target compound to 4-aminophenol [25].

An electrochemical biosensor was developed for the detection and determination of diacetylmorphine, morphine and their major metabolite morphine-3-glucuronide [25].

ACEI are drugs used in the treatment of hypertension. Several enantioselective biosensors have been developed for the analysis of this drug with chronoamperometric methods. They are constructed by immobilizing the enzyme L-aminoacid oxidase on a carbon paste electrode [25].

Nicotine can be found in many anti-tobacco pharmaceutical formulations. The inhibiting action of nicotine on enzymes can be used to determine nicotine. In order to determine this drug, a biosensor was constructed with butyryclolinesterase and choline oxidase immobilized, using butyrylcholine as substrate. The hydrogen peroxide involved in the reactions is detected with amperometric methods [25].

L-methotrexate, a drug reported to have antitumor activity, has been determined with multiple amperometric enzymatic biosensors. There are also biosensors capable of detecting the D-methotrexate using horseradish peroxidase. Glutamate oxidase, L-amino oxidase and horseradish peroxidase were used for the assay of the L-enantiomer. Horseradish peroxidase is also used in biosensors to monitor rifampicin, an antituberculosis drug, where the drug is reduced in the presence of hydrogen peroxide [25].

An enzymatic biosensor was developed for the determination of benzydamine, a drug with analgesic and anti-inflammatory activity. The biosensor was constructed using the enzyme monoamino oxidase [25].

1.4.2. Sensors and biosensors for determination of NSAIDs

As stated previously, NSAIDs are a widely used group of drugs that have been identified as emerging contaminants. The main problem regarding NSAIDs is that they are frequently over-the counter drugs and are sold without prescription, meaning that people can consume these drugs solely based on peer suggestions and previous experience [6], [26]. Despite their relatively low toxicity for humans, their presence in significant concentrations still represents a potential risk for the environment [6].

These group of drugs are one of the most consumed. According to a 2014 report from the National Authority for Medicines and Healthcare Products, Infarmed, more than 54 million packages of NSAIDs were sold in Portugal in that year. Acetylsalicylic acid, ibuprofen and naproxen were ranked 4th, 9th and 73rd respectively, in the top 100 active substances with the most packages in the Portuguese health system in 2014 [27]. Similar figures can be expected worldwide.

As a consequence, several environmental studies around the world, recurring to traditional analytical methods such as HPLC, have confirmed the presence of NSAIDs in water bodies [6, 26, 28–30].

In 2014, Shanmugam detected and determined five NSAIDs in the rivers of southern India, four of them being propionic acid derivatives (ketoprofen, ibuprofen, naproxen and diclofenac) and the other one being acetylsalicylic acid. [28].

Later, in 2017, a review article regarding the occurrence of pharmaceuticals in water bodies by Balakrishna et al. reported the presence of many pharmaceuticals and PCPs in the influents and effluents of wastewater treatment plants in India. Some of those contaminants included the NSAIDs ibuprofen, ketoprofen, naproxen and diclofenac [26].

In northwest Pakistan, Khan confirmed the presence of NSAIDs and anti-depressants in municipal wastewater and in rivers receiving untreated sewage. Acetaminophen, diclofenac, ibuprofen and codeine were found in the sewage and in surface water. Acetaminophen and ibuprofen levels in river samples were critical and represented the consequence of untreated disposal of sewage [29].

In Europe, NSAIDs were detected in influents and effluents of wastewater treatment plants in Rome, Italy [30]. Also, in Czech Republic, studies were performed to detect five widely used NSAIDs (ibuprofen, diclofenac, naproxen, ketoprofen and indomethacin) in the watercourses of the river Elbe basin [6].

NSAIDs were also found in Portugal water. The presence of NSAIDs in portuguese surface waters was first reported in 2013. Ibuprofen was found and determined in surface waters, landfill leachates, WWTP, and hospital effluents in the north of country [31]. In 2015, an assessment of NSAIDs and analgesic pharmaceuticals was performed in the North of Portugal, evaluating fourteen beaches in five different cities. Ketoprofen, acetaminophen and hydroxyibuprofen were detected in all fourteen locations [32].

Given the importance of NSAIDs, electrochemical sensors and biosensors have been extensively described in the literature for drugs such as diclofenac [33] acetaminophen [34] or acetylsalicylic acid [35].

NSAIDs derived from propionic acid have been also found frequently in the environment [6], [26], [28]. However, electrochemical sensors for propionic acid derivatives have been developed in lesser extent. Table 1.1 reviews some electrochemical (bio)sensors developed to determine and detect NSAIDs derived from propionic acid. Propionic acid derivatives include ibuprofen, naproxen, ketoprofen, oxaprozin, carprofen, fenoprofen, flurbiprofen and tiaprofenic acid [36].

Amongst propionic acid derivatives, ibuprofen and naproxen sensors have been heavily investigated when compared to the other drugs of the same class. A research has shown that most of the sensors developed for determination of these drugs are electrochemical sensors, with no biological recognition element. There are, however, reports of enzymatic and immune sensors developed for ibuprofen, naproxen, fenoprofen and flurbiprofen determination.

So far, ketoprofen electrochemical determination has been studied with different electrodes such as HMDE (Hanging Mercury Drop Electrode), ISE (Ion Selective Electrode) and GCE (Glassy Carbon Electrode). The applied electrochemical techniques in ketoprofen studies were potentiometry and amperometry. As shown in Table 1.1, the developed ketoprofen sensors have detection limits as low as 3.9×10^{-4} µM.

Table 1.1 – Sensors developed for determination of propionic acid derivatives.

Transducer	Electrochemical technique	Linear range (µM)	LOD (µM)	Sample	Reference
		Carprofen			
GCE	CV / DPV	0.717 - 89.8	0.262	Tablets	[37]
		Fenoprofen			
GCE/BSA	ADSPV / ACV	0.02 - 8.0	0.007	Tablets	[38]
HMDE	DPAdSV	1.73-215.7	0.334	Human urine/plasma	[39]
		Flurbiprofen			
PME	Potentiometry	0.015 - 1500	0.08	Tablets	[40]
PME/PVC	Potentiometry	70 - 10000	41	Tablets	[41]
		Ibuprofen			
SPCE/MWCNT	DPV	9.21-155	2.91	Tap water/wastewater	[42]
BDDE	DPV	0.95-67	0.41	Urine/Tablets	[43]
	SWV		0.93		
GCE/Poly-L-aspartic acid	SWV	1-150	0.22	Urine/Tablets	[44]
PVC/plasticizer/cyclodextrin	Potentiometry	3.87 - 10000	3.34	Tablets/Water	[45]
Au/DSP/antibody	Impedance	up to 0.04	1.2×10^{-6}	Drink water/River water	[46]

Table 1.1 – (Continued).

Transducer	Electrochemical technique	Linear range (µM)	LOD (µM)	Sample	Reference
		Ketoprofen			
HMDE	DPV	10-500	9.83	Tablets	[47]
HMDE	CV	9.83×10^{-4} - 0.983	0.002	Tablets	[48]
HMDE	SWAdSV	0.002-0.2	3.93×10^{-4}	Tablets/Plasma	[49]
ISE/PVC-Teflon	Potentiometry	100 – 100008	57.5	Tablets	[50]
ISE/PVC-IA	Potentiometry	100–10000	63	Tablets	[51]
GCE	DPV	9-5000	11.4	Tablets	[52]
		Naproxen			
GCE/CB-Polyacrylic acid-La_2O_3	DPV	0.05-884	0.035	Urine/serum	[53]
CPE – Graphite + SWCNT	DPV	0.291- 4.34	0.347	Tablets/Plasma	[54]
Au/rGO-AgNPs-β-cyclodextrin-nafion	DPV	0.4-80	0.023	Tablets/Urine	[55]
GCE/MWCNT	Amperometry	10-200	-	Tablets	[56]
GCE/MWCNT-β-cyclodextrin	Amperometry	1.74 - 99.9	1.61	Tablets	[57]
SPCE/MWCNT/CYP450	CV	up to 300	16	Tablets	[58]
SPCE/MWCNT/CYP450	CV	200-500	200	Serum	[59]
		Oxaprozin			
PAuE/BSA	CV	8.44 -32.78	-	Tablets	[60]
		Tiaprofenic acid			
SMDE	DCP / CV	0.01 - 0.06	0.006	Tablets	[61]

ACV – Affinity Cyclic Voltammetry; ADSPV – Affinity Differential Stripping Pulse Voltammetry; AgNPs – Silver Nano Particles; BDDE – Boron Doped Diamond Electrode; BSA – Bovine Serum Albumin; CB – Carbon Black; CPE – Carbon Paste Electrode; CV – Cyclic Voltammetry; DPAdSV – Differential Pulse Adsorptive Stripping Voltammetry; DPV – Differential Pulse Voltammetry; DSP – Dithiobis Succinimidyl Propionate; GCE – Glassy Carbon Electrode; HMDE – Hanging Mercury Drop Electrode; IA – Ion Associates; ISE – Ion Selective Electrode; MWCNT – Multi-Walled Carbon Nano Tubes; PAuE – Polycrystalline Gold Electrode; PME – Potentiometric Membrane Electrode; rGO – reduced Graphene Oxide; SMDE - Static Mercury Drop Electrode; SPCE – Screen Printed Carbon Electrode; SWAdSV – Square Wave Adsorptive Stripping Voltammetry; SWCNT – Single-Walled Carbon Nano Tubes; SWV – Square Wave Voltammetry

The use of alternative transducers in the electrochemistry field, especially electrochemical analysis, may bring some advantages regarding sensitivity, versatility, and material costs. A research made on Carbon Paper (CP) showed that this material is frequently used as electrode in Bio-Batteries and biofuel cells [62].

Toray Carbon Paper (TCP) (Figure 1.7) has been extensively used as a gas diffusion layer in fuel cells due to its low cost [63].

Figure 1.7 – Toray Carbon Paper. Source:[64].

Carbon Paper is frequently found in articles that aim to study the effect of electrode surface properties on enhanced electron transfer [65], the impact of nanoparticle density on electricity production and microbial community [66], the cost-effectiveness of biofuel cells for treatment of municipal sludge [67], the growth of gold, palladium and platinum nanoparticles [68], graphene nanosheets production [69], or even electrode modification with platinum nanoparticles [70], silicon nanoparticles [71] and other materials such as CNTs and graphene [72]. However, CP is not commonly used in sensors to detect hazardous substances. An extensive research on CP-based (bio)sensors found that these are not extensively reported in literature. The existing CP-based sensors to detect CECs are:

- potentiometric biosensor to detect urea with immobilized urease [73];
- amperometric biosensor to detect theophylline with immobilized theophylline oxidase [74];
- amperometric biosensors to detect pesticides such as atrazine [75] and malathion [76] modified with mithocondria;
- amperometric sensor to degrade and study diclofenac kinetics with immobilized CNTs in aqueous medium [77].

Non-related to hazardous substances, CP was also used as working electrode in an enzymatic sensor, for ester flavorants detection in food [78].

2. Research aims

The main goal of this project was to develop an electrochemical sensor for determination of ketoprofen, an anti-inflammatory drug used worldwide. In order to complete this work, the following specific objectives were established:

- Bibliographic research on electrochemical sensors to determine NSAIDs;
- Bibliographic research on carbon paper-based transducers used both in sensors and biofuel cell applications;
- Electrochemical characterization of Toray Carbon Paper and comparison with other conventional electrodes;
- Optimization of experimental parameters;
- Characterization of analytical performance;
- Application of the developed ketoprofen sensor in environmental samples.

3. Experimental

3.1. Apparatus

Voltammetric measurements were performed with the Metrohm Autolab potentiostat and GPES software for data acquisition. The measurements were conducted in a three-electrode system electrochemical cell with 10 mL volume. Toray Carbon Paper (Alfa Aesar, USA) was used as working electrode while a platinum electrode and a silver chloride electrode (Ag/AgCl, 3 M) were used as auxiliary and reference electrode, respectively. A copper mesh was added to one end of the Toray Carbon Paper substrate for a better connection to the equipment, as it can be observed in Figure 3.1.

Figure 3.1 – Example of a Toray carbon paper electrode used in the present work.

For comparison purposes, a Pyrolytic Graphite Electrode (PyrGE) 3 mm diameter, a GCE 3 mm diameter, Pencil Graphite Electrode (PGE) 2 mm diameter, Boron Doped Diamond Electrode (BDDE) 3 mm diameter, Screen-Printed Carbon Electrode (SPCE) 4 mm diameter and a Screen-Printed Gold-Electrode (SPAuE) 1.6 mm diameter were also tested as working electrodes. Before use, the PyrGE and GCE were mechanically polished with alumina (Buehler, Switzerland) 0.3 µm and 0.05 µm using a polishing cloth and then were ultrasonically cleaned in ethanol for 2 min and rinsed with water whereas PGE was first polished with sandpaper prior to alumina and BDDE was pre-treated electrochemically with 2-step a chronoamperometric method, at -3V and +3V for 45s each in sulfuric acid (H_2SO_4) 0.5 M. TCP was pre-treated through performing 50 cyclic voltammetric sweeps from -0.2 V to 1 V in a sulfuric acid (H_2SO_4) solution 0.1 M.

3.2. Reagents and solutions

Ethanol absolute ($CH_3CH_2OH \geq 99.5\%$) glacial acetic acid ($CH_3COOH > 99\%$), hydrochloric acid (HCl, 37%), ketoprofen ($C_{16}H_{14}O_3$, $\geq 98\%$), potassium chloride ($KCl > 99.8\%$), potassium hexacyanoferrate (III) ($K_3Fe(CN)_6 > 99\%$), potassium hexacyanoferrate (II) trihydrate ($K_4[Fe(CN)_6)].3H_2O > 98\%$), sodium hydroxide (NaOH; 98%), sodium phosphate dibasic (Na_2HPO_4), sodium phosphate monobasic (NaH_2PO_4) and sulfuric acid (H_2SO_4) were acquired from Sigma-Aldrich (Germany) whereas boric acid ($H_3BO_4 \geq 99.5\%$) and orthophosphoric acid (H_3PO_4, 85 wt. % in H_2O) were bought from Riedel-de-Haën (Germany).

All aqueous solutions were prepared with ultrapure water (Millipore Simplicity 185, ρ=18.2 MΩ cm^{-1}) The electrolyte used was a 0.1 M Britton-Robinson Buffer (BRB) prepared by weighting appropriate weights of CH_3COOH, NaOH, H_3PO_4, H_3BO_4 and KCl, according to BRB tables [79]. For pH optimization studies, aliquots of the BRB stock solution were taken, and pH was adjusted with previously prepared solutions of 1 M NaOH and 1 M HCl. Phosphate buffer solution, 0.1 M and pH 6, was also prepared using Na_2HPO_4 and NaH_2PO_4.

An equimolar (5 mM) aqueous solution of $K_3Fe(CN)_6$ and $K_4[Fe(CN)_6)].3H_2O$ with 0.1 M KCl was prepared to compare and assess electrodes performance.

Ketoprofen solutions were prepared dissolving it in the BRB (pH 5, 0.1 M) and keeping the solution in an ultrasonic bath (J. P. Selecta, Spain) for approximately 10 minutes until total dissolution.

3.3. Measurement Procedures

All experiments were performed at room temperature. The electrochemical characterization of ketoprofen was performed by voltammetric techniques. The different electrodes were compared through cyclic voltammetry using the electrochemical probe $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ in 0.1 M KCl. Three voltammetric cycles were carried out between -1000 and 900 mV at a scan rate of 100 mV/s.

DPV was used for the analytical determination of ketoprofen. The analysis was performed by the standard addiction method by adding a known volume of buffered ketoprofen solution to the electrochemical cell containing 10 mL of buffer solution. Previously, the technique parameters (pulse amplitude, pulse time and scan rate) were optimized in a Britton-Robinson buffer at pH 5. Before measurements, TCP was pre-treated, and a blank assay was performed in triplicate in order to activate and stabilize the TCP.

All ketoprofen solutions were deaerated prior to analysis by bubbling N_2 for 15 min.

3.4. Statistical Treatment

LOD and limit of quantification (LOQ) were calculated through the parameters of the calibration curve and with the equations 1 and 2 [80]:

$$\mathrm{LOD} = \frac{3.3 \times \mathrm{SD}}{\mathrm{m}} \quad (1) \qquad\qquad \mathrm{LOQ} = \frac{10 \times \mathrm{SD}}{\mathrm{m}} \quad (2)$$

Where SD is the standard deviation of the ordinate intercept and m is the slope of the regression line.

Standard deviation of the ordinate intercept was calculated with the expression 3 [81]:

$$SD = S_{y/x} \times \sqrt{\frac{\sum_i x_i^2}{n \times \sum_i (x_i - \bar{x})^2}} \qquad (3)$$

Where, $S_{y/x}$ is the standard deviation of the linear regression calculated using equation 4 [81], n is the number of points in the calibration curve, x_i are the concentration values for the calibration curve and $\bar{x}$ is the average of all concentrations.

$$S_{y/x} = \sqrt{\frac{\sum_i (y_i - \hat{y}_i)^2}{n-2}} \qquad (4)$$

Where y_i is the response for a certain level of concentration and $\hat{y}_i$ is the response predicted by the calibration curve.

4. Results and Discussion

4.1. Electrode Characterization

The experimental work began with the electrochemical characterization of TCP and other electrodes that are commonly used in electroanalysis. Cyclic voltammetric measurements were conducted at 100 mV/s in $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ in 0.1 M KCl. These preliminary assays aimed to compare the performance and kinetics of the different available electrodes. Thus, the electrochemical behaviors of the Toray Carbon Paper Electrode (TCPE), BDDE, PGE, PyrGE, SPCE and SPAuE were investigated and their representative cyclic voltammograms are represented in Figure 4.1. Generally, the anodic peak appears between potentials of 0.19 V and 0.39 V corresponding to the oxidation of Fe^{2+} to Fe^{3+} while the corresponding cathodic peak potential occurs between 0.05 V and 0.16 V during the reverse scan (Figure 4.1).

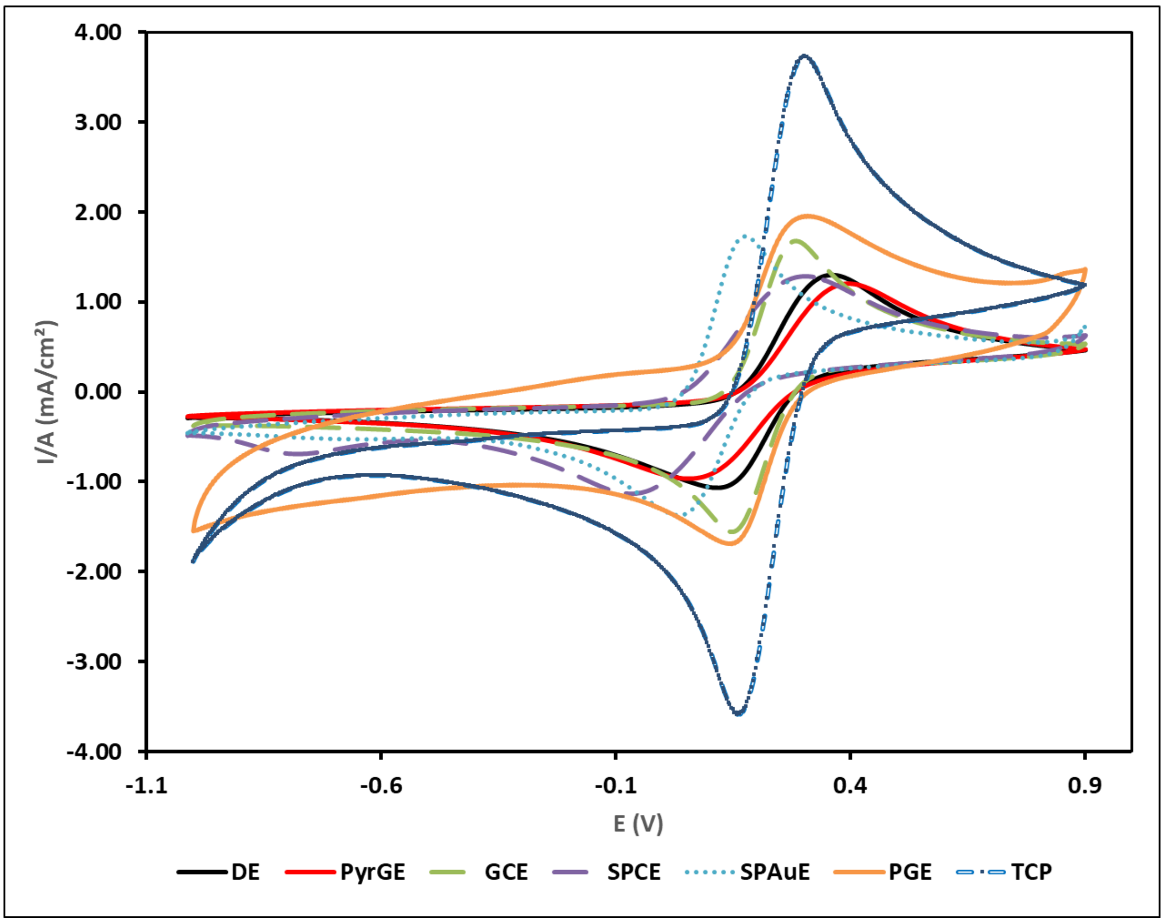

Figure 4.1 – Cyclic voltammograms of different electrodes in $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ probe in 0.1 M KCl at 100 mV/s.

The electrode performance was evaluated through the anodic peak (Ia) and cathodic peak (Ic) currents, Ic/Ia ratio, peak-to-peak separation (ΔEp) and the heterogeneous electron transfer rate (k^0). As it can be seen in Figure 4.1, all the electrodes showed well separated anodic and cathodic peaks, which indicates that the electron transfer is a heterogeneous process.

Heterogeneous electron transfer rate constants were determined using the method of Nicholson, according to Equation 5 [82].

$$k^0 = \theta.\left(\frac{D_r}{D_0}\right)^{\frac{\alpha}{2}}.\left(D_0.\pi.v.\left(\frac{nF}{RT}\right)\right)^{\frac{1}{2}} \tag{5}$$

Where θ is the kinetic parameter (dimensionless), D_0 is the diffusion coefficient for ferricyanide (7.6 $\times$ 10^{-6} cm^2/s), D_rr is the diffusion coefficient for ferrocyanide (6.3 $\times$ 10^{-6} cm^2/s), α is the transfer coefficient (0.5), v is the scan rate, (0.1 V/s), n is the number of transferred electrons ($n = 1$), F is the Faraday constant (96485 C), R is the universal gas constant (8.314 mol^{-1}), T is absolute temperature (298.15 K).

The kinetic parameter, θ, was calculated with the aid of a solver program that generated the plot of ΔEp vs θ [83]. The analytical data retrieved from the representative voltammograms (Figure 4.1) are presented in Table 4.1.

Table 4.1 – Intensity of anodic and cathodic peaks, peak-to-peak-separation and heterogeneous electron transfer rate constants in $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ *probe in 0.1 M KCl at 100 mV/s.*

Electrode	Anodic Peak (mA/cm^2)	Cathodic Peak (mA/cm^2)	Ic/Ia	ΔEp (mV)	K^0 (cm/s)
BDDE	1.37	1.25	0.912	237	8.62×10^{-4}
PyrGE	1.27	1.04	0.819	335	3.15×10^{-4}
GCE	1.72	1.75	1.02	143	2.27×10^{-3}
SPCE	1.32	1.27	0.958	354	2.59×10^{-4}
SPAuE	1.72	1.37	0.792	176	1.61×10^{-3}
PGE	1.25	1.56	1.25	140	2.34×10^{-3}
TCPE	4.08	3.95	0.968	122	2.81×10^{-3}

Of all the electrodes examined, TCPE exhibited the most reversible behavior, with a ΔEp value of 122 mV as compared to 140 mV and 143 mV for PGE and GCE. However, the ΔEp value for TCPE is not close to the ideal of 59 mV expected for Nernstian one-electron reactions [84]

All the electrodes examined, displayed perceptible anodic and cathodic peaks. However, the TCPE showed significantly higher anodic and cathodic peaks than the remaining electrodes, yielding current densities of 4.08 mA/cm^2 and 3.95 mA/cm^2, respectively. The GCE and SPAuE showed also acceptable anodic peaks (1.72 mA/cm^2). However, PGE and SPAuE presented Ic/Ia ratios of 1.25 and 0.792, which were the furthest away from 1 for all the electrodes, indicating poor reversibility. The Ic/Ia ratios closer to 1 were obtained for GCE and TCPE with these values being 1.02 and 0.968, respectively.

The electrodes evaluated in this study yielded k^0 values in magnitudes of 10^{-3} and 10^{-4} cm/s. TCPE yielded the best k^0 value of all the electrodes with values of 2.81 x 10^{-3} cm/s, whereas SPCE yielded the lowest k^0 value of all the electrodes with 2.59 x 10^{-4} cm/s. According to Aristov [85], the reversibility can be evaluated examining the k^0 value for each sensor, since at fast heterogeneous kinetics or low scan rate, the cyclic voltammogram will appear reversible. However, an increase of the scan rate, v, will gradually transform it into a quasi-reversible or irreversible process. The boundaries for heterogeneous kinetics are the following [85]:

- Reversible: $k^0 > 0.35 \times v^{\frac{1}{2}}$;
- Quasi-reversible: $0.35 \times v^{\frac{1}{2}} > k^0 > 3.5 \times 10^{-4} \times v^{\frac{1}{2}}$;
- Irreversible: $k^0 < 3.5 \times 10^{-4} \times v^{\frac{1}{2}}$.

Thus, it is possible to conclude that the reaction is characterized as quasi-reversible for all the electrodes studied.

Examining k^0, ΔEp and anodic and cathodic peaks, TCPE was shown to have the best behavior of all the electrodes, being therefore selected as a sensor for ketoprofen drug.

4.2. Optimization of Experimental Parameters

Differential pulse voltammetry was selected for quantitative determination of ketoprofen since it provides enhanced sensitivity and selectivity in the detection of low concentrations. Hence, electrolyte pH and DPV parameters such as modulation time (pulse time), modulation amplitude (pulse amplitude) and scan rate were optimized in order to enhance the signal response for ketoprofen reduction. All the measurements were carried out for a ketoprofen solution with a concentration of 50 µmol/L.

The pH value of the electrolyte is an important factor that may affect the electrochemical behavior of different molecules and drugs. To optimize the pH, a range of values between 1.0 and 10 was considered. BRB was used since it is appropriate in an extensive range of pH values. The influence of the pH in the DPV ketoprofen signal is shown in Figure 4.2.

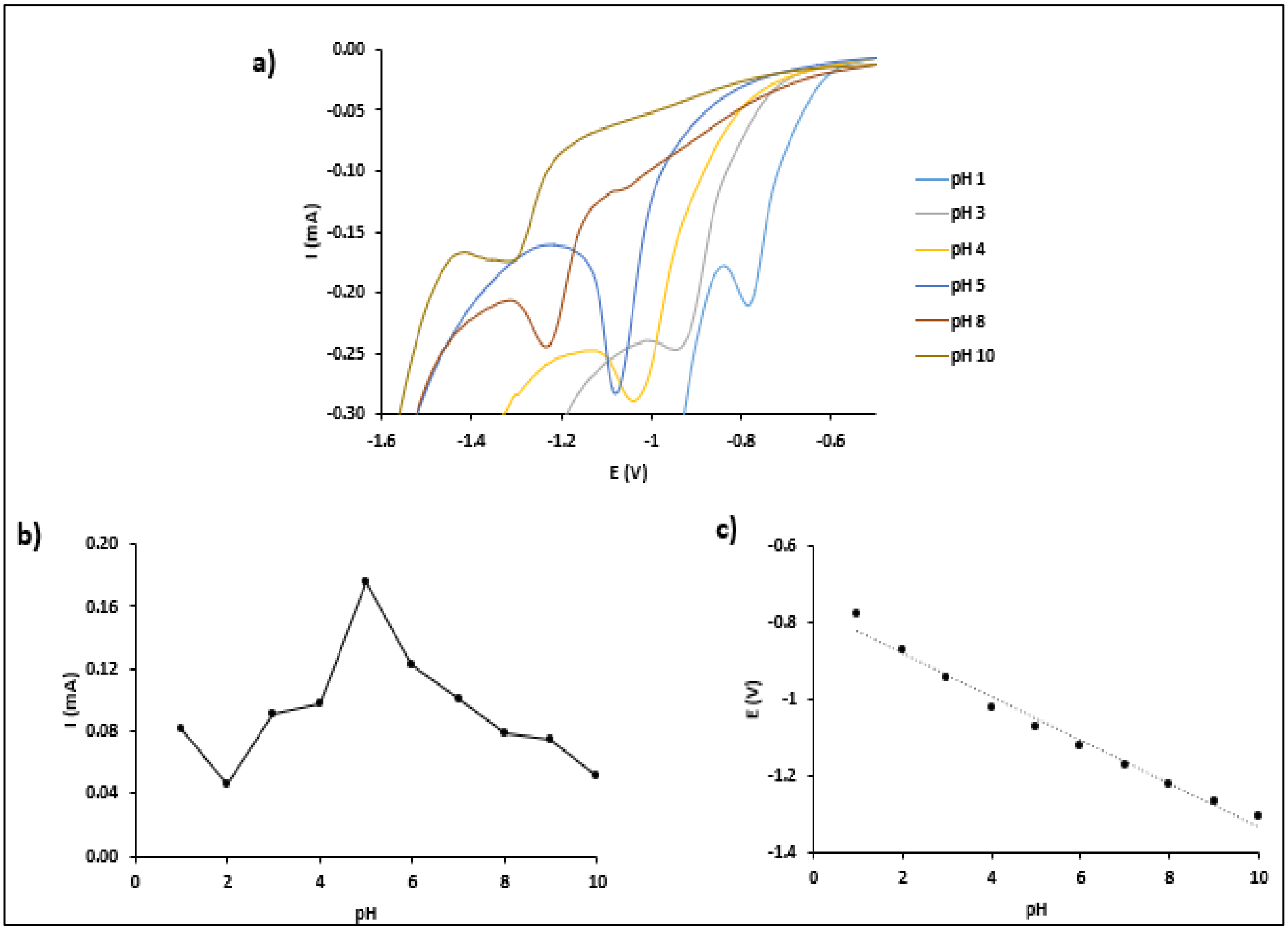

Figure 4.2 – a) Differential pulse voltammograms with TCPE at different pH values; b) Influence of pH in peak intensity; c) Influence of pH in peak potential. Measurements performed in 50 µM ketoprofen with scan rate = 20 mV/s, pulse amplitude = 50 mV and pulse time = 50 ms.

The maximum peak current is observable at pH 5 (Figure 4.2.b). It is also evidenced that for higher pH values, the peak potential displaces to more negative values (Figure 4.2.c), in agreement with the works carried out by Amankwa et al. [47] and Emara et al. [48] regarding the pH influence in the polarographic reduction behavior of ketoprofen. Therefore, pH 5 was selected as the optimum value and DPV parameters optimization was carried out using pH 5. Concerning the peak potential, the obtained results are consistent with the work of Ghoneim et al. in which ketoprofen reduction was reported to occur at a potential of -1.1 V at pH 5 [49]. Also, in all previous studies, ketoprofen cathodic peak appeared at potentials between -0.8 V and -1.3 V [47 - 48, 52].

To choose the best pulse amplitude, DPV measurements were carried out for different values of this parameter varying it from 10 to 200 mV. It is possible to observe that the peak intensity increases with increasing values of pulse amplification (Figure 4.3.a and Figure 4.3.b). In Figure 4.3.c it can be seen that the peak potential increased towards more positive values with the increasing of pulse amplitude accompanied by the peak width (Figure 4.3.d). When the pulse amplitude is higher than 10 mV, the peak intensity and width increase until 110 mV, and then both peak intensity and peak width stabilize for pulse amplitude values higher than 110 mV. However, it was found that there is a linear increase in the intensity value up to 70 mV (Figure 4.3.b). Thus, this value of pulse amplitude was chosen as the best value for ketoprofen determination.

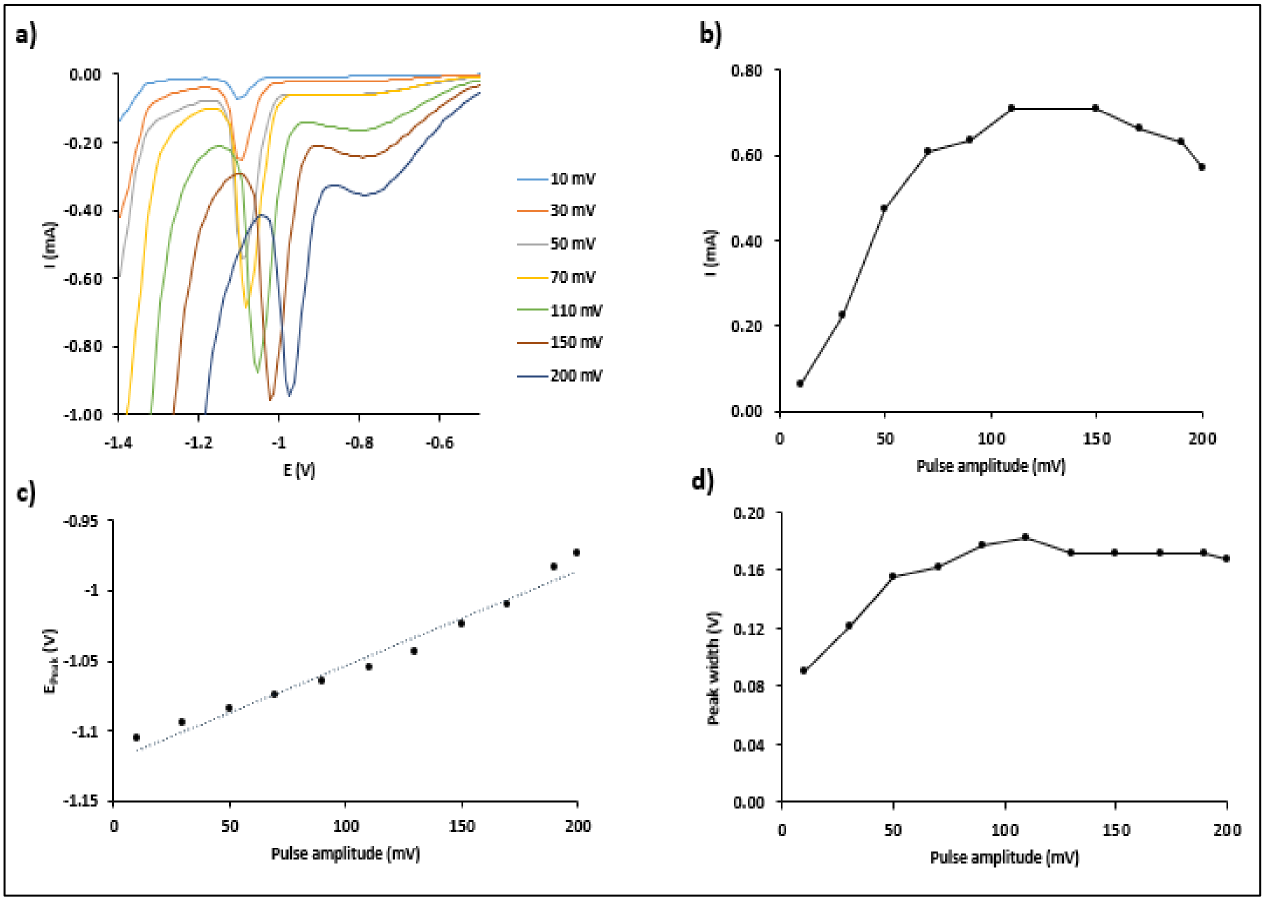

Figure 4.3 – a) Differential pulse voltammograms with TCPE at different pulse amplitudes; b) Pulse amplitude influence in peak intensity; c) Pulse amplitude influence on peak potential; d) Pulse amplitude influence on peak width for 50 µM ketoprofen (pH 5; Scan rate = 20 mV/s).

After pulse amplitude optimization, pulse time was investigated by varying this parameter from 3 to 30 ms (Figure 4.4.a). The intensity value was maximum for a pulse time of 5 ms, which was selected as optimum. From 5 and up to 30 ms, the intensity value decreased as the pulse time increased.

Observing Figure 4.4.b, it is also possible to observe that the peak potential shifts towards more negative values as pulse time increases.

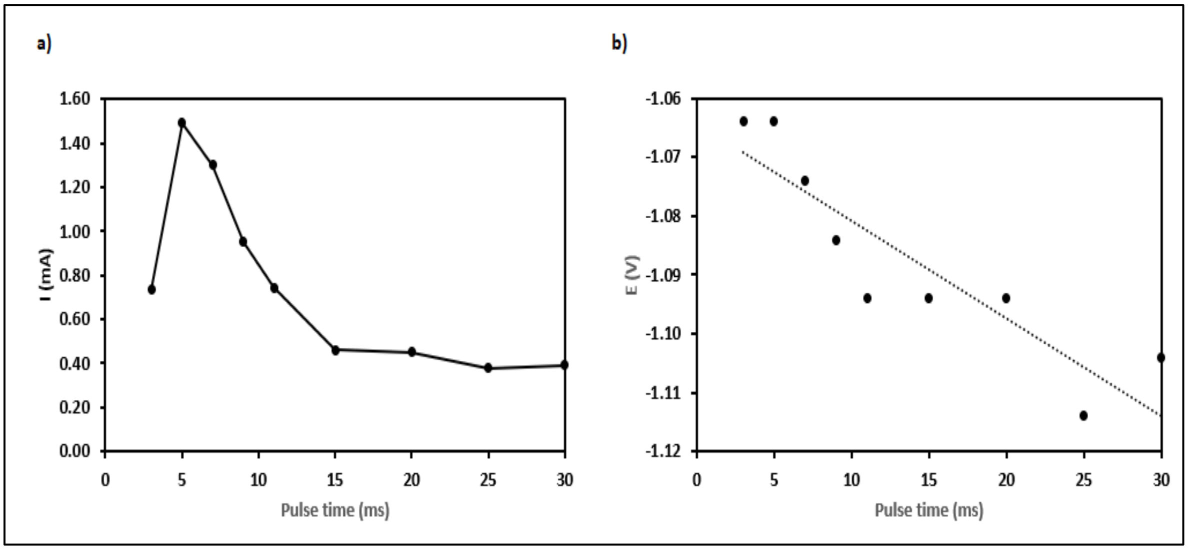

Figure 4.4 – a) Pulse time influence on peak intensity; b) Pulse time influence on peak potential for 50 µM ketoprofen with TCPE (pH 5; Scan rate = 20 mV/s; Pulse amplitude = 70 mV).

Finally, scan rate was also optimized. In DPV, scan rate is defined as the ratio between the potential step and the interval time. Thus, in order to optimize the scan rate, both step potential and interval time were evaluated. To choose the best step potential value, differential pulse voltammograms were conducted for step potential values between 1-25 mV, maintaining the previously optimized parameters and applying an interval time of 0.5 seconds. Figure 4.5.a shows the step potential influence on peak intensity, under the mentioned conditions. In order to keep the plot simple to read, only some of the obtained voltammograms are exhibited in Figure 4.5.b.

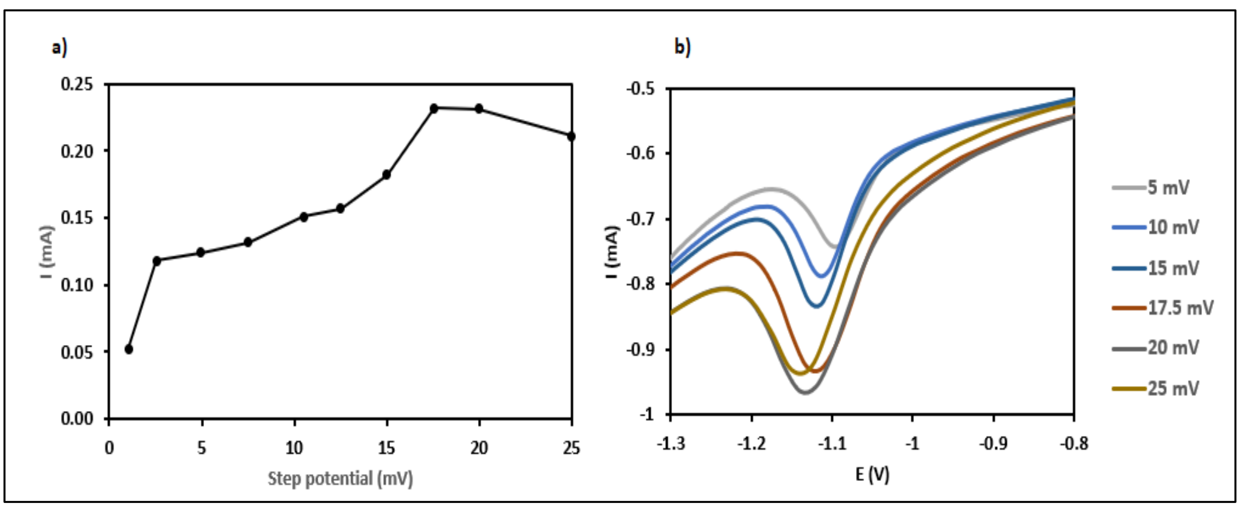

Figure 4.5 – a) Step potential influence on peak current; b) Differential pulse voltammograms for 50 µM ketoprofen with TCPE (pH 5; Interval time = 0.5 s; Pulse amplitude = 70 mV; Pulse time = 5 ms).

It was found that with the increase of the step potential, the peak current also increases, which relates to a faster change of potential and, consequently, a larger scan rate. The highest peak value was obtained for a step potential of 17.5 mV. Thus, this value was chosen as the optimum step potential since it yielded the highest peak current. It is shown in Figure 4.5.b that with the increase of the step potential, the peak potential shifts to more negative values, confirming the irreversible nature of the ketoprofen reduction process, in agreement with the studies carried out by Ghoneim et al. [49].

After the step potential optimization, the interval time was varied in order to find the optimum scan rate value. Differential pulse measurements were conducted with the previously stated conditions and step potential was set for 17.5 mV. Differential pulse voltammograms were performed for a multitude of scan rates by varying interval time from 8.77 seconds to 0.117 seconds (Figure 4.6) which, with a step potential of 17.5 mV, corresponds to a scan rate of 2 mV/s and 150 mV/s, respectively. In general, Figure 4.6 shows that peak intensity is higher for lower interval times and consequently higher scan rates, since scan rate and interval time are inversely proportional. The use of the optimum step potential (17.5 mV) and interval time (0.117 s) allowed for a scan rate of 150 mV/s, which was established as the optimum scan rate value and was used in the following experiments.

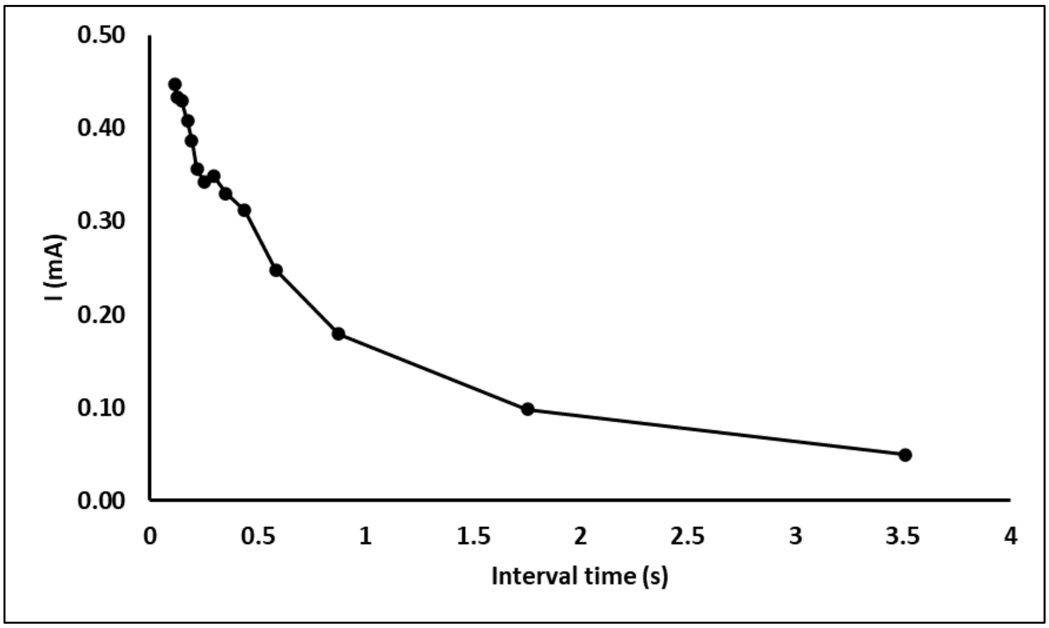

Figure 4.6 – Scan rate influence on peak current for 50 µM ketoprofen with TCPE (pH 5; step potential = 17.5 mV; pulse amplitude = 70 mV; pulse time = 5 ms).

Overall, the values of the optimized experimental parameters are the following:

- pH = 5;
- Pulse amplitude = 70 mV;
- Pulse time = 5 ms;
- Step potential = 17.5 mV;
- Interval time = 0.117 s (Scan rate = 150 mV/s).

Thus, these DPV parameters were then employed in the determination of ketoprofen at various concentrations.

4.3. Analytical Performance

The primary goal of this work is to develop an electrochemical sensor for ketoprofen determination. To do so, it is necessary to determine various electroanalytical parameters such as the LOD, LOQ, sensitivity and linear range. Thus, DPV measurements were performed at multiple ketoprofen concentrations from 0.125 µM to 8.60 µM in order to obtain a calibration curve (Figure 4.7). For each concentration value, measurements were performed three times with different TCPE and the standard deviation of the peak current was calculated. The curve ΔI=f([ketoprofen]) shows that the intensity of current increases linearly with the increase of ketoprofen concentration (I(µA/cm^2) = 24.015[ketoprofen, µM] + 7.6262); R^2=0.9942; n=19). Sensitivity was calculated based on the slope of the calibration curve, corresponding to 24.02 ± 4.01 µA/(µM.cm^2) (3 independent calibration curves), which compares favorably with those previously reported by Emara et al. [48] and Ghoneim et al. [49] with HMDEs.

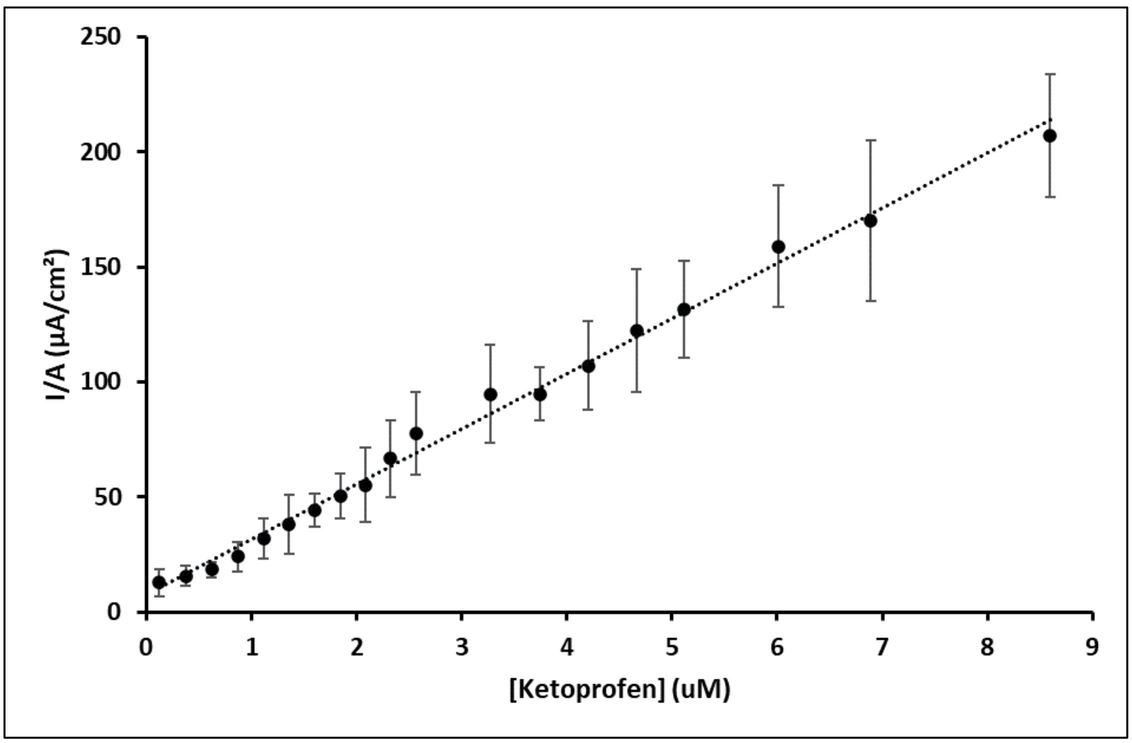

Figure 4.7 – Calibration curve obtained with TCPE from differential pulse voltammograms (pH 5; pulse time = 5 ms; pulse amplitude = 70 mVs; scan rate = 150 mV/s) in the presence of 0.125 – 8.60 µM of ketoprofen.

LOD and LOQ are very important parameters that must be considered when developing a sensor. Both parameters have related but distinct definitions. LOD is defined as the lowest concentration of an analyte in a sample that can be detected while LOQ represents the lowest concentration of an analyte in a sample that can be determined with suitable precision and accuracy [80].The attained LOD and LOQ were 0.233 µM and 0.755 µM, respectively. The developed electrochemical sensor showed a good detection limit when compared to other DPV-based sensors (LOD of 9.83 µM [63] and 11.4 µM [68)] and to potentiometric ion-selective sensors (LOD of 57.5 µM [66] and 63 µM [67]) found in literature for ketoprofen determination.

All reached analytical parameters are summarized in Table 4.2.

Table 4.2 – Analytical parameters of the developed ketoprofen sensor.

LOD (µM)	**LOQ (µM)**	**Sensitivity (µA/(uM.cm^2))**	**Linear range (µM)**
0.233	0.755	24.02	0.125 – 8.60

The reproducibility and repeatability of the electrochemical sensor were then assessed at two different ketoprofen spiking levels, 1 µM and 5 µM, in optimized conditions. Based on the attained data (Table 4.3), acceptable reproducibility and repeatability were reached.

Table 4.3 – Reproducibility and repeatability results at 1 and 5 µM spiking level of ketoprofen.

1 µM		**5 µM**	
RSD (%) of Reproducibility assays	**RSD (%) of Repeatability assays**	**RSD (%) of Reproducibility assays**	**RSD (%) of Repeatability assays**
8.8	6.1	11.7	8.2

The developed sensor was applied in the determination of ketoprofen in WWTP effluent samples. The assessment was performed by the standard addition in WWTP effluent samples at a concentration of 0.38 µM. It was possible to obtain recovery values between 86% and 99% for ketoprofen concentrations of 1 µM, 2 µM, 3 µM and 4 µM, with repeatability reaching a RSD value lower than 6.2%. The obtained results are satisfactory and confirm that the developed electrochemical sensor can be used for monitoring of ketoprofen in water samples and particularly in WWTP effluent samples.

5. Conclusions and future perspectives

The presence of CECs such as pharmaceutical compounds in the environment is an important issue since they can have a negative impact on the environment and in human health. Among these pollutants, ketoprofen, a propionic acid based NSAID, is one of the most prescribed drugs in Portugal and it has been reported to be present in the aquatic environment across the globe. Thus, the monitoring of pharmaceuticals, in aquatic matrices is an important measure for realistic risk assessment. However, until now, their determination is mostly performed by expensive conventional techniques such as HPLC-tandem mass spectroscopy. For this reason, it becomes important to develop quick, low cost and simple tools for the detection and quantification of these pollutants.

In this work, an electrochemical sensor based on Toray Carbon Paper was successfully developed for the determination of ketoprofen. Toray Carbon Paper is an innovative material that is usually employed in the construction of fuel cells. Specifically, paper-based electrodes are weightless, have a high surface to volume ratio and a porous structure that make them good candidates for small volume devices.

Considering the lack of data, the electrochemical behavior of Toray Carbon Paper was firstly evaluated using cyclic voltammetry and compared with conventional electrodes used in electrochemistry. The attained results showed that the Toray Carbon Paper yielded a better electrochemical behavior than the remaining electrodes, with a ΔEp of 122 mV, a k^0 of 2.81×10^{-3} cm/s and anodic and cathodic peak intensities of 4.08 mA/cm^2 and 3.95 mA/cm^2, respectively.

Ketoprofen drug determinations were performed using the highly sensitive DPV technique. Prior to calibration, the influence of pH was studied, and the optimum pH value was found to be 5. DPV parameters were also optimized in order to enhance the analytical response signal. The obtained electroanalytical data show that Toray Carbon Paper can be used without any other modification to detect low detection levels of ketoprofen. Overall, the proposed sensor exhibited adequate electroanalytical performance with a limit of detection of 0.233 µM, a sensitivity of 24.02 $\mu A/(\mu M.cm^2)$ and a linear range from 0.125 to 8.60 µM. Reproducibility and repeatability exhibited relative standard deviation values lower than 8.2% and 6.1%, respectively. Moreover, the electrochemical sensor was successfully applied in WWTP water samples, obtaining recoveries of 86% to 99%.

Altogether, the acquired results indicate that the developed carbon paper sensor can be used without modifications to obtain a ketoprofen signal and that the electrochemical sensor proposed is adequate to be applied in ketoprofen monitoring. However, further studies should be conducted to assess the possibility of further improving the sensor performance by incorporating a biological recognition element or even carbon-based nanostructures such as MWCNTs, graphene and others.

References

[1] G. Vandermeersch *et al.*, “Environmental contaminants of emerging concern in seafood - European database on contaminant levels,” *Environ. Res.*, vol. 143, pp. 29–45, 2015, doi: 10.1016/j.envres.2015.06.011.

[2] O. M. Rodriguez-Narvaez, J. M. Peralta-Hernandez, A. Goonetilleke, and E. R. Bandala, “Treatment technologies for emerging contaminants in water: A review,” *Chem. Eng. J.*, vol. 323, pp. 361–380, 2017, doi: 10.1016/j.cej.2017.04.106.

[3] N. S. Thomaidis, A. G. Asimakopoulos, and A. A. Bletsou, “Emerging contaminants: A tutorial mini-review,” *Glob. Nest J.*, vol. 14, no. 1, pp. 72–79, 2012, doi: 10.30955/gnj.000823.

[4] J. M. Galindo-Miranda, C. Guízar-González, E. J. Becerril-Bravo, G. Moeller-Chávez, E. León-Becerril, and R. Vallejo-Rodríguez, “Occurrence of emerging contaminants in environmental surface waters and their analytical methodology - A review,” *Water Sci. Technol. Water Supply*, vol. 19, no. 7, pp. 1871–1884, 2019, doi: 10.2166/ws.2019.087.

[5] K. W. Raghav, Madhumitha; Eden, Susanna; Mitchell and Becky, “Contaminants of Emerging Concern in Water and Wastewater,” *Contam. Emerg. Concern Water Wastewater*, 2013, doi: 10.1016/c2016-0-05074-x.

[6] P. Marsik, J. Rezek, M. Židková, B. Kramulová, J. Tauchen, and T. Vaněk, “Non-steroidal anti-inflammatory drugs in the watercourses of Elbe basin in Czech Republic,” *Chemosphere*, vol. 171, pp. 97–105, 2017, doi: 10.1016/j.chemosphere.2016.12.055.

[7] D. Rosenbloom and M. A. Craven, “A Review of Non-Steroidal Anti-Inflammatory Drugs,” 1963.

[8] S. Wongrakpanich, A. Wongrakpanich, K. Melhado, and J. Rangaswami, “A comprehensive review of non-steroidal anti-inflammatory drug use in the elderly,” *Aging Dis.*, vol. 9, no. 1, pp. 143–150, 2018, doi: 10.14336/AD.2017.0306.

[9] C. Carbone, P. Rende, P. Comberiati, D. Carnovale, and M. Mammì, “Case Review The safety of ketoprofen in different ages,” vol. 4, no. December, pp. 99–104, 2013, doi: 10.4103/0976-500X.120967.

[10] H. Adachi, M. Paoloni, and V. Santilli, “properties and clinical efficacy of the ketoprofen patch : a new patch formulation,” pp. 823–830, 2011.

[11] N. Bolong, A. F. Ismail, M. R. Salim, and T. Matsuura, “A review of the effects of emerging contaminants in wastewater and options for their removal,” *Desalination*, vol. 239, no. 1–3, pp. 229–246, 2009, doi: 10.1016/j.desal.2008.03.020.

[12] M. Bilal, M. Adeel, T. Rasheed, Y. Zhao, and H. M. N. Iqbal, “Emerging contaminants of high concern and their enzyme-assisted biodegradation – A review,” *Environ. Int.*, vol. 124, no. October 2018, pp. 336–353, 2019, doi: 10.1016/j.envint.2019.01.011.

[13] T. Rasheed, M. Bilal, F. Nabeel, M. Adeel, and H. M. N. Iqbal, “Environmentally-related contaminants of high concern: Potential sources and analytical modalities for detection, quantification, and treatment,” *Environ. Int.*, vol. 122, no. September 2018, pp. 52–66, 2019, doi: 10.1016/j.envint.2018.11.038.

[14] N. J. Ronkainen, H. B. Halsall, and W. R. Heineman, “Electrochemical biosensors,” *Chem. Soc.*

Rev., vol. 39, no. 5, pp. 1747–1763, 2010, doi: 10.1039/b714449k.

[15] A. A. Ensafi, *An introduction to sensors and biosensors*. Elsevier Inc., 2019.

[16] L. M. D. C. Silva, “Desenvolvimento de biossensores eletroquímicos para fenol e uréia com foco na aplicação ambiental,” *Tese Doutorado, Univ. Fed. do Rio Janeiro*, p. 154, 2011.

[17] B. Rezaei and N. Irannejad, *Electrochemical detection techniques in biosensor applications*. Elsevier Inc., 2019.

[18] D. Grieshaber, R. MacKenzie, J. Vörös, and E. Reimhult, “Electrochemical Biosensors - Sensor Principles and Architectures,” *Sensors*, vol. 8, no. March, pp. 1400–1458, 2008, doi: 10.1016/j.talanta.2014.01.002.

[19] A. Chen and B. Shah, “Electrochemical sensing and biosensing based on square wave voltammetry,” *Anal. Methods*, vol. 5, no. 9, pp. 2158–2173, 2013, doi: 10.1039/c3ay40155c.

[20] R. A. Osteryoung and M. T. Carter, “Pulse voltammetry,” *J. Chem. Educ.*, vol. 60, no. 4, pp. 296–298, 2006, doi: 10.1201/9781439807842.ch8.

[21] K. Scott, *Electrochemical Principles and Characterization of Bioelectrochemical Systems*. Elsevier Ltd., 2016.

[22] G. Denuault, “Electrochemical techniques and sensors for ocean research,” *Ocean Sci.*, vol. 5, no. 4, pp. 697–710, 2009, doi: 10.5194/os-5-697-2009.

[23] F. Lisdat and D. Schäfer, “The use of electrochemical impedance spectroscopy for biosensing,” *Anal. Bioanal. Chem.*, vol. 391, no. 5, pp. 1555–1567, 2008, doi: 10.1007/s00216-008-1970-7.

[24] E. de S. Gil and G. R. de Melo, “Electrochemical biosensors in pharmaceutical analysis,” *Brazilian J. Pharm. Sci.*, vol. 46, no. 3, pp. 375–391, 2010, doi: 10.1590/S1984-82502010000300002.

[25] S. Girousi, “Electrochemical Biosensors; A Promising Tool in Pharmaceutical Analysis,” *Pharm. Anal. Chem.*, vol. 02, no. 01, pp. 2–4, 2016, doi: 10.4172/2471-2698.1000e104.

[26] K. Balakrishna, A. Rath, Y. Praveenkumarreddy, K. S. Guruge, and B. Subedi, “A review of the occurrence of pharmaceuticals and personal care products in Indian water bodies,” *Ecotoxicol. Environ. Saf.*, vol. 137, no. April 2016, pp. 113–120, 2017, doi: 10.1016/j.ecoenv.2016.11.014.

[27] INFARMED I.P., “Medicine and healthcare products statistics,” pp. 1–95, 2014.

[28] G. Shanmugam, S. Sampath, K. K. Selvaraj, D. G. J. Larsson, and B. R. Ramaswamy, “Non-steroidal anti-inflammatory drugs in Indian rivers,” *Environ. Sci. Pollut. Res.*, vol. 21, no. 2, pp. 921–931, 2014, doi: 10.1007/s11356-013-1957-6.

[29] A. Khan *et al.*, “Prevalence of selected pharmaceuticals in surface water receiving untreated sewage in northwest Pakistan,” *Environ. Monit. Assess.*, vol. 190, no. 6, 2018, doi: 10.1007/s10661-018-6683-6.

[30] L. Patrolecco, S. Capri, and N. Ademollo, “Occurrence of selected pharmaceuticals in the principal sewage treatment plants in Rome (Italy) and in the receiving surface waters,” *Environ. Sci. Pollut. Res.*, vol. 22, no. 8, pp. 5864–5876, 2015, doi: 10.1007/s11356-014-3765-z.

[31] P. Paíga *et al.*, “Pilot monitoring study of ibuprofen in surface waters of north of Portugal,” *Environ. Sci. Pollut. Res.*, vol. 20, no. 4, pp. 2410–2420, 2013, doi: 10.1007/s11356-012-1128-1.

[32] A. Lolić, P. Paíga, L. H. M. L. M. Santos, S. Ramos, M. Correia, and C. Delerue-Matos, “Assessment of non-steroidal anti-inflammatory and analgesic pharmaceuticals in seawaters of North of Portugal:

Occurrence and environmental risk," *Sci. Total Environ.*, vol. 508, pp. 240–250, 2015, doi: 10.1016/j.scitotenv.2014.11.097.

[33] G. Y. Aguilar-Lira *et al.*, "New insights on diclofenac electrochemistry using graphite as working electrode," *J. Electroanal. Chem.*, vol. 794, no. January, pp. 182–188, 2017, doi: 10.1016/j.jelechem.2017.03.050.

[34] A. Cernat, M. Tertiş, R. Săndulescu, F. Bedioui, A. Cristea, and C. Cristea, "Electrochemical sensors based on carbon nanomaterials for acetaminophen detection: A review," *Anal. Chim. Acta*, vol. 886, pp. 16–28, 2015, doi: 10.1016/j.aca.2015.05.044.

[35] V. Supalkova *et al.*, "Electrochemical sensors for detection of acetylsalicylic acid," *Sensors*, vol. 6, no. 11, pp. 1483–1497, 2006, doi: 10.3390/s6111483.

[36] M. J. Parnham, *Compendium of Inflammatory Diseases*. 2014.

[37] I. G. David *et al.*, "Voltammetric Determination of Carprofen," *Curr. Anal. Chem.*, vol. 14, no. 1, pp. 13–18, 2016, doi: 10.2174/1573411012666160725162348.

[38] A. K. Youssef and D. A. El-Hady, "Using of In-Situ Mercury Film Sensor Hyphenated with Affinity Voltammetry for High Throughput Drug-Protein Binding Studies," *Am. J. Anal. Chem.*, vol. 04, no. 04, pp. 159–165, 2013, doi: 10.4236/ajac.2013.44021.

[39] S. Abbasi and S. Zadkhast, "A new method for determination of fenoprofen in urine and human plasma by differential pulse adsorptive stripping voltammetry," *Anal. Chem. an indian J.*, vol. 13, no. 1, pp. 69–76, 2016.

[40] R. I. Stefan-Van Staden, R. G. Bokretsion, K. I. Ozoemena, J. F. Van Staden, and H. Y. Aboul-Enein, "Enantioselective, potentiometric memberane electrodes based on different cyclodextrins as chiral selectors for the assay of S-flurbiprofen," *Electroanalysis*, vol. 18, no. 17, pp. 1718–1721, 2006, doi: 10.1002/elan.200603574.

[41] A. A. Bunaciu, A. Grasu, and H. Y. Aboul-Enein, "Pharmaceutical applications of a flurbiprofen sensor," *Anal. Chim. Acta*, vol. 311, no. 2, pp. 193–197, 1995, doi: 10.1016/0003-2670(95)00165-V.

[42] N. Serrano, Ò. Castilla, C. Ariño, M. S. Diaz-Cruz, and J. M. Díaz-Cruz, "Commercial screen-printed electrodes based on carbon nanomaterials for a fast and cost-effective voltammetric determination of paracetamol, ibuprofen and caffeine in water samples," *Sensors (Switzerland)*, vol. 19, no. 18, 2019, doi: 10.3390/s19184039.

[43] Ľ. Švorc, I. Strežová, K. Kianičková, D. M. Stanković, P. Otřísal, and A. Samphao, "An advanced approach for electrochemical sensing of ibuprofen in pharmaceuticals and human urine samples using a bare boron-doped diamond electrode," *J. Electroanal. Chem.*, vol. 822, pp. 144–152, 2018, doi: 10.1016/j.jelechem.2018.05.026.

[44] B. Mekassa, M. Tessema, B. S. Chandravanshi, and M. Tefera, "Square wave voltammetric determination of ibuprofen at poly(L-Aspartic Acid) modified glassy carbon electrode," *IEEE Sens. J.*, vol. 18, no. 1, pp. 37–44, 2018, doi: 10.1109/JSEN.2017.2769137.

[45] T. F. A. Sousa, C. G. Amorim, M. C. B. S. M. Montenegro, and A. N. Araújo, "Cyclodextrin based potentiometric sensor for determination of ibuprofen in pharmaceuticals and waters," *Sensors Actuators, B Chem.*, vol. 176, no. May 2014, pp. 660–666, 2013, doi: 10.1016/j.snb.2012.09.016.

[46] V. J. Nagaraj, M. Jacobs, K. M. Vattipalli, V. P. Annam, and S. Prasad, "Nanochannel-based electrochemical sensor for the detection of pharmaceutical contaminants in water," *Environ. Sci.*

Process. Impacts, vol. 16, no. 1, pp. 135–140, 2014, doi: 10.1039/c3em00406f.

[47] L. Amankwa and L. G.Chatten, "Electrochemical Reduction Of Ketoprofen And Its Determination In Pharmaceutical Dosage Forms By D.C. Polarography," *Analyst*, vol. 19, no. 3–4, pp. 453–459, 1984, doi: 10.1080/00032718608064510.

[48] K. M. Emara, A. M. M. Ali, and N. Abo El Maali, "The polarographic behaviour of ketoprofen and assay of its capsules using spectrophotometric and voltammetric methods," *Talanta*, vol. 41, no. 5, pp. 639–645, 1994, doi: 10.1016/0039-9140(94)80042-1.

[49] M. M. Ghoneim and A. Tawfik, "Voltammetric studies and assay of the anti-inflammatory drug ketoprofen in pharmaceutical formulation and human plasma at a mercury electrode," *Can. J. Chem.*, vol. 81, no. 8, pp. 889–896, 2003, doi: 10.1139/v03-092.

[50] J. Lenik, C. Wardak, and B. Marczewska, "Ketoprofen ion-selective electrode and its application to pharmaceutical analysis," *Acta Pol. Pharm. - Drug Res.*, vol. 63, no. 4, pp. 239–244, 2006.

[51] Z. Kormosh, I. Hunka, Y. Bazel, and O. Matviychuk, "Potentiometric determination of ketoprofen and piroxicam at a new PVC electrode based on ion associates of Rhodamine 6G," *Mater. Sci. Eng. C*, vol. 30, no. 7, pp. 997–1002, 2010, doi: 10.1016/j.msec.2010.04.015.

[52] Z. Cebula, P. Niedziałkowski, and T. Ossowski, "Electrochemical behavior and determination of ketoprofen at glassy--carbon electrode," *Appl. Biosci.*, vol. 1, no. 3, pp. 7–8, 2018, doi: 10.31708/spi3.2018/ceb.cns18.

[53] B. Mutharani, P. Ranganathan, S. M. Chen, and C. Karuppiah, "Simultaneous voltammetric determination of acetaminophen, naproxen, and theophylline using an in-situ polymerized poly(acrylic acid) nanogel covalently grafted onto a carbon black/La2O3 composite," *Microchim. Acta*, vol. 186, no. 9, 2019, doi: 10.1007/s00604-019-3752-7.

[54] H. A. M. Hendawy, W. M. Salem, M. S. Abd-Elmonem, and E. Khaled, "Nanomaterial-Based Carbon Paste Electrodes for Voltammetric Determination of Naproxen in Presence of Its Degradation Products," *J. Anal. Methods Chem.*, vol. 2019, 2019, doi: 10.1155/2019/5381031.

[55] S. Tarahomi, G. H. Rounaghi, and L. Daneshvar, "A novel disposable sensor based on gold digital versatile disc chip modified with graphene oxide decorated with Ag nanoparticles/Β-cyclodextrin for voltammetric measurement of naproxen," *Sensors Actuators, B Chem.*, vol. 286, no. January, pp. 445–450, 2019, doi: 10.1016/j.snb.2019.01.131.

[56] R. H. O. Montes *et al.*, "Size effects of multi-walled carbon nanotubes on the electrochemical oxidation of propionic acid derivative drugs: Ibuprofen and naproxen," *J. Electroanal. Chem.*, vol. 775, pp. 342–349, 2016, doi: 10.1016/j.jelechem.2016.06.026.

[57] F. Afzali, G. Rounaghi, M. H. A. Zavar, and N. Ashraf, "Supramolecular β-cyclodextrin/multi-walled carbon nanotube paste electrode for amperometric detection of naproxen," *J. Electrochem. Soc.*, vol. 163, no. 3, pp. B56–B61, 2016, doi: 10.1149/2.0521603jes.

[58] C. Baj-Rossi, T. Rezzonico Jost, A. Cavallini, F. Grassi, G. De Micheli, and S. Carrara, "Continuous monitoring of Naproxen by a cytochrome P450-based electrochemical sensor," *Biosens. Bioelectron.*, vol. 53, pp. 283–287, 2014, doi: 10.1016/j.bios.2013.09.058.

[59] A. Cavallini, S. Carrara, G. De Micheli, and V. Erokhin, "P450-mediated electrochemical sensing of drugs in human plasma for personalized therapy," *6th Conf. Ph.D. Res. Microelectron. Electron. PRIME 2010*, no. August, 2010.

[60] B. D. Bozic, M. L. Avramov Ivic, N. P. Trisovic, S. D. Petrovic, and G. S. Uscumlic,

"Electrochemical characterization of oxaprozin on bare gold electrode and electrode modified with bovine serum albumin," *Int. J. Electrochem. Sci.*, vol. 7, no. 11, pp. 11609–11616, 2012.

[61] A. M. M. Ali, K. M. Emara, and M. Khodari, "Quantification of tiaprofenic acid using voltammetric and spectrophotometric techniques," *Analyst*, vol. 119, no. 5, pp. 1071–1074, 1994, doi: 10.1039/AN9941901071.

[62] A. M. Kannan, V. Renugopalakrishnan, S. Filipek, P. Li, G. F. Audette, and L. Munukutla, "Bio-batteries and bio-fuel cells: Leveraging on electronic charge transfer proteins," *J. Nanosci. Nanotechnol.*, vol. 9, no. 3, pp. 1665–1678, 2009, doi: 10.1166/jnn.2009.SI03.

[63] V. K Mathur and J. Crawford, "Fundamentals of Gas Diffusion Layers in PEM Fuel Cells," in *Recent Trends in Fuell Cell Science and Technology*, S. Basu, Ed. Anamaya Publishers, New Delhi, India, 2007, pp. 116–128.

[64] Fuel Cell Store, "Toray Carbon Paper." [Online]. Available: https://www.fuelcellstore.com/toray-carbon-paper-060. [Accessed: 15-Jun-2020].

[65] A. Choudhury *et al.*, "Effect of electrode surface properties on enhanced electron transfer activity in microbial fuel cells," *Eng. Life Sci.*, vol. 17, no. 2, pp. 186–192, 2017, doi: 10.1002/elsc.201600063.

[66] F. A. Z. a. Alatraktchi, Y. Zhang, and I. Angelidaki, "Nanomodification of the electrodes in microbial fuel cell: Impact of nanoparticle density on electricity production and microbial community," *Appl. Energy*, vol. 116, pp. 216–222, 2014, doi: 10.1016/j.apenergy.2013.11.058.

[67] A. Nandy, M. Sharma, S. V. Venkatesan, N. Taylor, L. Gieg, and V. Thangadurai, "Comparative evaluation of coated and non-coated carbon electrodes in a microbial fuel cell for treatment of municipal sludge," *Energies*, vol. 12, no. 6, pp. 1–15, 2019, doi: 10.3390/en12061034.

[68] Y. Holade, D. P. Hickey, and S. D. Minteer, "Halide-regulated growth of electrocatalytic metal nanoparticles directly onto a carbon paper electrode," *J. Mater. Chem. A*, vol. 4, no. 43, pp. 17154–17162, 2016, doi: 10.1039/c6ta08288b.

[69] A. Koushanpour, N. Guz, M. Gamella, and E. Katz, "Graphene-Functionalized 3D-Carbon Fiber Electrodes – Preparation and Electrochemical Characterization," *Electroanalysis*, vol. 28, no. 9, pp. 1943–1946, 2016, doi: 10.1002/elan.201600110.

[70] J. Ledesma-García, I. L. E. García, T. W. Chapman, F. J. Rodríguez, and L. A. Godínez, "Preparation Of Carbon-Fiber Electrodes Modified With Platinum Nanoparticles Encapsulated In PAMAM Dendrimers," *Electrochem. Soc. Prep.*, 2006, doi: 10.1149/1.2392929.

[71] Y. Fu and A. Manthiram, "Silicon nanoparticles supported on graphitic carbon paper as a hybrid anode for Li-ion batteries," *Nano Energy*, vol. 2, no. 6, pp. 1107–1112, 2013, doi: 10.1016/j.nanoen.2013.09.004.

[72] S. D. Minteer, P. Atanassov, H. R. Luckarift, and G. R. Johnson, "New materials for biological fuel cells," *Mater. Today*, vol. 15, no. 4, pp. 166–173, 2012, doi: 10.1016/S1369-7021(12)70070-6.

[73] M. J. Syu and Y. S. Chang, "Ionic effect investigation of a potentiometric sensor for urea and surface morphology observation of entrapped urease/polypyrrole matrix," *Biosens. Bioelectron.*, vol. 24, no. 8, pp. 2671–2677, 2009, doi: 10.1016/j.bios.2009.01.036.

[74] C. J. McNeil, J. M. Cooper, and J. A. Spoors, "Amperometric enzyme electrode for determination of theophylline in serum," *Biosens. Bioelectron.*, vol. 7, no. 5, pp. 375–380, 1992, doi: 10.1016/0956-5663(92)85034-8.

[75] S. L. Maltzman and S. D. Minteer, "Mitochondrial-based voltammetric sensor for pesticides," *Anal. Methods*, vol. 4, no. 5, pp. 1202–1206, 2012, doi: 10.1039/c2ay05946k.

[76] T. Wang, R. C. Reid, and S. D. Minteer, "A Paper-based Mitochondrial Electrochemical Biosensor for Pesticide Detection," *Electroanalysis*, vol. 28, no. 4, pp. 854–859, 2016, doi: 10.1002/elan.201500487.

[77] M. Ferreira *et al.*, "Electrochemical degradation of Diclofenac on catalysts based on CNT and M / CNT modified electrodes," pp. 499–500, 2018.

[78] M. C. Radulescu, M. P. Bucur, B. Bucur, and G. L. Radu, "Ester flavorants detection in foods with a bienzymatic biosensor based on a stable Prussian blue-copper electrodeposited on carbon paper electrode," *Talanta*, vol. 199, no. February, pp. 541–546, 2019, doi: 10.1016/j.talanta.2019.02.094.

[79] V. Cerdrá, "A Britton-Robinson buffer of known ionic strangth," no. January 1974, p. 5, 2017.

[80] A. Shrivastava and V. Gupta, "Methods for the determination of limit of detection and limit of quantitation of the analytical methods," *Chronicles Young Sci.*, vol. 2, no. 1, p. 21, 2011, doi: 10.4103/2229-5186.79345.

[81] J. N. Miller and J. C. Miller, *Statistics and Chemometrics for Analytical Chemistry*, no. 6. 2010.

[82] R. S. Nicholson, "Theory and Application of Cyclic Voltammetry for Measurement of Electrode Reaction Kinetics," *Anal. Chem.*, vol. 37, no. 11, pp. 1351–1355, 1965, doi: 10.1021/ac60230a016.

[83] S. U. Pedersen and K. Daasbjerg, "Electrochemical Techniques," in *Electron Transfer in Chemistry*, V. Balzani, Ed. 2001, pp. 422–502.

[84] N. Elgrishi, K. J. Rountree, B. D. McCarthy, E. S. Rountree, T. T. Eisenhart, and J. L. Dempsey, "A Practical Beginner's Guide to Cyclic Voltammetry," *J. Chem. Educ.*, vol. 95, no. 2, pp. 197–206, 2018, doi: 10.1021/acs.jchemed.7b00361.

[85] N. Aristov and A. Habekost, "Cyclic Voltammetry - A Versatile Electrochemical Method Investigating Electron Transfer Processes," *World J. Chem. Educ. Vol. 3, 2015, Pages 115-119*, vol. 3, no. 5, pp. 115–119, 2015, doi: 10.12691/WJCE-3-5-2.