Electrochemical Analysis of the Biorremediation of Pharmaceutical Drugs

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ABSTRACT

Abuse of pharmaceutical drugs has led to wastewater contamination due to their presence in human feces. These waters eventually get to sources of potable water and may present a hazard to humans. In addition, pharmaceutical companies must constantly regulate their disposal of drugs wastes to minimize pollution. These companies use methods of detection of contaminants such as High-Performance Liquid Chromatography, Gas Chromatography, etc. This research is aimed to develop a new detection limit (LOD) method for pharmaceutical drugs using Cyclic Voltammetry (CV), an Electroanalytical technique that use a Glassy Carbon (GC) as a working electrode. CV is a more economically viable method of lower detection limits than previously mentioned methods. During this study, various drug sample solutions, such as Clonazepam and a combination medication of Acetaminophen, Pamabron and Pyrilamine, were prepared in a 0.1M potassium chloride (KCl) solution used as the solvent. Solutions with a range of concentrations from 10^{-2} to 10^{-6} were prepared for each drug and the variation in the anodic or cathodic peaks was observed. Afterwards, a calibration curve of current vs. concentration was prepared for each drug. Preliminary results show that reduction or oxidation signals may be obtained even at parts per million concentrations.

INTRODUCTION

Pollution with organic wastewater contaminants is a major concern to environmental health. These contaminants have been found persistently in sewage treatment plants and drinking treatment facilities, which can further have a direct impact in humans and the environment. A previous study, which determined the persistence of contaminants in drinking water facilities, consisted in taking samples of raw water, settled water, filtered water and finished water, collected over 4 consecutive weeks during November and December 2001. Results show that contaminants present in potable water survive conventional drinking water treatment processes. As a way to contribute and find a solution to reduce pollution of organic contaminants, this research project seeks to come up with a solution for analgesics and anti-inflammatory drugs present in organic wastewater contaminants and physiological waste by achieving two essential procedures: 1) Develop a new method for detection of commonly used analgesics and anti-inflammatory drugs using cyclic voltammetry (CV) and 2) Usage of Escherichia coli (E.coli) as the degrading organism for the bioremediation of the drugs particles. Cyclic voltammetry is an electrochemical technique which measures the current that develops in an electrochemical cell under conditions where voltage is in excess of that predicted by Nernst's equation**: E = Eo –RT/nF ln Red/Ox**; where E is the cell potential, Eo is the cell potential at standard conditions, R is the universal gas constant, T is the temperature, n is the number of electrons exchanged in the process, F is Faraday's constant, [Red] is the molar concentration of the reduced species and [Ox] is the molar concentration of the oxidized species. This technique is a more affordable and unexplored technique for pharmaceutical drug determination compared to other techniques such as Gas Chromatography (GC) and High-Performance Liquid Chromatography (HPLC). Detection of contaminants using CV will provide information in the range of parts per billion (ppb), which is the range of concentration most contaminants are present. Having information of contaminants present in the samples, the bioremediation will play an important role as a contaminant removal technique. Bioremediation involves the use of organisms to remove or neutralize pollutants from a contaminated site. The current scientific literature does not provide information on bioremediation of commonly used analgesics and anti-inflammatory drugs using Escherichia coli (E. coli). However, preliminary studies have shown the potential of E. coli to degrade Clonazepam (a benzodiazepine used to prevent and control seizures) and a combination medication containing Acetaminophen, Pamabrom and Pyrilamine Maleate (used to treat symptoms of premenstrual syndrome (PMS), tension, muscle pain, cramps and irritability). Bioremediation with E. coli will provide information of new degradation mechanisms of commonly used drugs, such as Clonazepam and combination medication containing Acetaminophen, Pamabrom and Pyrilamine Maleate, present in pharmaceutical drug wastewater.

OBJECTIVES

- Develop a new method for the detection and electrochemical analysis of drugs using Cyclic Voltammetry.
- Determine the effectiveness of E. coli for the bioremediation of pharmaceutical drug solutions.
- Raman Spectroscopy analysis of solutions after bioremediation.

METHODOLOGY

To carry out the cyclic voltammetry process, the instrument and model used for the analysis was BAS CV-50 W and the working electrode was made of glassy carbon. All solutions under analysis were made with 0.1 M KCl, which was also used as the blank solution. First, to prepare the stock solution of the respective pharmaceutical drug, the mass of the active ingredient in one tablet was determined to obtain the amount tablets necessary to produce a 50mL solution with a concentration of 1.0 x 10^{-3} M. The stock solution was then purged with N_2 to remove any trace of O_2 and analyzed in order to verify if the active ingredient was susceptible to reduction and/or oxidation and demonstrated a signal. If it did, then five solutions at decreasing concentrations were prepared by dilution using the stock solution as the initial one. These were then analyzed at a scan rate of 500 mV/s in order to obtain various voltammograms that should demonstrate how current signals vary in intensity with respect to the concentration change in the solutions. With these voltammograms a calibration curve was constructed with which a LOD can be determined. The blank solution was also analyzed in order to compare with the solutions containing the pharmaceutical drugs.

RESULTS AND DISCUSSION

Figure 1 shows the adjusted voltammogram of Clonazepam. This voltammogram corresponds to the stock dilution, which presented a Clonazepam concentration of 3.2 x 10-5 M, and four dilutions with concentrations of 1.6 x 10-5 M, 8.0 x 10-6 M, 4.0 x 10-6 M, and 2.0 x 10-6 M, respectively. When observing the graph, two signals can be detected from the stock solution: a cathodic peak (reduction) with a value of -5.98 x 10-6 A between -5.00 x 10-1 V and -1.00 V and an anodic peak (oxidation) with a value of 1.03 x 10-5 A between 1.00 V and 5.00 x 10-1 V. However, the first dilution graph, which is colored in orange, as shown in Figure 1, appears to portray the disappearance of the anodic peak signal between 1.00 V and 5.00 x 10-1 V. Also, this graph shows a new anodic signal at approximately 1.69 x 10-1 V, which is consistent throughout the other dilution graphs.

On the other hand, Figure 2 shows the calibration curve for Clonazepam. The signals used for the calibration curve were the anodic peak signals since they showed better visible patterns. The data points do not appear to have an efficient linear tendency. This is due to the third dilution data point, since it presents a smaller current than the fourth dilution. Therefore, it does not go along with the stipulated theory: as the concentration of Clonazepam decreases with each dilution, the signal's amplitude (current) decreases as well. However, the data points corresponding to the first, second, and fourth dilution appear to be consistent with the expected results. Nevertheless, a subsequent analysis of the dilutions is recommended in order to obtain a better linear tendency in the calibration curve.

Figure 3 shows the adjusted voltammogram of acetaminophen, pamabrom, and pyrilamine maleate. A major signal can be seen at approximately 0 V. This signal is indicative of a reduction process, which is represented by a cathodic peak. Interestingly, the dilutions present a greater current value than the stock solution. Therefore, the tendency between current and concentration is not well established, as shown in Figure 4. For this reason, these signals are not quite useful since the current increases as the concentration of the active ingredients decreases, which is the opposite of the expected results.

Disturbances between the obtained results and the expected ones can be attributed to volumetric pipette contamination with other pharmaceutical drug previously analyzed, personal error when cleaning the instrumentation prior to its use, or interferences caused by the blank used

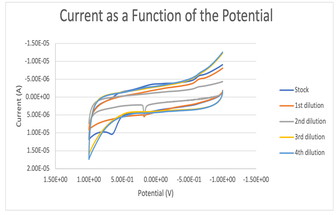

Figure 1. Adjusted Voltammogram of Clonazepam

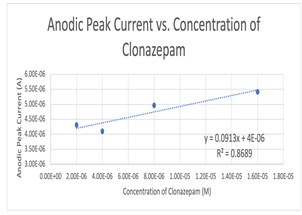

Figure 2. Calibration curve for Clonazepam

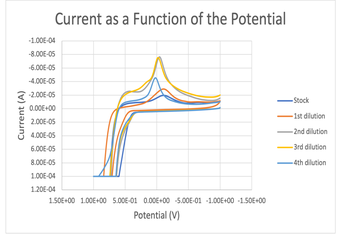

Figure 3. Adjusted Voltammogram of Acetaminophen, Pamabrom and Pyrilamine.Maleate

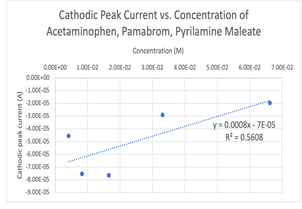

Figure 4. Calibration Curve of Acetaminophen, Panabrom and Pyrilamine Maleate

CONCLUSIONS

The results from this research demonstrate that cyclic voltammetry is a feasible method to analyze pharmaceuticals drugs that exhibit an electrochemical behavior. Therefore, all objectives regarding cyclic voltammetry analysis were accomplished. Clonazepam and a second drug with active ingredients such as acetaminophen, pamabrom and pyrilamine maleate were two of the many drugs analyzed by this method. Clonazepam showed oxidation and reduction signals, but since its calibration curve did not exhibit an efficient linear tendency, further research is needed to correct this discrepancy. The second drug exhibited reduction signals, in addition, it also presented unexpected results with its current increasing with dilution. The dilutions also had higher values than the stock solution. Just as clonazepam, further analysis for the dilution of this drug is necessary to validate the data and make sure these results are not product from cross contamination or other types of errors.

FUTURE WORKS

- Find more pharmaceutical drugs that exhibit electrochemical properties for cyclic voltammetry analysis.
- Perform bioremediation of the drugs previously analyzed by cyclic voltammetry using E. Coli.
- Bioremediation product will be analyzed using Raman Spectroscopy.

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