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Electrochemical sensors for therapeutic drug monitoring of antibiotics

Final degree thesis

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Acknowledgments

This work is a reflection of several months of work not only mine, but also of the people around me. That is why I wanted to thank each one of them for their support and effort, which have been fundamental for the completion of this thesis.

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In conclusion, I would like to thank the people who have been there during the development of this project. Your collaboration has been fundamental.

Abstract

The electrochemical analysis of samples to detect the presence of antibiotics plays an important role nowadays. Antibiotics are key drugs for our society since they have helped to cope with and reduce the impact of infections. More and more people are developing resistance to some antibiotics due to their overuse or misuse, and this is causing a great problem for treating infections. This is why the potential to quantify antibiotics almost in real-time by electrochemical detection is so important. This study focuses on assessing the performance of electrochemical sensors for the detection of two antibiotics, such as vancomycin and meropenem. Next, preliminary results are shown for measurements performed in both artificial sweat and artificial interstitial fluid, including the linear dynamic range for each case. The study concludes by discussing the limitations observed for vancomycin detection in buffer, artificial sweat and artificial interstitial fluid, and the promising perspectives for meropenem detection in artificial sweat.

Keywords: *antibiotics, vancomycin, meropenem, electrochemical measurements, artificial sweat and artificial interstitial fluid*

Resumen

El análisis electroquímico de muestras para detectar la presencia de antibióticos desempeña un papel importante en la actualidad. Los antibióticos son medicamentos clave para nuestra sociedad, ya que han ayudado a hacer frente y reducir el impacto de las infecciones. Cada vez más personas están desarrollando resistencia a algunos antibióticos debido a su uso excesivo o incorrecto, lo que está causando un gran problema para el tratamiento de las infecciones. Por eso es tan importante la posibilidad de cuantificar antibióticos casi en tiempo real mediante detección electroquímica. Este estudio se centra en evaluar el rendimiento de sensores electroquímicos para la detección de dos antibióticos, la vancomicina y el meropenem. A continuación, se muestran los resultados preliminares de las mediciones realizadas tanto en sudor artificial como en fluido intersticial artificial, incluyendo el rango dinámico lineal para cada caso. El estudio concluye discutiendo las limitaciones observadas para la detección de vancomicina en tampón, sudor artificial y fluido intersticial artificial, y las perspectivas prometedoras para la detección de meropenem en sudor artificial.

Palabras clave: *antibióticos, vancomicina, meropenem, medidas electroquímicas, sudor artificial y fluido intersticial artificial.*

Outline

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Structure of the project

This study is focused on the electrochemical detection of two antibiotics, vancomycin and meropenem. The work has been divided into five main parts, the first part of introduction, the second part of materials and methods, the third part of results and discussion, the fourth part of conclusions, and the last part of future work.

In the Introduction, we find an overview of the two antibiotics, the group to which they belong, their modus operandi and the importance of both and antibiotics in general.

As a second section, we find the Materials and methods used in this work. Both, the devices, and the chemical compounds used can be found. In addition, it is accompanied by a description of the three electrochemical methods, cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV) used in this study.

For the third section, Results and discussion, we have the discussion of the different processes carried out and the results in each section. Results for the analysis of both vancomycin and meropenem in phosphate buffer saline (PBS), artificial sweat and artificial interstitial fluid are discussed.

For the Conclusion, the objectives are answered with the results obtained in each section. In addition, there is also a general conclusion of the work.

Finally, Future work, allows us to present possible ways to move forward in the detection of both vancomycin and meropenem once this study has been completed.

1 Introduction

The discovery and introduction of antibiotics in our lives marked a before and after in our society. Indeed, these are very necessary drugs nowadays since they allowed us to face and reduce the impact of many bacterial infections. Their objective is to fight against bacterial infections by killing them or blocking their reproduction by helping the immune system to fight them. However, they have been overused and misused, what has contributed to the emergence of antimicrobial resistance (AMR). To fight AMR, detecting antibiotics in bodily fluids to guide the administration of the right doses, is key. This project is focused on the detection of two antibiotics, vancomycin and meropenem, which are commonly used to treat a wide range of bacterial infections.

On the one hand, there is vancomycin. It is an antibiotic belonging to the glycopeptide group. In this same group, telavancin, oritavancin, teicoplanin, and dalbavancin can also be seen. Their principal function is to inhibit the synthesis of the bacterial cell wall [1].

Vancomycin is mainly used to treat infections of the intestine caused by bacteria (colitis). In fact, it only attacks bacteria found in the intestine, which is its only site of action if orally administered. Figure 1 shows the chemical structure of this antibiotic.

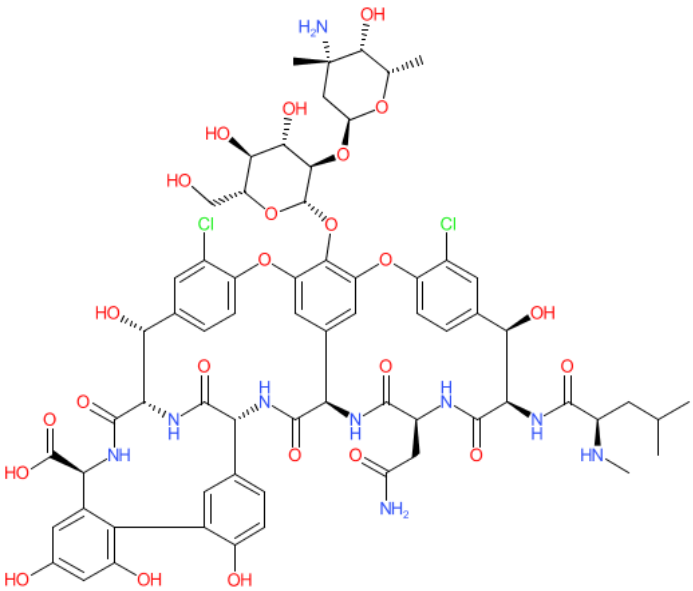

Figure 1: Chemical structure of Vancomycin. Extracted from: *[2]*

Bacteria to maintain their shape and protect themselves from external factors, have a cellular layer of peptidoglycan, an exclusive element of these organisms and a key element in their structure. Indeed, peptidoglycan is a compound whose function is to ensure the rigidity and configuration of the bacterial cell wall. Its composition is based on sugars and amino acids. Depending on whether the bacterium is Gram-positive or Gram-negative, it will be found in one place or another. That is, for Gram-positive bacteria this layer is located above the plasma membrane while for Gram-negative bacteria it is located between the outer membrane and the plasma membrane [3].

In more detail, a peptidoglycan is composed of long polymers of N-acetylmuranic acid (NAM) and N-acetylglucosamine (NAG), which are sequences of two sugars [4] and of a tetrapeptide (a compound classified as an oligopeptide since it has only four amino acids linked by peptide bonds [5]). The tetrapeptide is composed of the following amino acids: L-alanine, D-glutamic acid, L-lysine (for Gram positive) or meso-diamino-pimelic acid (for Gram negative and for certain Gram positive) and D-alanine. In short, their conformation is based on: NAG and NAM covalently bound, and NAM also bound to the tetrapeptide. In some cases, depending on the bacterial group, a linkage may appear between the different tetrapeptides. In other cases, the linkage is made directly between the amino acids [3]. All this can be seen in Figure *2*, in which a scheme represents the peptidoglycan layer of bacteria.

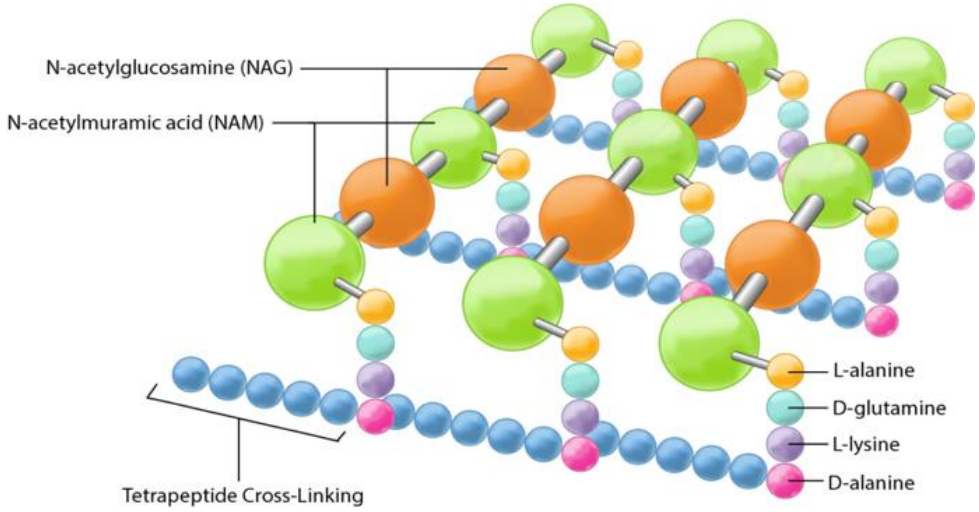

Figure 2: Scheme representing the peptidoglycan layer. Extracted from: *[6]*

The mechanism of action of vancomycin is based on affecting the peptidoglycan. As seen, this layer is a unique compound in this type of organism. Therefore, exerting an effect on this structure is very useful as it ensures no collateral damage. In order to affect the bacterial cell wall, the antibiotic binds to the D-alanyl D-alanine of the growing cell wall and inhibits the enzyme glucosyltransferase (peptidoglycan synthase) and the phospholipid transporter [4] (Figure 3). This causes the bacterium to become aware that synthesis is not occurring normally. To resolve this, the cell increases the peptidoglycan precursors which in turn accelerates a feedback loop that activates enzymes that may also contribute to cell disintegration [7]. As a result of all these processes the cell wall loses rigidity. As soon as the cell wants to divide, since the peptidoglycan layer is so weakened, the cell itself is flooded with external liquid and thus eventually disintegrates.

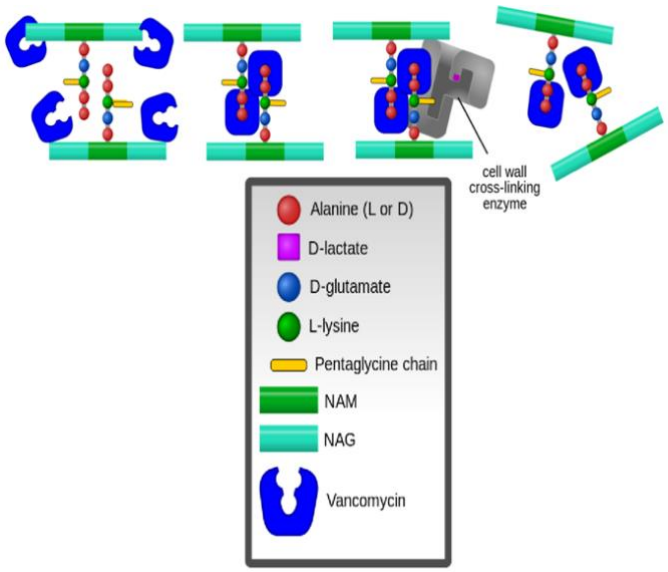

Figure 3: Scheme representing the mechanism of action of vancomycin. Extracted from: *[8]*

An important point to control the effect of this antibiotic by quantifying changes in its concentration is that it can be found in various bodily fluids. But, in each of them it will have a different concentration. Vancomycin concentration in plasma ranges from 3.24 to 5.86 μM [9], for serum and interstitial fluid from 10.3 to 13.8 μM [10] [11], for urine 3.66 μM [12], and for sweat 0.0076 to 0.0815 μM [9].

On the other hand, meropenem belongs to the carabapenem group. Other antibiotics that belong to the same group are: doripenem, ertapenem and imipenem (among others) [13]. The chemical structure of Meropenem is represented in Figure *4*.

This antibiotic, unlike the previous one, can act against a wide range of organisms, whether Gram-positive or Gram-negative. The way it works is the same as vancomycin, it directly affects the synthesis of bacterial cell wall [14].

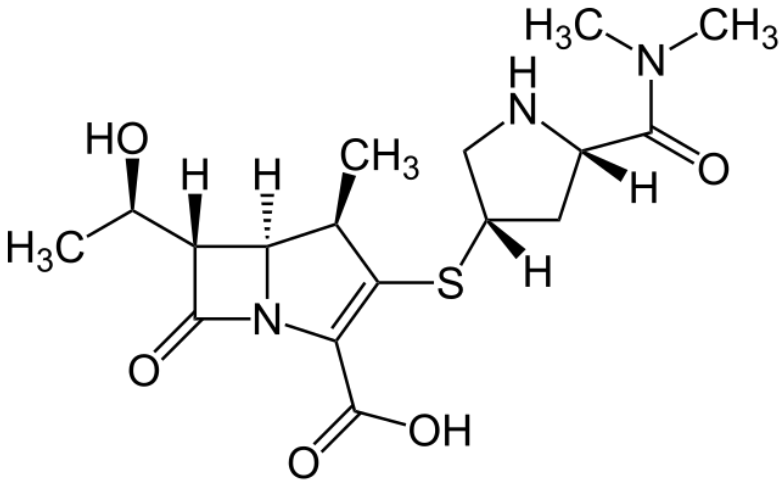

Figure 4: Chemical structure of meropenem. Extracted from: *[15]*

Indeed, this group of antibiotics has the same target of action, attacking the peptidoglycan layer to destabilize the cell. But unlike Vancomycin, this antibiotic also affects gram-negative bacteria.

As has been seen, the peptidoglycan layer in GRAM-positive bacteria is located on the outside of the cell, whereas in GRAM-negative bacteria, it is located under the outer membrane, making access more difficult. In order to reach its destination, the antibiotic uses membrane proteins (OMPs) called porins, anchored to the outer membrane, which allow the entry and exit of the substances *[16]* . Once the antibiotic has crossed the periplasmic space, it inhibits enzymes called penicillin binding proteins (PBP), which are responsible for catalysing peptidoglycans (transglycosylation) and cross-linking between glycan chains (transpeptidation) [17]. By inhibiting this enzyme, the peptidoglycan layer is weakened, and the bacteria eventually die [18] (Figure 5).

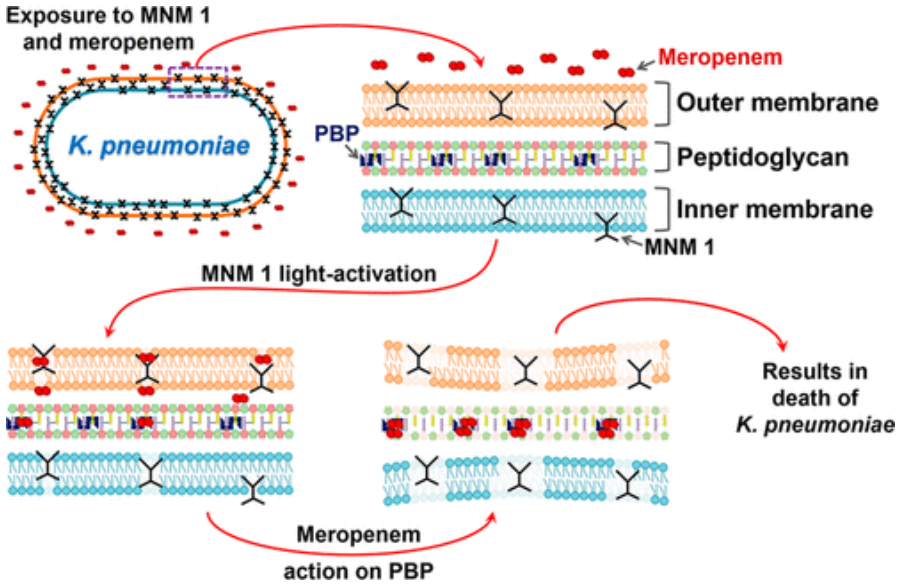

Figure 5: Scheme representing the process action of Meropenem. Extracted from: *[19]*

For the concentration range of Meropenem in different body parts, we have, for plasma and serum 73 to 78,3 µM [20, 21], for interstitial fluid from 0,00836 to 0,0225uM [22] and in sweat is between 0.2 to 2 µM (0.2 to 2.5% of its concentration in serum) [23].

The problem with these substances is that they can directly affect the liver or kidney. In addition, abusive or inappropriate use is creating more and more resistance. Antibiotic resistance means when bacteria mutate in response to an inadequate supply of them. This leads an evolution of the bacteria resulting in no affectation of antibiotics [24]. This problem affects not only the health care system, but also the economy, since it increases the under-supply of drugs, the cost of medical care and designing a new drug, and the length of hospital stay [24].

In order to manage this, there are a number of traditional techniques that allow quantification of antibiotic levels in the individual. Examples include gas chromatography (GC), high-performance liquid chromatography (HPLC) and mass spectroscopy (MS) among others. These techniques offer very good results but have some drawbacks. Most of them require a prepared and sterile laboratory, laborious methods, and expensive instruments. In addition, professional equipment and usually sample pretreatment are also needed [25].

One of these possible solutions would be quantifying the levels of this substances in people with the help of electrochemical sensors. This type of sensors are defined as chemical sensors, which use an electrode as transducer and a compound as analyte. They allow the analysis of redox reactions in solutions [26].

In recent years, the electrochemical quantification methods have shown enormous potential for measuring antibiotic concentrations. That is why these techniques have been used to quantify the antibiotics in this study.

These are based on generating electrical stimulation in solutions to analyse their chemical reactivity. A potential is applied into the solution and the resulting current is measured to obtain the reaction rates [27].

The benefits of these techniques are numerous. Among them, we can highlight the speed with which samples are analysed, the ease with which the method can be used, and the low cost involved [28]. Another advantage of this technique is that it allows the analysis of different analytes at the same time. It provides both quantitative and qualitative information as it determines the compounds that have undergone redox reactions and the amount that has reacted [29].

Nevertheless, it also has some limitations. One of them is that most electroactive antibiotics have a very similar oxidation potential, making it difficult to differentiate between them. An electroactive compound is a composite that can show some activity when stimulated by an electroactive potential [30].

Another disadvantage may be the occurrence of the passivation phenomenon. Passivation happens when a film is formed, in our case, around the electrodes due to a fouling agent. This film means that the process of applying potential cannot be repeated if we want to obtain the same result as the previous one with the same electrode. This will cause the current values read, to be lower with each repetition. In short, this phenomenon generates electrodes with less sensitivity, non-reproducible and, above all, unreliable results. Therefore, an electrode for each measurement had to be uses for doing measurements.

The focus of this study is on recent achievements in the development of electrochemical sensors for antibiotic detection. The following sections will provide the methods used such as Electrochemical methods such as Cyclic Voltammetry (CV), Differential Pulse Voltammetry (DPV) and Square Wave Voltammetry, with the antibiotics in different matrixes. Finally, an explanation of the results obtained.

1.1 Motivation

To understand the motivation of this work, it is first necessary to put ourselves in context with our current situation. Approximately 33,000 people die each year in Europe because of an untreatable disease due to antibiotic resistance. In Spain, a total of 4,000 people per year. Indeed, they say it is a silent pandemic [31].

In order to address this, a national plan called the *National Antibiotic Resistance Plan* (PRAN) was created. This was put in place in 2014 and thanks to it Spain ranks as the sixth country with the greatest reduction in antibiotic use of the 25 countries that have reported results to the *European Centre Disease Prevention and Control* (ECDPC). Even so, the mortality rate is still remarkable. This is why new technologies are being used to reduce this rate to a minimum.

But, not only does it affect avoiding antibiotic resistance, but it could also help people with other anomalies. For example, with liver problems. In order to eliminate antibiotics from the body, they must pass through this organ. Antibiotics are toxic compounds so, if the liver is not normal, an abusive or increased use could lead to serious complications. [32].

Also, an excessive administration of antibiotics could promote the development of colon cancer [33], being the third most deadly type of cancer in both men and women. [34].

This new tool would make it possible to obtain patient diagnoses in real time and to be able to react to the situation by offering a treatment adapted to the patient. It would also be possible to determine bacterial sensitivity by analyzing the concentration needed to inhibit bacterial growth and thus see if it is higher than usual. Another possible option would be to monitor the evolution of antibiotics in a person and be able to determine if it is being effective. In addition, it can also help to determine the concentration in different tissues after administration to subsequently optimize the dose depending on the site of infection.

To be able to solve this, the sensors would use the concept of *Therapeutic Drug Monitoring* (TDM) which refers to the fact of measuring concentrations of a drug (either antibiotics or heart drugs among others) in blood [35]. Thanks to the combination of therapeutic drug monitoring and medicine we will help people involved with this problem. In addition, it will reduce the number of citizens involved.

1.2 Objectives

In this work electrochemical sensors were developed for direct and rapid electrochemical detection of Vancomycin and Meropenem. For this aim, carbon screen printed electrodes were used and measurements were performed in buffer, artificial sweat and artificial interstitial fluid. The main objectives of this work are as follow:

1. Direct and rapid electrochemical quantification of Vancomycin and Meropenem in buffer
2. To study the effect of electrode fouling on electrochemical quantification of both antibiotics
3. To study the matrix effect on the electrochemical quantification of both antibiotics by performing measurements in artificial sweat and interstitial fluid
4. To check the feasibility of using the developed sensors for quantification of both antibiotics in biofluids considering the concentration level of the antibiotic in different biofluids

2 Materials and methods

2.1 Materials and chemicals

Sodium hydroxide (NaOH), potassium ferrocyanide ($C_6FeN_6^{-4}$), potassium ferricyanide ($C_6FeN_6^{-3}$), Phosphate Buffer Saline (PBS), urea, lactic acid, sodium chloride (NaCl), calcium chloride ($CaCl_2$), 4-(2-Hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES), potassium chloride (KCl), magnesium sulphate ($MgSO_4$), sodium phosphate monobasic (NaH_2PO_4) and sucrose ($C_{12}H_{22}O_{11}$) were provided by Sigma-Aldrich. Vancomycin and meropenem were supplied by Lab. Reig Jofre, S.A.

2.2 Instrumentation

A CompactStat.h potentiostat device from Ivium Technologies was employed for electrochemical measurements, connected to a PC with IviumSoft software. Carbon screen-printed electrodes (C-SPE) were used, which consisted of a three-electrode system with a carbon working electrode, a carbon counter electrode and a silver reference electrode. They were supplied by Quasense. A pH meter from XS instruments was used for adjusting the pH values of the solutions investigated.

2.3 Preparation of artificial bodily fluids

In this project, two different artificial fluids, artificial sweat, and artificial interstitial fluid, were prepared to study the effect of different matrixes on the quantification of the two antibiotics.

2.3.1 Preparation of artificial sweat

Antibiotics metabolites are excreted by different ways, the two principles are through the urine (thanks to the kidney) or through the feces (thanks to the liver) [36]. But they have also been found that sweat can be another way. [37]

The main function of sweat is to regulate temperature through transpiration since evaporation from the skin reduces the temperature. But it also has other functions such as eliminating toxins [38], and as it has been said, antibiotics are toxins, so, it is interesting to analyze the evolution of antibiotics in sweat.

Sweat is a bodily fluid which composition depends on each person. Not everybody's sweat has the same components and pH, it can change from genres, ages or even emotions [39]. This is why, in this work, a general composition has been adopted. On the one hand, it has been determined that generally sweat is composed of water, minerals (sodium, potassium, calcium...), metabolites (lactate, ammonia, urea...) and some non-metabolized pharmaceutical compounds [40].On the other hand, it has been found in numerous studies, that for a man the pH can be equal to 7, while for a woman it can be around 5,6 [41]. Therefore, in this study artificial sweat solutions with pH of 5 or 7 were used. Artificial sweat solutions were prepared by adding 0.1% w/w urea, 0.1% w/w lactic acid and 0.5% w/w NaCl to milliQ (MQ) water [42]. Then the pH was adjusted to either pH 5 or pH 7, using concentrated sodium hydroxide solution.

2.3.2 Preparation of artificial interstitial fluid

Interstitial fluid is the liquid that surrounds the cells. It helps to bring nutritional supply to the cells, either oxygen or nutrients [43] (Figure *6*). In fact, it has several functions, such as bringing compounds into the cells, eliminating wastes, or allowing inter-cellular communication, among others [44]. This fluid is part of what is called extracellular fluid which, added to plasma, makes up 20% of the total body weight [45].

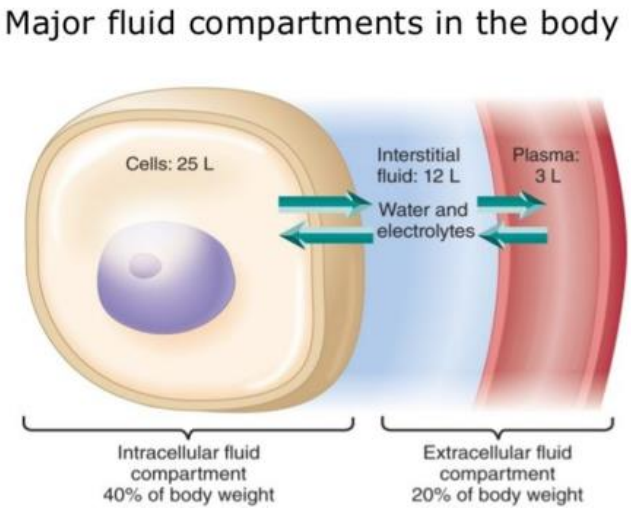

Figure 6: Scheme representing the cell, the interstitial fluid, and plasma and their exchanges. Extracted from: *[45]*

Most infections are found in the extracellular fluid compartment, so analyzing antibiotic concentrations in the interstitial fluid can be very effective. [46].

Generally, interstitial fluid is composed of water, amino acids, sugars, fatty acids, coenzymes, hormones, neurotransmitters, mineral salts and cell products [44]. Regarding pH, there is no variation in interstitial fluid, being always pH 7, so neutral pH. Therefore, it was decided to use the following chemicals to prepare artificial interstitial fluid: 2.5 mM $CaCl_2$, 10 mM HEPES, 3.5 mM KCl, 0.7 mM $MgSO_4$, 123 mM NaCl, 1.5 mM NaH_2PO_4 and 7.4 mM sucrose [47]. Finally, pH was adjusted with NaOH to 7.

2.4 Measurements and techniques

The electrochemical behaviour of vancomycin and meropenem was studied by different voltametric techniques. To achieve this, a measurement system based on an electrochemical cell was used. An electrochemical cell is a device that its able to produce electrical energy from chemical reactions. Some electrochemical cells are supplied by an external energy (e.g., voltage) for increasing and facilitating the reactions. Indeed, the principle of these devices is to transform chemical energy to electrical energy [48].

Our electrochemical cell was composed of three different electrodes: the reference, the working, and the counter electrode (Figure 9). Indeed, through the working and reference electrodes, a potential is applied to the solution. This generates either an oxidation and/or a reduction of the electroactive species present in the solution, measured as a current change between the counter and working electrodes. The electrodes used in this work were single use but offered suitable properties for in the electrochemical analysis of antibiotics.

There are several methods for electrochemical quantification, but Cyclic Voltammetry (CV), Differential Pulse Voltammetry (DPV) and Square Wave Voltammetry (SWV) were selected for this study.

2.4.1 Cyclic Voltammetry

Cyclic voltammetry (CV) is a method that linearly sweeps the voltages applied to the solution and measures the resulting electric current. It is normally used to analyse oxidation and reduction processes in a solution.

Two possible conventions are normally used, US Convention and IUPAC Convention. For the US Convention, it will go from the higher potential to the lower. Nevertheless, the IUPAC Convention will go from the lower to the higher potential [49]. In this project, IUPAC Convention is used.

CV measurements are plotted as cyclic voltammograms (Figure 7 image a). The x-axis of the plot shows the potential applied (chosen by the user, both minimum and maximum), and the y-axis shows the current resulting. The rate with which the potential is swept is called scan rate. The key parameters in a cyclic voltammogram include the anodic peak current (Ipa), the cathodic peak current (Ipc) the oxidation potential (Epa) and the reduction potential (Epc). Ipa refers to the highest current value from the anodic part. This current value is proportional to the concentration of the electroactive species being oxidized. On the other side, Ipc, is the lowest current point from the cathodic part. This current value is proportional to the concentration of the electroactive species being reduced. Regarding to Epa and Epc, Epa indicates at which voltage the analyte becomes oxidized, while Epc indicates the voltage at which the analyte is reduced [50].

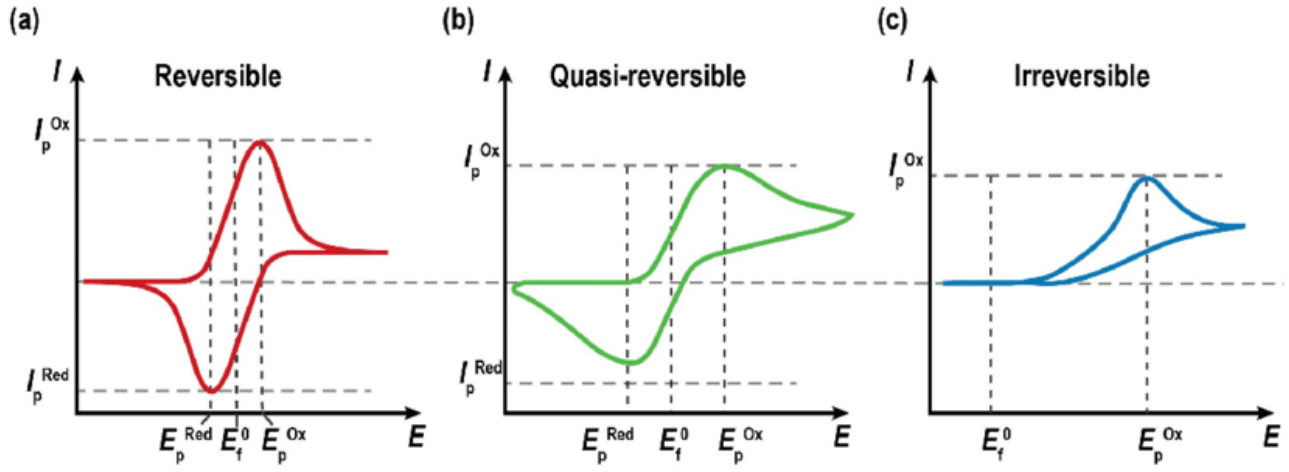

Figure 7. Cyclic voltammograms depicting (a) a reversible, (b) quasi-reversible and (c) irreversible electrochemical reaction. Extracted from: *[51]*

A reversible reaction allows oxidation and reduction. In this case, Ipa and Ipc will have the same value but with opposite signs [52]. An irreversible reaction only allows oxidation or reduction. Vancomycin and meropenem are compounds that undergo irreversible electrochemical reactions.

2.4.2 Differential Pulse Voltammetry

Differential Pulse Voltammetry (DPV) is an electrochemical technique based on applying potential pulses following a linear ramp. This technique uses the combination of linear scans of continuous potential current with pulses that disturb the system's equilibrium. For the anodic part, the potential increases with every pulse, and for the cathodic part, it decreases [53] (Figure 8). In order to extract the final current values, the current is measured at two different points each time. The first measurement is taken at the end of the pulse (S1) and the second at the end of the decay (S2) (Figure 8). The final current value corresponds to the difference between these two points (Equation (1)).

$$\Delta i = S1 - S2 \tag{1}$$

The benefit of this technique over CV is its greater sensitivity. Thanks to the difference of the current values in each case (Δi), the so-called capacitive current is eliminated. In fact, the current of interest, the one generated by electrochemical reactions, is called faradaic current. The capacitive current is known as non-faradaic or double-layer current. It is generated by an accumulation of ions on the electrode surface. The sum of the ions and the charged surface of the electrodes forms a capacitor. The relationship of these is given by the following equation:

$$C = \frac{Q}{E} \tag{2}$$

In this equation, Q is the charge of the ions, E the electrode potential and C the capacitance. [54].

In cases where the voltage applied to the solution is constant (CV), the capacitive current is constant. This can be seen in equation (3).

$$I = \frac{\partial Q}{\partial t} = C * \frac{\partial E}{\partial t} \tag{3}$$

S1 from equation (1) corresponds to the non-faradaic and anodic current, and S2 corresponds to the non-faradaic and cathodic current [55]. By subtracting both, only the anodic and cathodic values are maintained as a result. So, in DPV there is no non-faradaic current.

2.4.3 Square Wave Voltammetry

The last electrochemical method used in this study is Square Wave Voltammetry (SWV). This technique combines a square wave with a pulse from one potential to another, both set by the user (like the two techniques discussed previously). In more detail, this technique contains a staircase waveform in a direct (positive) manner that is followed by a double symmetrical pulse in a reverse (negative) manner. That is, for each step (advancing on the abscissa axis), there is a symmetric double pulse (decreasing on the ordinate axis) [56].

Therefore, SWV follows the same principle as DPV, based on the subtraction of the current at two points (equation (1)). But in this case, the first measurement is at the end of the step (S1) and the second measurement is at the end of the inverse double pulse (S2). (Figure 8). The two are subtracted and the anodic and cathodic currents are obtained as a result [55].

These last two techniques are very similar, both have high sensitivity. The difference is that SWV allows to oxidize and/or reduce the solution with a lower potential range. That is, the vertex2 (maximum voltage value, as far as you want to go, imposed by the user, seen in Table *1*) for SWV is lower than the DPV value. By having a lower maximum value, the analysis in SWV is faster (because it has less range of potential) than in DPV [57].

Each electrochemical technique has its benefits and disadvantages. Depending on the process to be carried out, it is convenient to use one or the other. For this reason, in this study the three methods were used in different situations. Below, in Figure 8 these three methods are presented, how they are executed, and the result expected in each case.

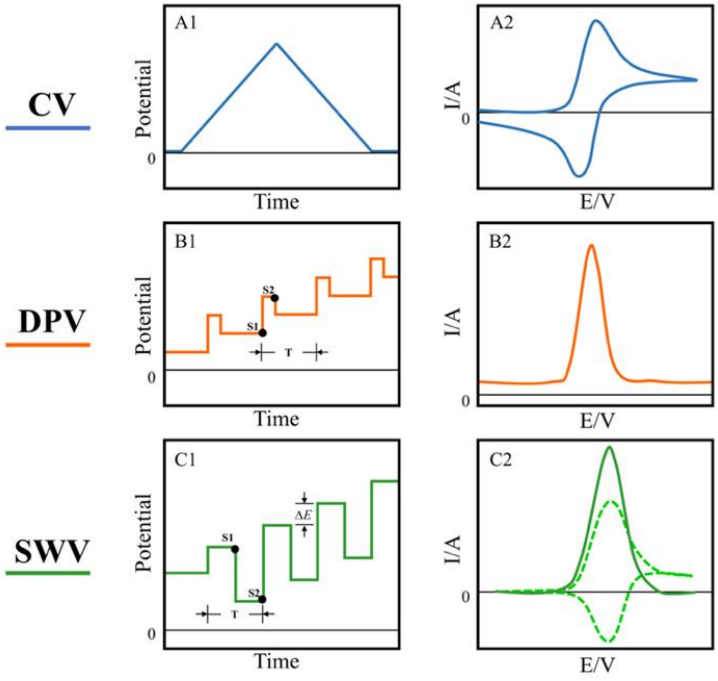

Figure 8: Experimental parameters and expected results for CV, DPV and SWV. (A1) Potential applied over time in CV, (A2) cyclic voltammogram, (B1) potential pulses applied over time in DPV, (B2) differential pulse voltammogram, (C1) potential pulses applied over time in SWV, and (C2) square wave voltammogram. Extracted from: [57]

2.5 Carbon screen-printed electrodes

In this work, carbon screen-printed electrodes were used for all measurements. Figure 9 shows a representation of the electrodes used in this study. In order to carry out all the procedures, carbon electrodes (working and counter) and silver electrodes (reference) were used.

This type of electrode has numerous benefits, such as its low cost, ease of use, good reproducibility, fast manufacturing [58], its speed in extracting the electron from the solution, minimal sample usage (only 100 µL of solution are required) and speed in performing the analysis [59].

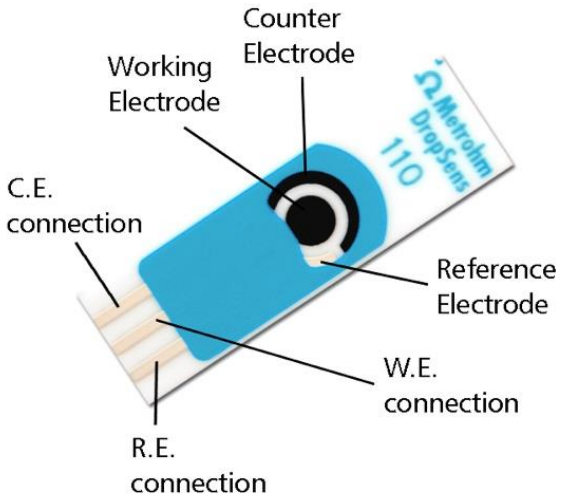

Figure 9: Image representing the electrode used.

Extracted from: [60]

2.6 Activation of electrodes and general procedure

In order to carry out all electrochemical measurements with the electrodes, they must be first subjected to an activation step. The activation of carbon screen-printed electrodes allows improving their sensitivity and reproducibility among electrodes.

For electrode activation, the three electrodes were covered with a 0.5M NaOH solution and 5 CV cycles were applied to the electrode. The activation parameters are given in the **Error! Reference source not found.**, second column. Secondly, once the electrodes were activated, the NaOH solution was removed, and the electrodes were rinsed with water so that no residue remains. To check the electrochemical behaviour and reproducibility of the activated CSPEs, CV measurements were performed in a solution containing 2 mM of ferro/ferricyanide salts prepared in PBS. The CV measurement parameters are given in Table 1 in second column.

Table 1: Parameters for the NaOH activation of CSPEs and their electrochemical characterization in a 2 mM ferro/ferricyanide solution

	0.5 M NaOH	2 mM ferro/ferricyanide
E start (V)	0.0	-0.3
Vertex 1 (V)	2.0	0.8
Vertex2 (V)	0.0	-0.3
E step (mV)	5	5
N scans (cycles)	5	3
Scan rate (mV/s)	100	100
Current Range (mA)	1	1
Scan time (s)	324.1	96.7

3 Results and discussion

3.1 Activation of carbon screen-printed electrodes

As mentioned above, the CSPEs must be activated before use, to improve their sensitivity and reproducibility. Figure *10* shows the cyclic voltammograms obtained from a CSPE in a 2 mM ferro/ferricyanide solution before and after activation in NaOH. Table *2* summarizes the analysed data from the cyclic voltammograms shown in Figure *10*. As this table shows, the anodic and cathodic peak currents increased with electrode activation, while the peak-to-peak potential separation decreased. This confirms the improvement of electrochemical behaviour and therefore sensitivity of the electrodes after activation.

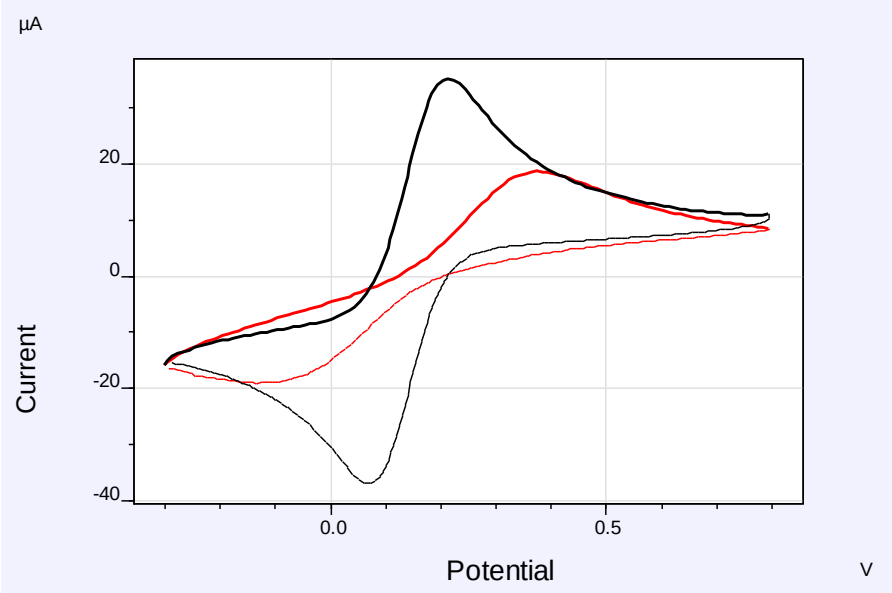

Figure 10: Cyclic voltammograms from an activated CSPE (black) and a non-activated CSPE (red)

Table 2: Oxidation and reduction current and voltages extracted from the cyclic voltammograms shown in Figure 10, for a CSPE before and after activation

	Ipc (µA)	Ipa (µA)	Epc (V)	Epa (V)	ΔEp (V)
before activation	4.75	16.13	-0.12	0.38	0.50
After activation	32.45	39.20	0.06	0.21	0.14

Also, *Figure 11* and *Figure 12* show the cyclic voltammograms of 3 different activated CSPEs measured in a 2 mM ferro/ferricyanide solution before (*Figure 11*) and after (*Figure 12*) activation.

Table *3* shows the analysed data for these measurements. As it can be seen, there is a decrease in RSD% values after activation of the electrodes. This confirms the improvement of the reproducibility of the electrodes using this activation method.

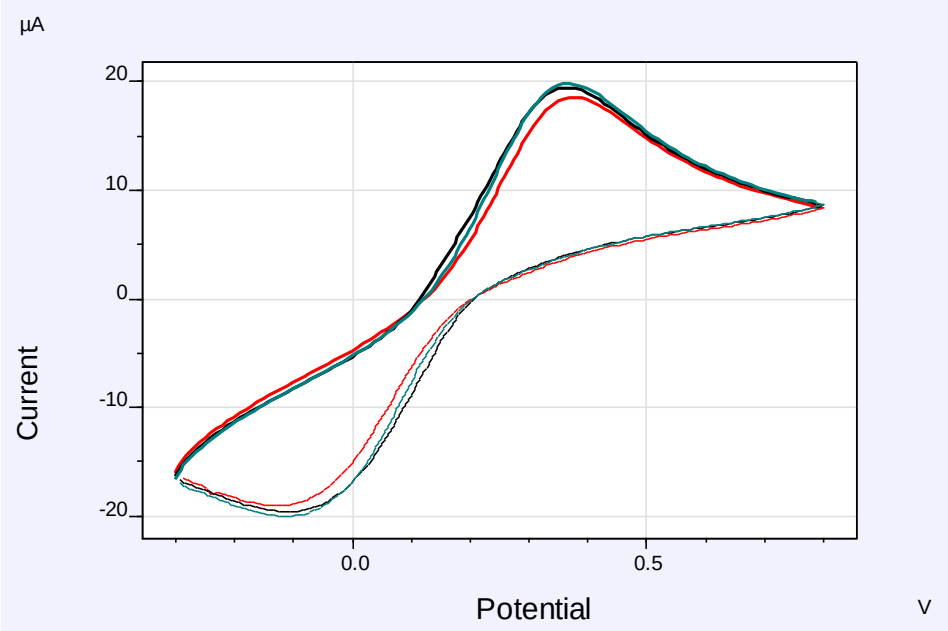

Figure 11: Cyclic voltammograms measured in a 2 mM ferro/ferricyanide solution using 3 different non-activated electrodes

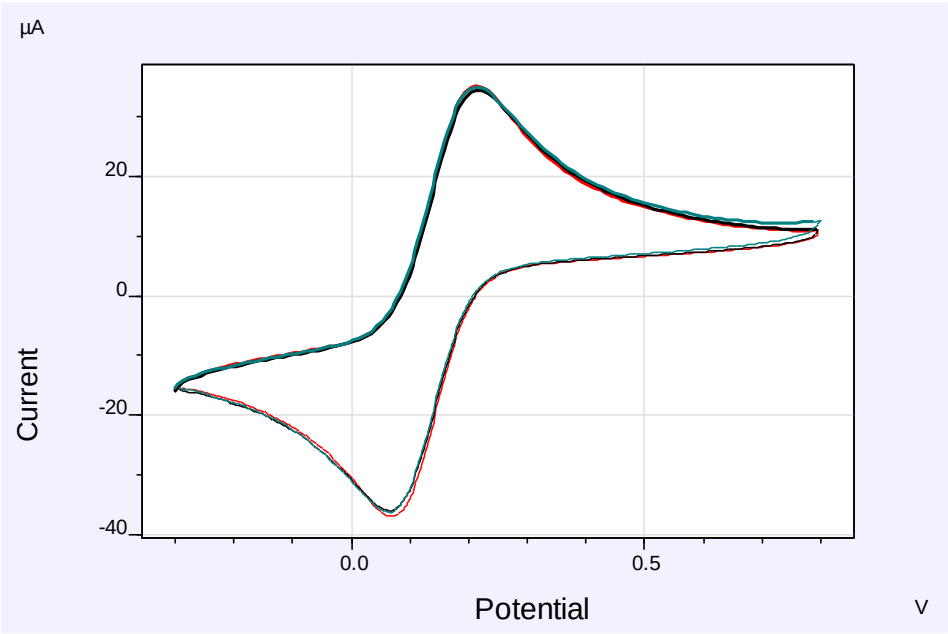

Figure 12: Cyclic voltammograms measured in a 2 mM ferro/ferricyanide solution using 3 different activated electrodes

Table 3: Anodic and cathodic peak current values, and peak-to-peak potential separation values extracted from the cyclic voltammograms in Figure 11 and Figure 12 obtained using three different electrodes, and the relative standard deviation associated to each parameter

	Ipc (μA)		Ipa (μA)		ΔEpa (V)	
	before activation	after activation	before activation	after activation	before activation	after activation
electrode 1	4.75	33.42	16.13	36.63	0.50	0.14
electrode 2	9.62	32.91	17.36	36.87	0.44	0.15
electrode 3	8.61	33.59	18.21	37.01	0.46	0.15
RSD %	2.570	0.354	1.046	0.192	0.030	0.005

3.2 Vancomycin

3.2.1 Optimization of parameters and choosing the electrochemical technique

The first stage in electrochemical quantification of vancomycin, entails selecting the optimum electroanalytical methods and optimizing the measuring parameters to maximize the electrochemical signal.

DPV and SWV are highly sensitive electroanalytical techniques mainly used to detect species present at very small concentrations. For this reason, in this study, both were assessed, in order to determine which of them offered the best results.

For this aim, electrochemical measurements were performed in a 500 μM solution of vancomycin prepared in PBS, using both DPV and SWV techniques. To obtain the best electrochemical signal for each approach, these measurements were made using different parameters. This section describes the outcomes of these optimization steps and the discussion that follows.

3.2.1.1 Differential pulse voltammetry

For the DPV measurements, important parameters are: potential range, pulse time, amplitude, step potential (Estep) and scan rate.

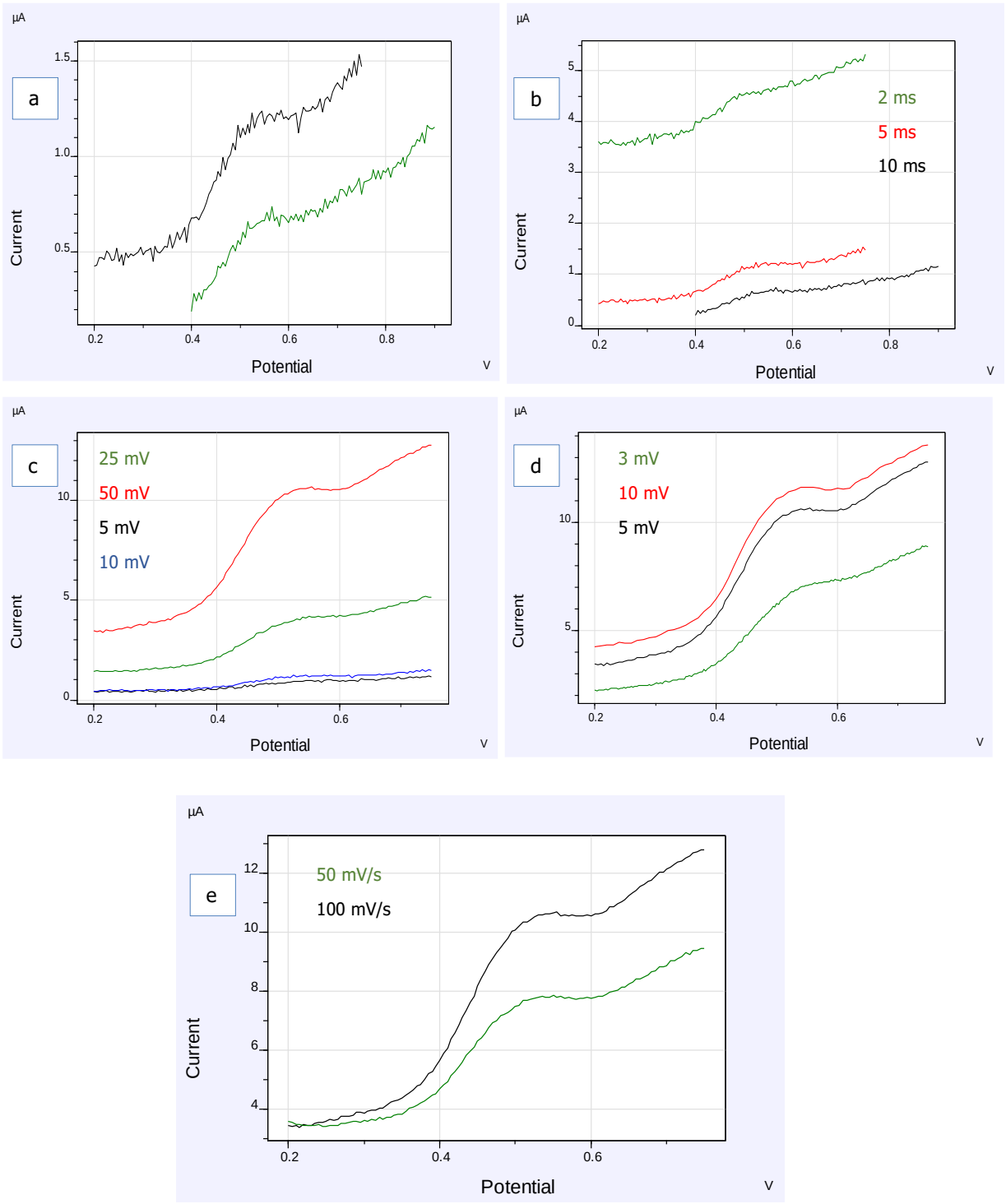

Figure 13: Differential pulse voltammograms obtained for the analysis of a 500 µM vancomycin solution using a CSPE, varying the following parameters: (a) potential range, (b) pulse time, (c) amplitude, (d) step potential, and (e) scan rate

Table *4* summarizes the variables studied and the optimized values of each parameter for DPV measurements in a 500 μM solution.

Different aspects were considered when choosing the optimized parameters. For example, the height and shape of the peak, as well as the baseline current. For each case, the parameter showing the best-defined peak with the highest current value and the lowest baseline current was the one chosen.

Table 4: Different parameters assessed for DPV measurements in a 500 μM vancomycin solution and their optimal values

DPV in Vancomycin	Values assessed	Optimized parameters
Potential range (V)	0.2 - 0.7	0.2 - 0.7
	0.4 – 0.9	
Pulse time (ms)	2	5
	5	
	10	
Amplitude (mV)	5	50
	10	
	25	
	50	
Step potential (mV)	3	5
	5	
	10	
Scanrate (mV/s)	50	100
	100	

3.2.1.2 Square wave voltammetry

Regarding the SWV technique, other parameters were found important and, therefore, analysed: amplitude, step potential and frequency.

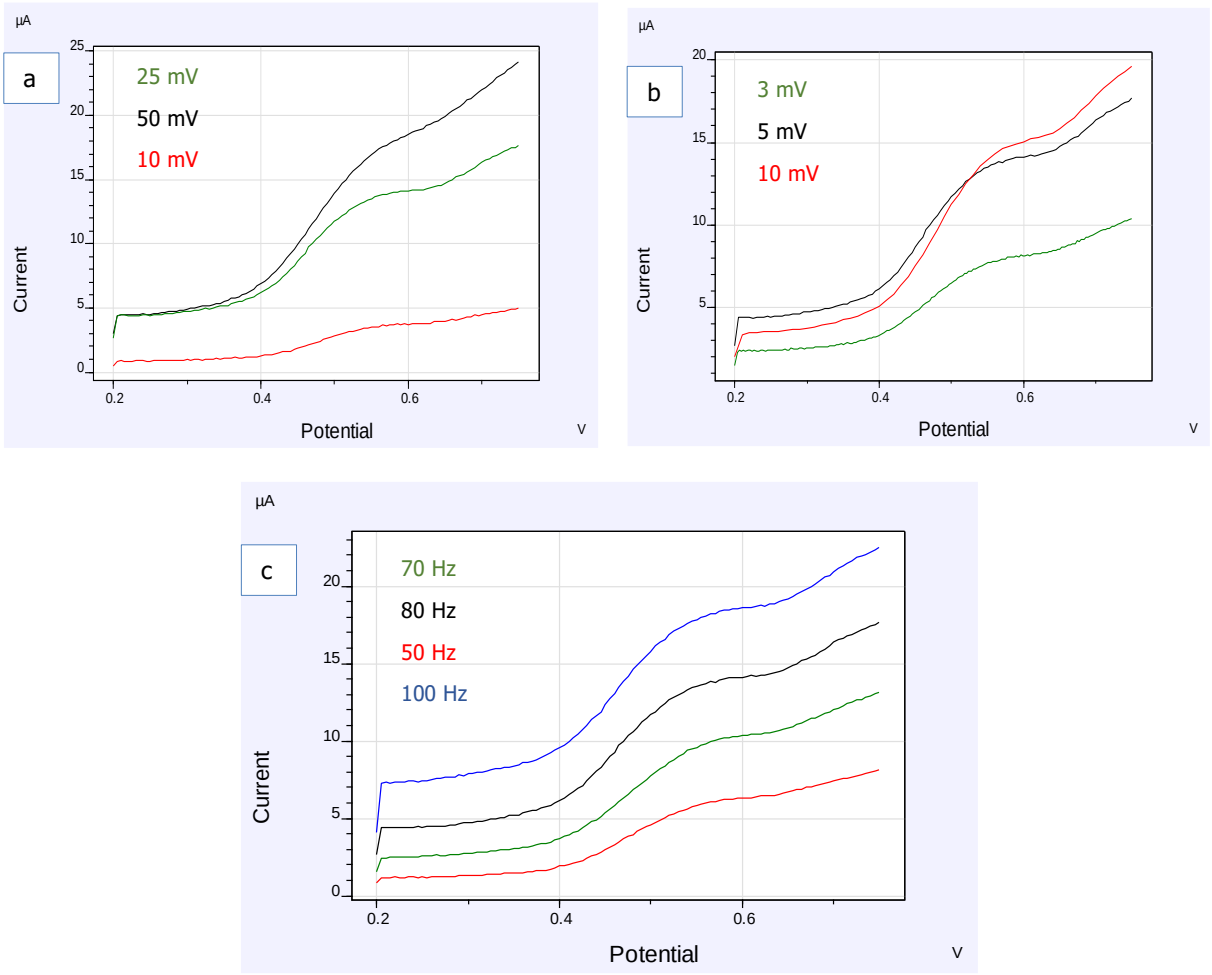

Figure 14: Square wave voltammograms obtained for the analysis of a 500 μM vancomycin solution using a CSPE, varying each parameter analyzed: (a) amplitude, (b) step potential, and (c) frequency

Table 5 provides an overview of the mentioned parameters together with the optimal values for SWV measurements in a 500 μM vancomycin solution.

As for DPV, the value for each parameter that displayed the most clearly defined peak, the highest current value, and the lowest base-line current was the one chosen.

Table 5: Different parameters assessed for SWV measurements in a 500 µM vancomycin solution and their optimal values

SWV in Vancomycin	Values assessed	Optimize parameters
Potential range (V)	0.2 – 0.7	0.2 – 0.7
Amplitude (mV)	10	25
	25	
	50	
Step potential (mV)	3	5
	5	
	10	
Frequency (Hz)	50	80
	70	
	80	
	100	

3.2.1.3 Comparison of DPV and SWV

Once the optimized parameters for each technique were selected, results using both techniques were compared to decide which technique offered the best results. Therefore, the results from both techniques were plotted with their respective parameters. Results are shown in Figure *15*.

At first glance, SWV offered higher current values. However, the peak in this case was not well-defined and the base-line current was higher. As a result, the DPV method was determined to be the best method for sensing vancomycin.

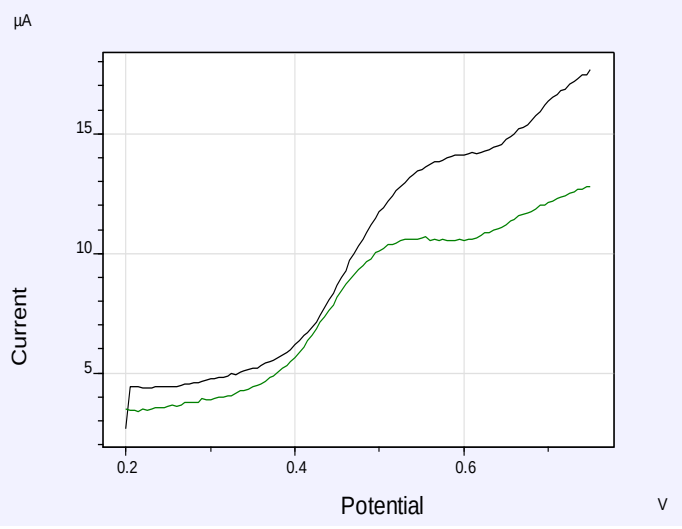

Figure 15: Measurement of vancomycin using CSPE with two techniques, DPV (green) and SWV (black)

3.2.2 Sensing in buffer

To determine the optimal working range of vancomycin concentrations, discarding concentrations where the results were not relevant, a calibration curve was plotted. In addition, this step aimed at determining whether the passivation phenomenon was present. In order to do this, three different strategies were carried out.

Electrochemical measurements for the three approaches were performed in different concentrations of vancomycin prepared in PBS, using DPV method with the optimized parameters. The concentrations were: 1, 2, 5, 10, 25, 50, 75, 100, 250 and 500 µM. This section explains the outcomes of the three different strategies.

3.2.2.1 Strategy 1: one new electrode for each concentration

For the first strategy, the aim was to measure the current value for each concentration. For this purpose, a new activated electrode was used for measuring the current at each vancomycin concentration tested. This process was repeated three times to assess reproducibility and to see how relevant the results were. Results are shown in Figure *16*, where current values in µA are plotted as a function of the logarithmic concentration of vancomycin in µM.

This graph allowed us to analyze several aspects. Firstly, the linearity of the results in the range between 1 µM and 25 µM. Secondly, it was observed that current values decreased from 50 µM onwards. This was attributed to the significant chemical passivation of the electrode, that occurs for vancomycin solutions at concentrations higher than 50 µM.

Figure *17* displays three differential pulse voltammograms obtained for vancomycin concentrations of 2 µM, 100 µM and 500 µM. As figure shows, the oxidation potential shifted towards higher values by increasing vancomycin concentration. This means the electron transfer reaction was becoming more and more difficult. This could be due to the passivation and fouling of the electrode surface at high concentrations of vancomycin. Therefore, the optimal working range of vancomycin concentration was chosen as from 1 µM to 25 µM.

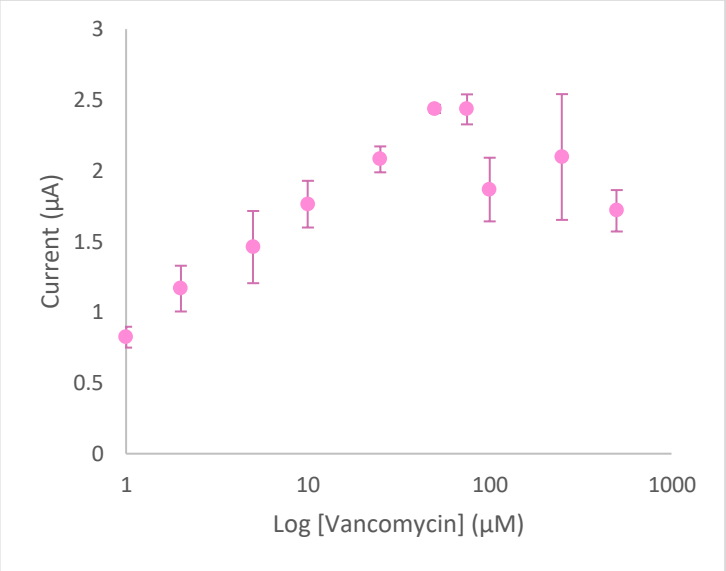

Figure 16: Calibration curve that plots the current measured (µA) as a function of the logarithmic concentration of vancomycin solution (µM). The values are the average of three measurements using three different electrodes, and error bars are SD values for these measurements.

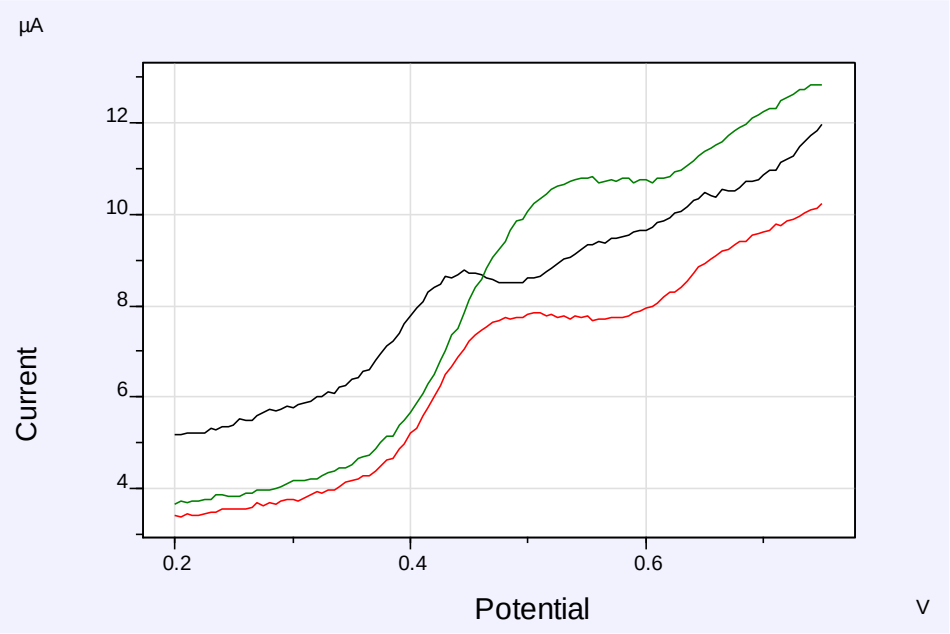

Figure 17: Three DPV measurements using CSPE at three different vancomycin concentrations, 2 µM (black), 100 µM (red) and 500 µM (green).

3.2.2.2 Strategy 2: one electrode for all concentrations

For the second strategy, the objective was to see if there was electrochemical passivation phenomenon. As a mean to achieve this, a single activated electrode was used for measurements at all concentrations. The process was repeated three times using three different electrodes to obtain more relevant results and analyze the reproducibility.

Figure *18* depicts the measured values of the current in µA as a function of the logarithmic concentration of vancomycin in µM when a single electrode was used.

As figure shows, there was a decrease in the measured current values when the vancomycin concentration increased, which confirmed the strong electrochemical passivation that arises when performing consecutive measurements in vancomycin solutions. These results confirmed that it is not possible to re-use electrodes for electrochemical measurements of vancomycin.

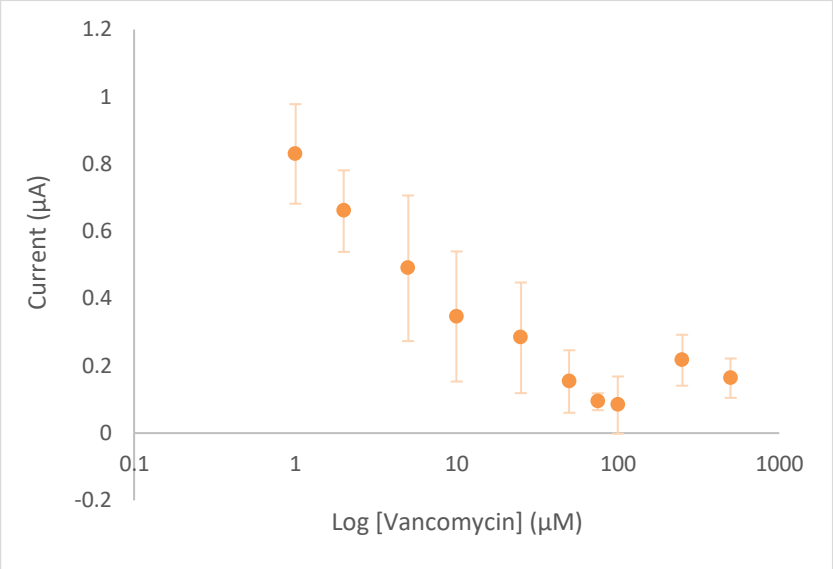

Figure 18: Calibration curve that plots the current measured (µA) as a function of the logarithmic concentration of vancomycin solution(µM). The values are the average of three measurements using the same electrode, and error bars are SD values for these measurements.

3.2.2.3 Strategy 3: one electrode for all concentrations with cleaning

The previous sections demonstrated the presence of both chemical and electrochemical passivation in vancomycin measurements. Therefore, here the study focused on removing this phenomenon.

Previous studies in the group confirmed that the passivating layer formed during the electrochemical measurements in vancomycin solution, is negatively charged. Considering that an electrostatic repulsion might arise when a negative potential is applied, this concept was used to attempt the removal of the passivating layer formed on the electrodes during vancomycin electrochemical measurements.

To this aim, after each measurement in vancomycin solution, a negative potential of -0.5 V was applied for 2 min with the electrodes immersed in PBS solution. Then, this electrode was used for measuring the next vancomycin solution.

Figure *19* shows the evolution of the current measured in µA for increased vancomycin concentrations in µM, using the same CSPE and applying PBS cleaning between consecutive measurements. As figure shows, current values increased by increasing the vancomycin concentration, confirming that the cleaning method can partly remove the passivating layer. Nonetheless, the current values were not the same as those obtained from approach 1 (Figure *16*). For example, for strategy 1 the maximum peak at 50 µM was 2.436 µA while for this approach, the current value obtained at 50 µM was 0.295 µA, which is 87% lower. These results confirmed that the cleaning process was not successful. It is worth mentioning that different cleaning methods for different electrodes, using different applied potentials and time, were also examined, but the results showed that passivation could not be successfully eliminated.

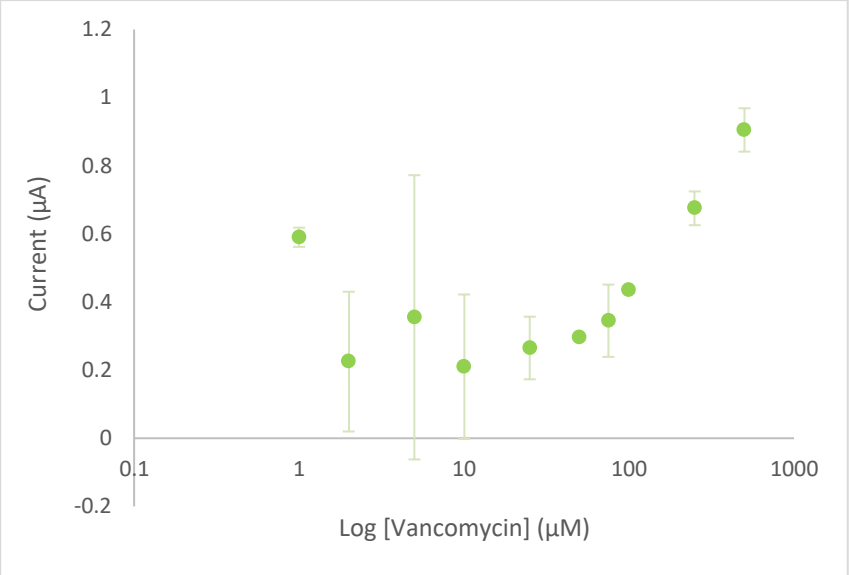

Figure 19: Calibration curve that plots the current measured (µA) as a function of the logarithmic concentration of vancomycin solution (µM). The values are the average of three electrodes, each one doing all the measurements with a cleaning in between, and error bars are SD values for these measurements.

3.2.2.4 Comparison of the three previous approaches

The results comparing the three previous strategies are presented in Figure *20*. Results showed that using the same electrode for consecutive vancomycin electrochemical measurements was not an option, because the effect of electrode passivation cannot be neglected.

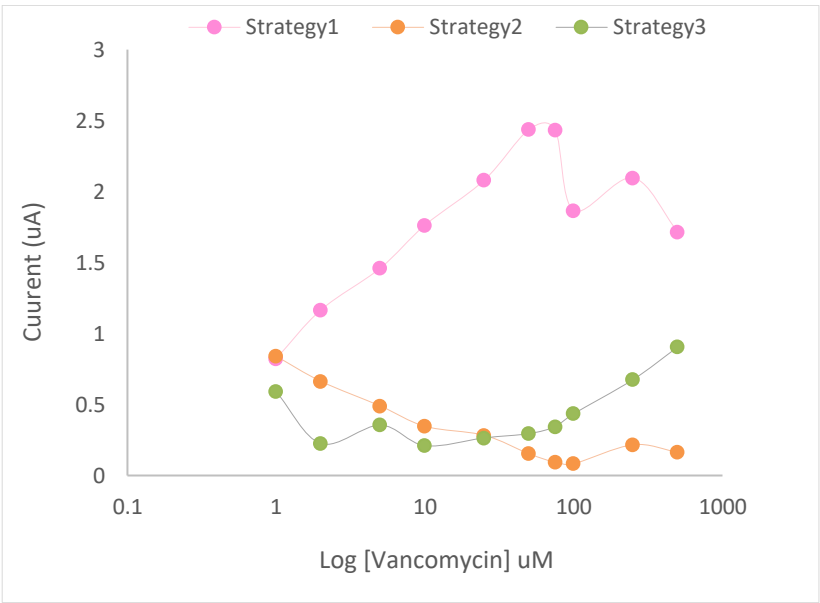

Figure 20: Calibration plots obtained using each one of the three strategies described, (1) using one new electrode for each measurement, (2) using the same electrode for all vancomycin concentrations, and (3) using one single electrode for all measurements but with a cleaning step between them.

3.2.2.5 Analytical performance of the sensor in buffer

In order to find the linear dynamic range for vancomycin electrochemical detection, DPV measurements were performed in different vancomycin solutions (500, 1000, 5000, 10000 and 25000 nM) prepared in PBS, using a new electrode for each measurement. Figure *21* shows the results obtained from these measurements. The linear dynamic range for the electrochemical detection of vancomycin ranges from 500 to 25000 nM, with a logarithmic relation of $I = 0.4028 \ln(C_{Vancomycin}) - 1.9734$, and R^2 value of 0.9988. The detection limit (LOD) was calculated using the following equation:

$$LOD = \frac{(3*SD)}{slope} \quad (4)$$

Knowing that SD refers to standard error and slope to sensitivity. The result of this equation corresponds to LOD= 344 nM.

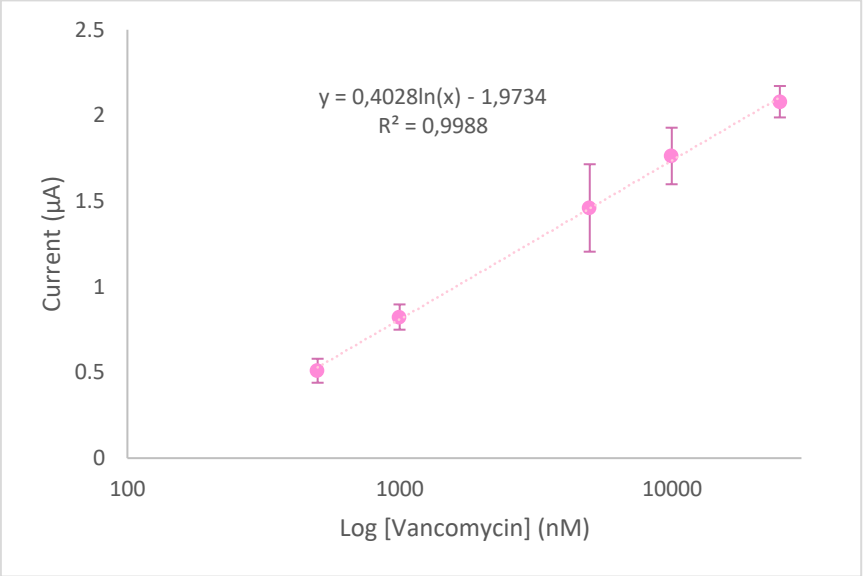

Figure 21: Calibration curve that plots the current measured (µA) as a function of the new logarithmic range of concentration of vancomycin solution (nM). The values are the average of three measurements using a new electrode for each concentration, and error bars are SD values for these measurements.

3.2.3 Sensing in artificial sweat

In order to evaluate the performance of the electrochemical sensor in artificial sweat, and assessing any potential matrix effects that might arise when analysing sweat, different vancomycin solutions (500, 1000, 5000, 10000 and 25000 nM) were prepared in artificial sweat at two pH values (5 and 7). The electrochemical measurements using these solutions were performed using one new electrode for each measurement.

Figure *22* compares the calibration curves obtained when measuring vancomycin in PBS and in sweat at pH 5 and pH 7. The peak current values obtained for different vancomycin concentrations in artificial sweat in both pH 5 and 7, were much lower than the values obtained in PBS. This can also be seen in Table *6* that summarizes the values of current measured for vancomycin concentrations of 500 and 25000 nM, and the difference between them. These results reflect the effect of the ionic strength and pH of the matrix on the vancomycin

electrochemical signal. Therefore, it was concluded that analyzing the concentration of vancomycin in sweat through the optimized electrochemical measurements is not feasible.

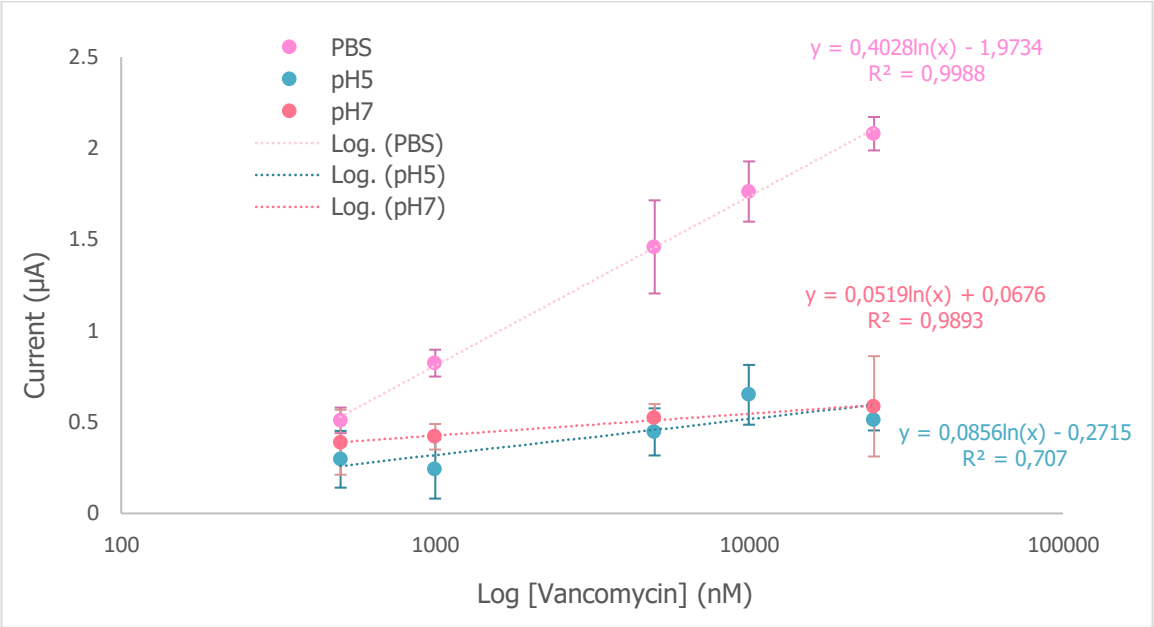

Figure 22: Calibration curves obtained from DPV measurements performed in vancomycin solutions prepared in PBS, and artificial sweat at pH 5 and pH 7.

Table 6: Current values obtained for vancomycin concentrations of 500 nM and 25000 nM prepared in PBS, and artificial sweat at pH 5 and pH 7.

	PBS	pH=5	pH=7	Difference in current values between pH 5 and PBS (%)	Difference in current values between pH 7 and PBS (%)
Current for 500 nM (µA)	0.51	0.29	0.39	43% lower	24% lower
Current for 25000 nM (µA)	2.08	0.51	0.58	75% lower	72% lower
Increased in current between 500 and 25000 nM (µA)	1.57	0.22	0.19	86% lower	88% lower

3.2.4 Sensing in artificial interstitial fluid

The matrix effect of artificial interstitial fluid was also studied. Differential pulse voltammograms were recorded using different vancomycin solutions (500, 1000, 5000, 10000 and 25000 nM) prepared in artificial interstitial fluid, using a new electrode for each measurement.

The performed electrochemical measurements did not show any quantifiable electrochemical signal for vancomycin, leading to the conclusion that the measurements in this body fluid are not an option. The lack of response could be attributed to the change in the ionic strength in artificial interstitial fluid when compared to the other tested solutions.

3.3 Meropenem

3.3.1 Optimization of parameters and choosing the electrochemical technique

As for vancomycin, first, the working parameters for both DPV and SWV were optimized. In this case, measurements were performed in a 1000 µM meropenem solution prepared in PBS using activated CSPEs. A new activated electrode was used for each measurement.

3.3.1.1 Differential pulse voltammetry

The different parameters analysed in this section for DPV are: potential range, pulse time, amplitude, step potential, and scan rate.

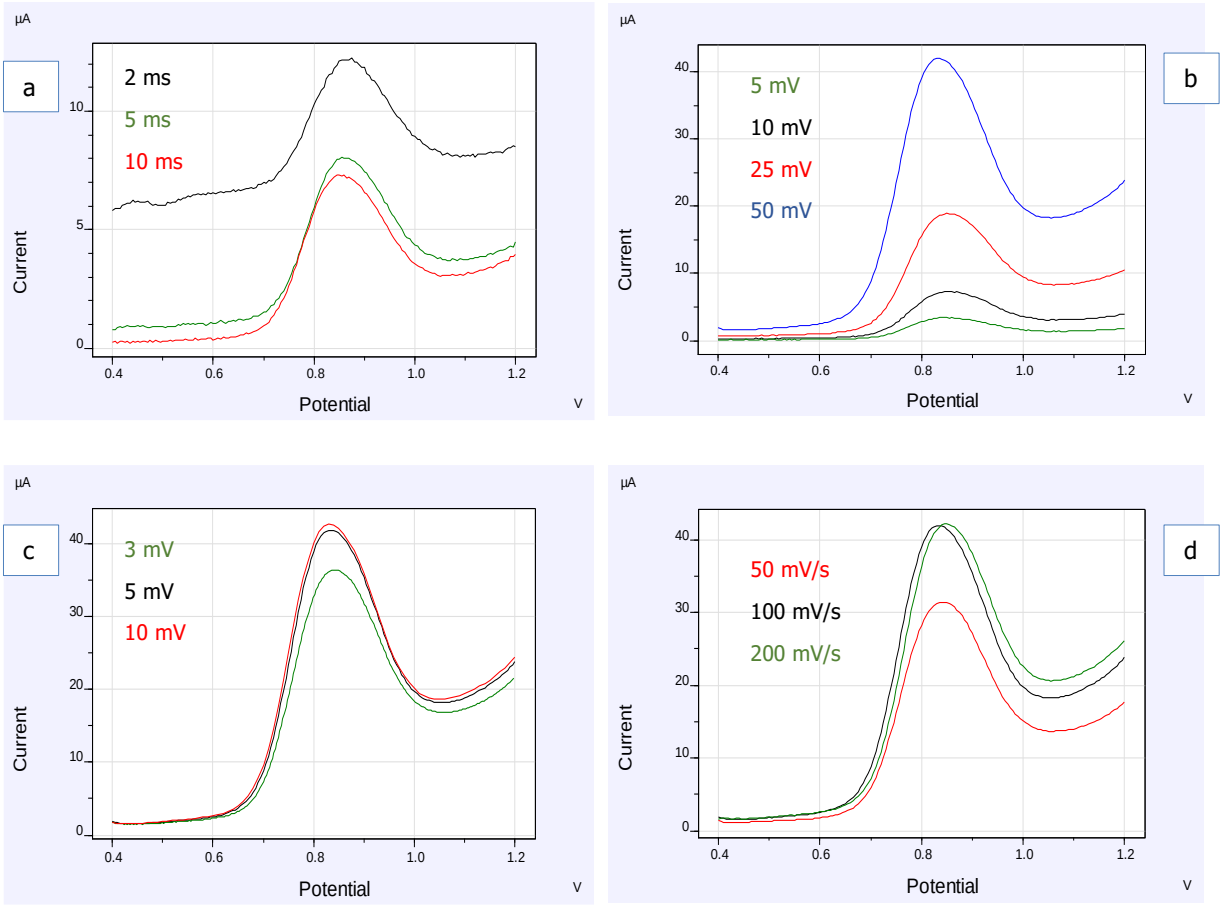

Figure 23: Differential pulse voltammograms obtained for the analysis of a 1000 µM meropenem solution using a CSPE, varying the following parameters: (a) pulse time, (b) amplitude, (c) step potential, and (d) scan rate

The optimal values for each parameter in the DPV study for meropenem (of 1000 μM) detection are listed in *Table 7* . As for vancomycin, the peak height and shape, as well as the baseline current were taken into account while determining the optimum values. The best parameter was determined to be the one that displayed the most clearly defined peak, the highest current value, and the lowest baseline current.

Table 7: Different parameters assessed for DPV measurements in a 1000 μM meropenem solution and their optimal values

DPV in meropenem	Values assessed	Optimized parameters
Potential range (V)	0.4 – 1.2	0.5 – 1.2
	0.5– 1.2	
Pulse time (ms)	2	10
	5	
	10	
Amplitude (mV)	5	50
	10	
	25	
	50	
Step potential (mV)	3	5
	5	
	10	
Scanrate (mV/s)	50	100
	100	
	200	

3.3.1.2 Square wave voltammetry

Regarding the SWV technique, other parameters were found important: amplitude, step potential and frequency.

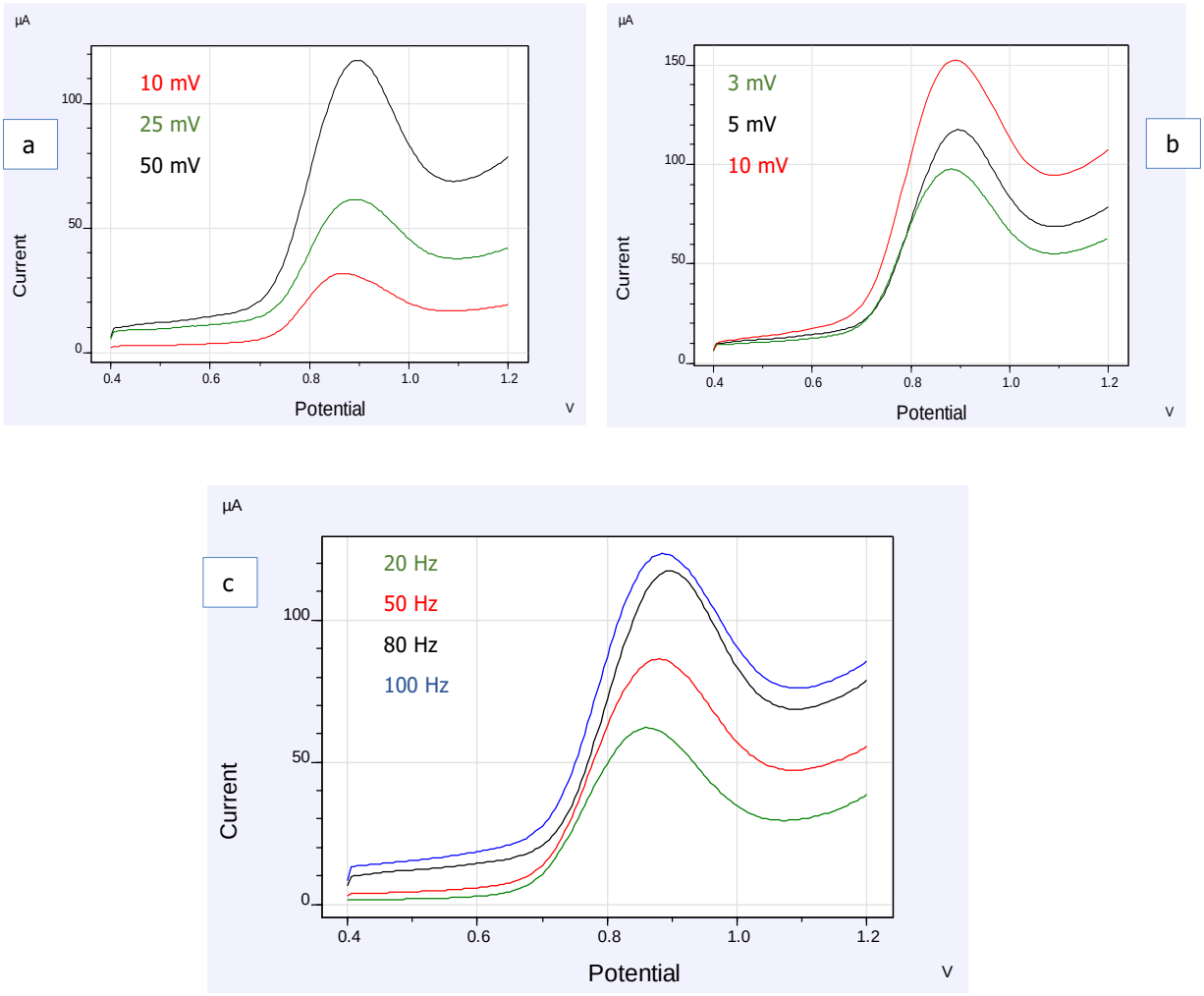

Figure 24: Square wave voltammograms obtained for the analysis of a 1000 µM meropenem solution suing a CSPE, varying each parameter analyzed: (a) amplitude, (b) step potential, and (c) frequency

A summary of the prior parameters and the optimized values for SWV measurements in a 1000 µM meropenem solution are shown in Table *8*. The criteria followed was the same as for the previous optimizations.

Table 8: Different parameters assessed for SWV measurements in a 1000 µM meropenem solution and their optimal values

SWV in meropenem	Values assessed	Optimized parameters
Potential range (V)	0.2 – 0.7	0.2 – 0.7
Amplitude (mV)	10	50
	25	
	50	
Step potential (mV)	3	5
	5	
	10	
Frequency (Hz)	20	50
	50	
	80	
	100	

3.3.1.3 Comparison of DPV and SWV

Results for the two methods, DPV and SWV, are shown in Figure *25* when using their final optimized parameters. As it can be observed, the value of the oxidation peak for SWV (85 µA) was much higher than the one obtained for DPV (42 µA). Indeed, the value of the SWV peak is approximately twice as large as that of the DPV peak. Moreover, the shape of both peaks is well defined. Because of this, SWV was selected as the best method for sensing meropenem.

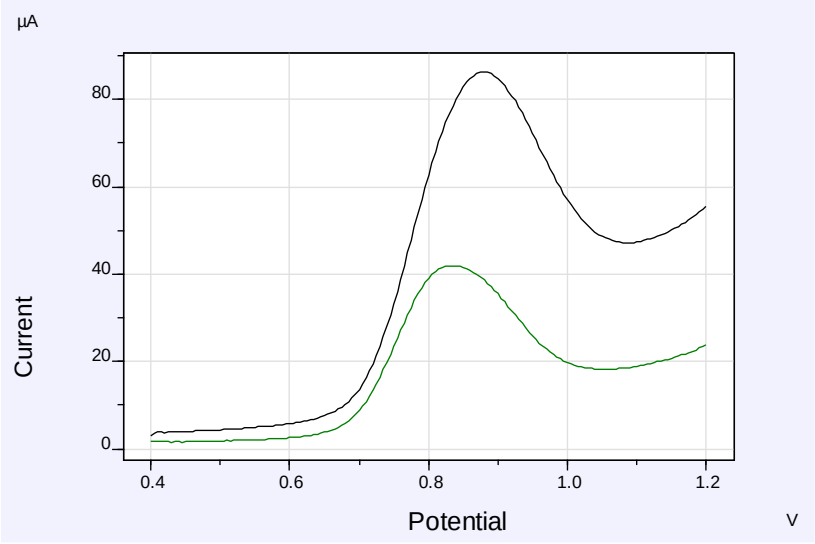

Figure 25: Measurement of 1000 µM meropenem solution using CSPE with two techniques, DPV (green) and SWV (black)

3.3.2 Sensing in buffer

As for vancomycin, this section had the objective of determining the optimal working range of meropenem concentrations to be electrochemically quantified via SWV. Also, an analysis of the effect of passivation, both chemical and electrochemical, on our measurements was carried out. To this purpose, two strategies were followed, either using one new electrode to measure each meropenem concentration or using the same electrode to measure all meropenem concentrations.

Using SWV and the previously optimized parameters, electrochemical measurements following those two approaches were carried out in meropenem solutions prepared in PBS at various concentrations: 1, 2, 5, 10, 25, 50, 75, 100, 250, 500 and 750 µM. The outcomes of the two alternative strategies are explained in this section.

3.3.2.1 Strategy1: one electrode for each concentration

For the first strategy, as it was done for vancomycin, oxidation current values were measured for each concentration, and results were analyzed to determine the presence of chemical passivation. In order to achieve this, one new electrode was used for each concentration. In this way, the current value could be obtained in each case.

Results are plotted in Figure 26, as current values (µA) as a function of meropenem concentration (µM). The measured current increased when the meropenem concentration also increased. However, it can be observed that when the meropenem concentration reached 750 µM, results were no longer linear. This was attributed to the presence of chemical passivation, which affected measurements performed in meropenem solution at high concentrations.

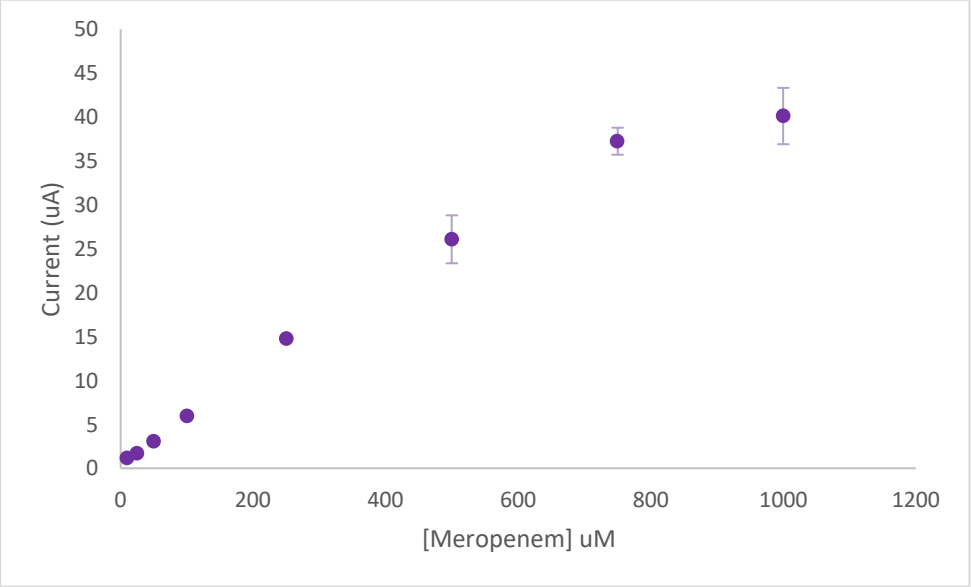

Figure 26: Calibration curve that plots the current measured (µA) as a function of the concentration of meropenem solution (µM), using a new CSPE for each concentration tested. The values are the average of three measurements and error bars are SD values for these measurements.

3.3.2.2 Strategy2: one electrode for all concentrations

The second strategy wanted to prove the existence of electrochemical passivation. In order to achieve this, an electrode was used to measure all meropenem concentrations. In this way, it could be clearly seen if this phenomenon existed.

Results are shown in Figure *27*, where a practically linear increase of the current values can be observed, when excluding the last concentration value. By comparing the results shown in Figure *27* with those shown in Figure 26, it can be observed that the current values are a little lower than those obtained when using a new electrode for each measurement. Therefore, it can be concluded the existence of some electrochemical passivation on the electrode surface.

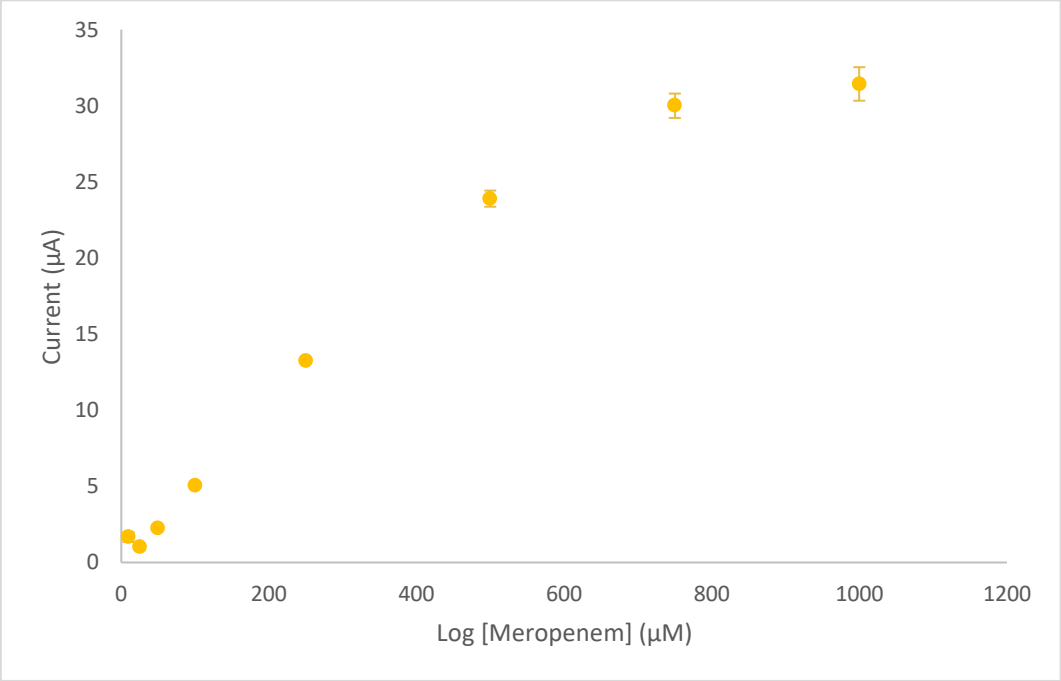

Figure 27: Calibration curve that plots the current measured (µA) as a function of the concentration of meropenem solution (µM), using the same electrode for all measurements. The values are the average of three measurements and error bars are SD values for these measurements.

3.3.2.3 Comparison of strategy1 and strategy2

Figure 28 compares the results obtained for the two strategies by showing the evolution of the current. In both cases the current had the same pattern and increased linearly until it reached 750 µM. However, the current values obtained for high concentrations of meropenem were a bit lower when the same electrode was used, effect that was attributed to the chemical passivation of the electrode that occurs when measurements are performed at high meropenem concentrations.

Table 9 summarizes the current values obtained for the highest meropenem concentration tested following strategies 1 (new electrode for each concentration) and 2 (same electrode for all concentrations), as well as their difference (%).

Thanks to these results, it could be concluded that at low meropenem concentrations, no passivation was present. However, the passivation effect increased as the concentration increased. This was the reason why it was decided to use one electrode for each measurement in the next steps.

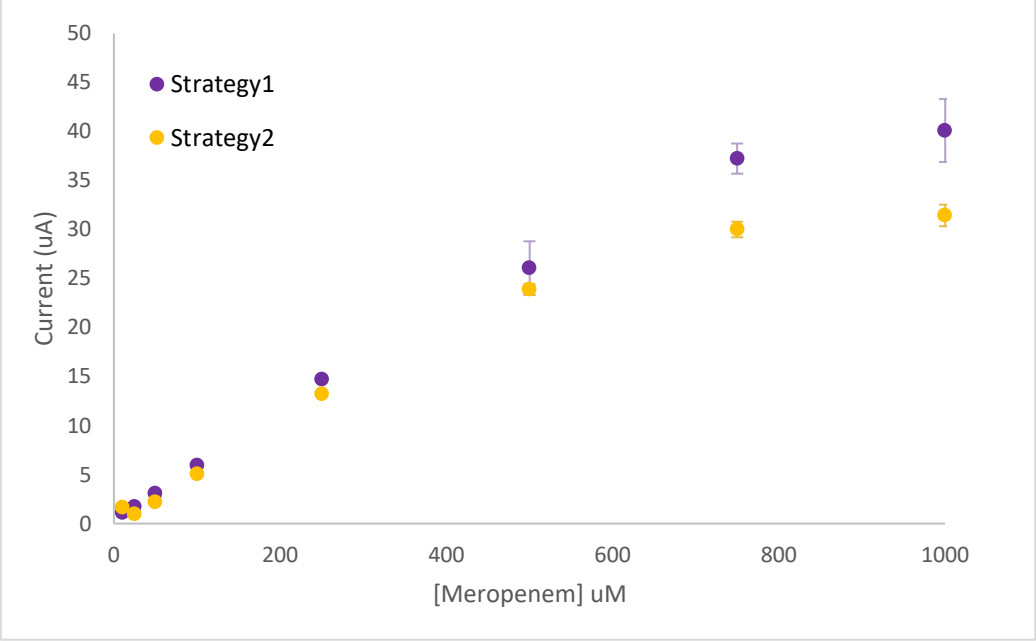

Figure 28: Calibration plots obtained using each one of the two strategies described, (1) using one new electrode for each measurement, and (2) using the same electrode for all meropenem concentrations.

Table 9: Current values obtained for meropenem concentrations of 10 µM and 1000 µM using new electrodes or a single electrode for all measurements.

	Strategy1	Strategy2	Difference in current (%)
Current for 1000 µM (µA)	40.1	31.4	22% lower

3.3.2.4 Analytical performance of the sensor in buffer

The linearity observed at low meropenem concentrations, suggested the study of a new range of meropenem concentrations to determine the linear dynamic range. The new range of concentrations included: 10, 25, 50, 100, 250, 500, 750 and 1000 µM. Electrochemical measurements were performed via SWV in meropenem solutions prepared in PBS, using approach 1 (i.e., a new electrode for each measurement).

Figure *29* shows the results obtained from these SWV measurements. The plotted calibration curve shows the linear dynamic range for the electrochemical detection of meropenem ranged from 10 to 750 µM, and the linear regression equation was $I = 0.0493C_{meropenem} + 0.9696$, with R^2 value of 0.9972.

The calculated LOD value (using equation (4)) for the electrochemical quantification of meropenem in PBS was 5.4 µM.

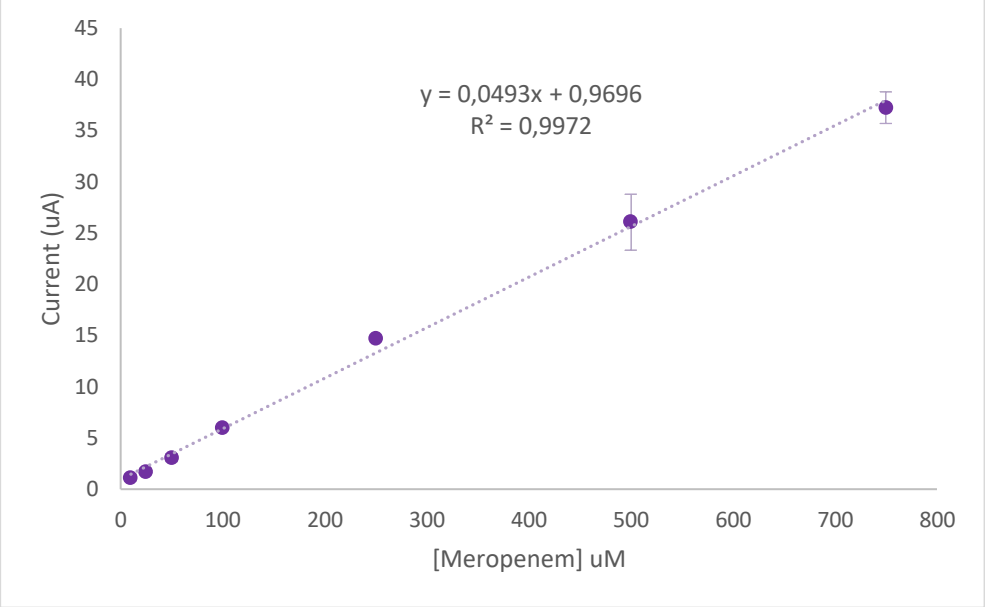

Figure 29: Calibration curve that plots the current measured (µA) as a function of the concentration of meropenem solution (µM) using a new electrode for each measurement. The values are the average of three measurements and error bars are SD values for these measurements.

3.3.3 Sensing in artificial sweat

Meropenem's electrochemical behaviour was studied using two solutions of artificial sweat, at pH 5 and pH 7. SWV measurements were performed using the optimized parameters. The concentrations of meropenem tested were: 10, 25, 50, 100, 250, 500, 750 and 1000 µM. Results from the measurements obtained for each concentration prepared in artificial sweat at both pH 5 and pH 7, are explained in this section.

To analyze the sensitivity of meropenem detection in artificial sweat at pH 5, the current results were extracted from the corresponding SWV measurements and plotted in Figure *30*.

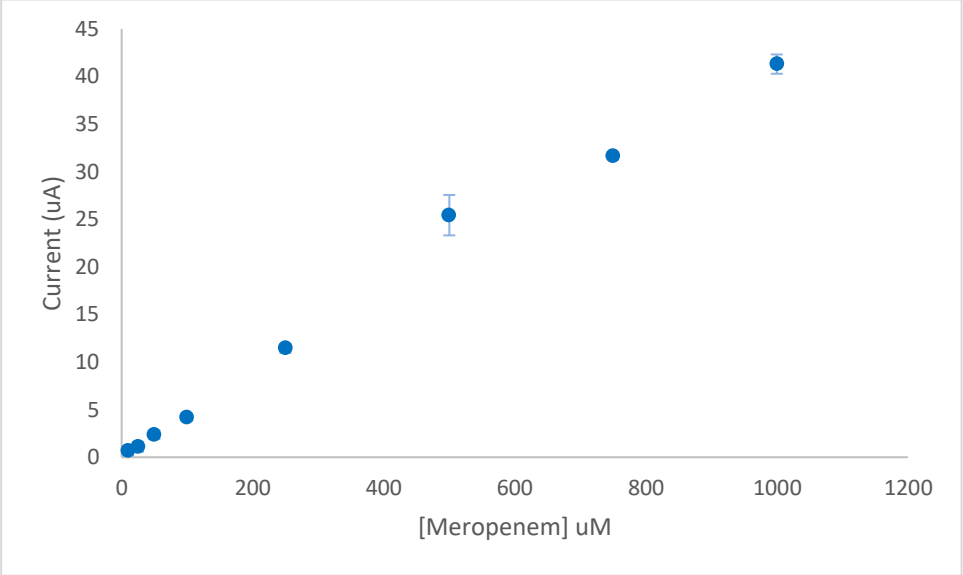

Figure 30: Calibration curve that plots the current measured (μA) as a function of the concentration of meropenem solution (μM) prepared in artificial sweat at pH 5. The values are the average of three measurements and error bars are SD values for these measurements.

For the next step, the same experiment was performed with meropenem solutions prepared in artificial sweat at pH 7. Figure *31* depicts the peak current measured for each meropenem concentration extracted from the corresponding square wave voltammograms.

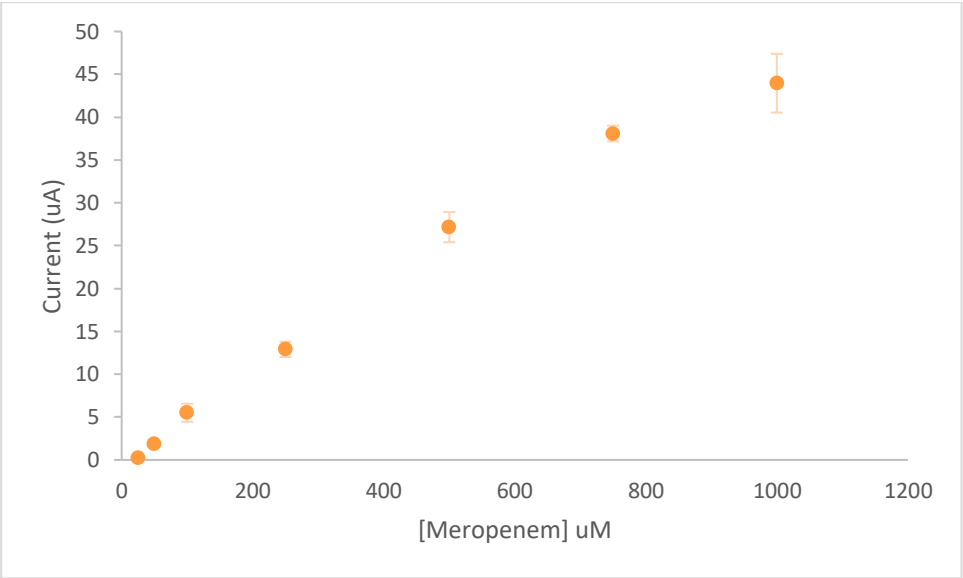

Figure 31: Calibration curve that plots the current measured (μA) as a function of the concentration of meropenem solution (μM) prepared in artificial sweat at pH 7. The values are the average of three measurements and error bars are SD values for these measurements.

Figure *32* compares the results obtained from the electrochemical detection via SWV of meropenem solutions prepared in PBS, and in artificial sweat at pH 5 and pH 7. Several conclusions can be drawn from this figure. It can be seen that the values obtained in the three cases were very similar, all following a linear trend. Nonetheless, the linear dynamic range in PBS was wider than that determined from measurements in both artificial sweat solutions (pH 5 and pH 7).

The pH of PBS was adjusted to 7. Therefore, when performing the analysis in PBS and in artificial sweat at pH 7, the only difference was the ionic strength of the solution. Results in PBS and artificial sweat at pH 7 showed almost the same sensitivity (slopes and trend lines). As a result, it was concluded that the ionic strength did not affect the sensitivity of the measurements.

On the other hand, when comparing the results obtained when analyzing meropenem prepared in artificial sweat at pH 5 and in PBS, a small variation was observed. Knowing that the ionic strength did not have any effect on the electrochemical response to meropenem, the difference was attributed to the pH change.

Results are summarized in Table 10, showing a quite similar electrochemical response both in PBS and in artificial sweat at pH 5 and pH 7. In short, it could be seen that neither the ionic strength nor the change in pH have an effect on the sensitivity of the electrochemical detection of meropenem. Therefore, artificial sweat was identified as a suitable bodily fluid to monitor the concentration of meropenem administered to a patient.

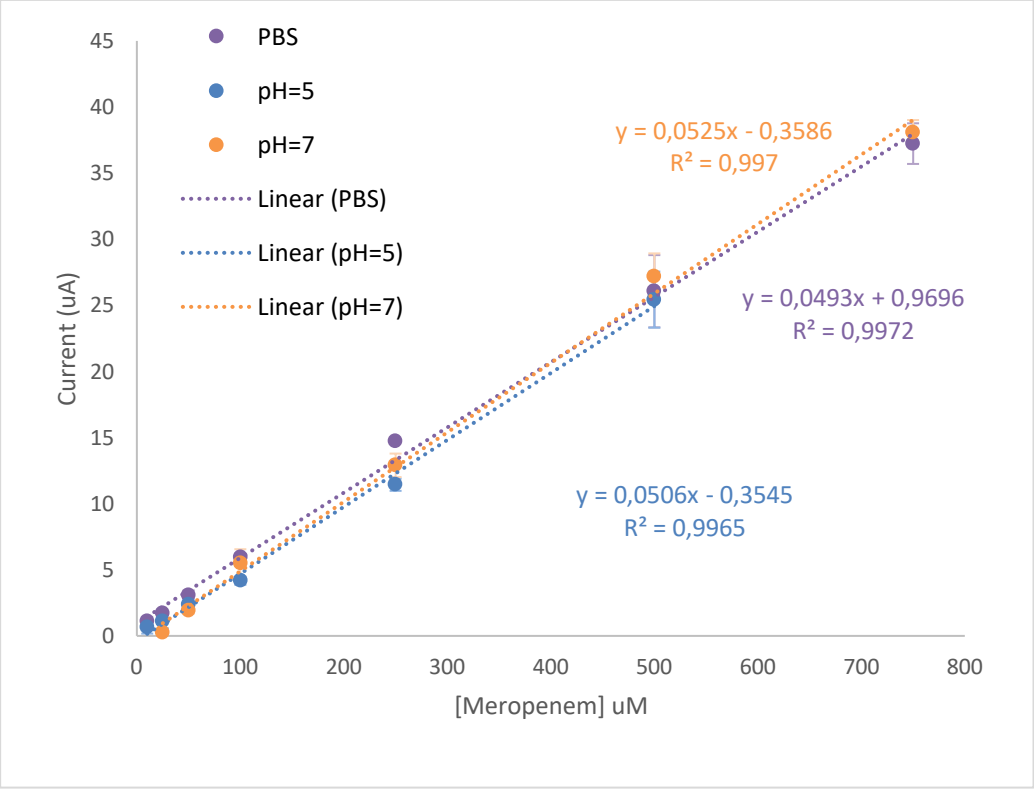

Figure 32: Calibration curves obtained from SWV measurements performed in meropenem prepared in PBS, and artificial sweat at pH 5 and pH 7.

Nonetheless, the best LOD value and the widest dynamic range was achieved for the measurements in solutions of meropenem prepared in PBS.

Table 10: Analytical parameters obtained from the electrochemical detection of meropenem prepared in PBS, and in artificial sweat at pH 5 and pH 7

	PBS	pH=5	pH=7
sensitivity ($\frac{\mu M}{\mu A}$)	0.049	0.051	0.053
R^2	0.997	0.996	0.997
linear range	10 to 750 µM	10 to 500 µM	25 to 750 µM
detection limit µM	5.42	42.2	22.9

3.3.4 Sensing in artificial interstitial fluid

The objective of this section was to study the electrochemical response from the SWV analysis of meropenem solutions prepared in artificial interstitial fluid, using CSPEs. In order to analyze it, the following concentrations of meropenem were used: 10, 25, 50, 100, 250, 500, 750 and 1000 µM. The same process as for artificial sweat was carried out.

In this case, measurements were performed, but no signal was detected. Knowing that the pH of the artificial interstitial fluid was 7, the same as the PBS, it can be said that the reason for not obtaining a signal could be due to the ionic strength of this artificial solution. This allowed us to conclude that no electrochemical measurements via SWV under the previously optimized conditions can be performed for meropenem solutions prepared in interstitial fluid.

4 Conclusion

This study allowed us to analyze the electrochemical behavior of both vancomycin and meropenem antibiotics, in different situations. Thanks to the electrochemical methods, either DPV, SWV or CV, the two antibiotics could be electrochemically detected at different concentrations, and in different matrices. The reproducibility of the measurements carried out was analyzed in each case, determining their usefulness.

In response to the first objective, quantification of both antibiotics in buffer was successfully carried out. For each of them, a linear dynamic range and a detection limit were determined. It was demonstrated the capacity to detect and quantify the concentrations of each antibiotic in PBS, assuring reproducibility in a determined range, being for vancomycin: 500, 1000, 5000, 10000 and 25000 nM and for meropenem 10, 25, 50, 100, 250, 500, and 750 µM.

Regarding the second objective, the presence of the passivation phenomenon was observed for both, vancomycin and meropenem. For vancomycin, the effect of both chemical and electrochemical passivation was much higher, preventing to obtain reproducible results for any of the concentrations tested. In addition, the method tested to clean the electrode surface by applying a negative potential was not successful. As a result, the passivation phenomena continued to make it difficult to obtain accurate values for vancomycin over the concentration range determined. For meropenem, passivation was observed but, in this case, it was not very present and only for high concentrations. However, in both cases, the use of a single electrode for each measurement was determined to prevent the effect of electrode fouling.

As to the third objective, the behavior of each antibiotic was analyzed with antibiotic solutions prepared in different matrixes, the first one artificial sweat at two pH (5 and 7), and the second one in artificial interstitial fluid. For vancomycin, results were not satisfactory. In both cases, artificial sweat and artificial interstitial fluid, matrix effects were too significant, considerably affecting the reproducibility and accuracy of the results. The ionic strength and the variation of pH affected directly to the solution. For meropenem, results for solutions prepared in interstitial fluid were again not accurate, nor reproducible, the ionic strength of artificial interstitial fluid affected directly to the solution. Nevertheless, those obtained for solutions prepared in sweat showed almost no matrix effects, neither ionic strength nor ph effect. Meropenem prepared in artificial sweat at both pH 5 and pH 7 could be quantified, giving results very similar to the ones obtained for meropenem prepared in PBS. These results confirmed the possibility to analyze meropenem concentrations in this body fluid.

Finally, referring to the last objective, taking into account the analytical performance of meropenem detection via SWV (linear dynamic range from 10 to 750 µM) and that meropenem detection was demonstrated in PBS and sweat, its potential detection in various bodily fluids was assessed. As already stated, the reported meropenem concentrations in body fluids include: 73 to 78.3 µM in plasma and serum, from 0.00836 to 0.0225 µM in interstitial fluid, and from 0,2 to 2 µM in sweat. Therefore, with the current system meropenem could be analyzed in plasma and serum, so analysis in these bodily fluids should be attempted. However, regarding the results for the electrochemical detection of meropenem in sweat, the linear range obtained is above the expected concentrations in sweat. In order to achieve this result, a possible avenue for future work would be to obtain a more sensitive system with a lower LOD, and thus be able to quantify a lower concentration range of meropenem and therefore suitable for this body fluid. Nevertheless, the results are very promising and as soon as a system with these modifications is obtained, the quantification of this antibiotic in sweat could be another option.

In conclusion, this study has provided valuable information regarding the electrochemical detection of vancomycin and meropenem. For vancomycin, the most optimal electrochemical technique to quantify this antibiotic and its linear dynamic range were determined. It was also concluded that passivation for this antibiotic is a major limitation for its electrochemical quantification, and thus new methods to clean the electrode surface should be sought. For meropenem, also the optimal electrochemical technique for its quantification, as well as its dynamic linear range were determined. Finally, the electrochemical quantification of meropenem in sweat was demonstrated, which has significant implications for non-invasive monitoring and future therapeutic uses.

In essence, the promising results of this study support a new avenue of research towards the quantification of antibiotics by electrochemical detection, demonstrating the reproducibility, accuracy and sensitivity of this technique.

5 Future work

Our study showed that when applying potential to trigger redox reactions in vancomycin, the phenomenon of passivation appears on the surface of the electrodes. A potential solution to avoid such passivation could be through the use of aptasensors. Aptasensors rely on a bioreceptor, in this case an aptamer, with affinity towards the analyte, in this case vancomycin [61]. Aptasensors do not rely on the electrochemical reaction of vancomycin, and therefore do not trigger its oxidation. Thus, it is expected that by using aptasensors no chemical or electrochemical passivation occur. In addition, aptasensors offer high sensitivity and affinity. Therefore, it could be a good option to quantify low vancomycin concentrations. [62].

Regarding meropenem, successful results have been achieved for its detection in artificial sweat. However, the drawback for meropenem detection is that the concentration range of meropenem in sweat that can be electrochemically detected, is lower than the linear range determined. Therefore, the main focus for future analysis should be on increasing the sensitivity of the system so that reproducible current values can be obtained for lower concentrations.

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