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DEVELOPING CYCLIC VOLTAMMETRY INTERPRETATION SOFTWARE FOR TUBERCULOSIS PATIENTS

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INTRODUCTION

Conventionally, tuberculosis is diagnosed in patients using one of three methods: skin sampling, blood testing, and sputum testing. In a skin test, or Mantoux tuberculin skin test, the skin on a patient's arm is injected with a small amount of a purified protein derivative (PPD) which contains a mixture of antigens commonly found in *Mycobacterium tuberculosis*. After 48 to 72 hours, the patient's arm is evaluated for any reactions that would indicate the presence of *M. tuberculosis*. These tests are limited by both time scale and efficacy, as they rely on the presence of antibodies in a patient's system. That is, the patient may show positive results if they have recovered from a tuberculosis infection in the past or if they have an infection of a non-tuberculosis mycobacterium. In a blood test, a blood sample from the patient is applied to an antigen assay. Results from blood tests can be retrieved as soon as 24 hours after sampling, and the assays used are less likely to react with non-tuberculosis mycobacterium.[1] Sputum testing, like blood testing, involves the use of an assay, like Cepheid Xpert, which is exposed to samples of patient sputum.[2] Sputum testing can provide results even faster than blood or skin testing, with results being available as soon as two hours after testing.[2] However, all currently-available tests still require a significant amount of time between sampling and diagnosis and depend on patients having consistent access to healthcare services. These issues are further exacerbated when patients are in lower-income regions.

It would be ideal for a method of tuberculosis diagnosis to both be inexpensive and offer results only minutes after testing. Such a method has been proposed in the form of volatile organic compound (VOC) detection. *M. tuberculosis* have been found to regularly release several specific VOC's. These include methyl nicotinate and methyl p-anisate, among others. [3] It has been proposed that these VOC's can be detected in the vapor phase using several methods. One of these methods is gas chromatography mass spectrometry (GC-MS). GC-MS is used to isolate and identify the components of unknown mixtures of compounds. Previous work has already provided some indication that breath from patients suffering from tuberculosis may have a unique composition distinguishable from tuberculosis-negative patients.[4] In Dr. Mohanty's lab group, work is currently being done to confirm the VOC fingerprint of tuberculosis in the breath of both adult and pediatric patients from Kampala, Uganda. GC-MS, then, may provide an accurate way to diagnose patients with this illness. However, it is still not an ideal solution for lower-income regions. GC-MS equipment is expensive, bulky, and can be difficult to operate. It also requires a significant amount of time to analyze samples.

A better alternative has been proposed in the form of gas-phase compound-specific VOC detection. Using TiO_2 nanotubes functionalized with cobalt chloride, Mohanty et al. have been able to detect methyl nicotinate, a biomarker for *M. tuberculosis*, in mimic patient breath samples[5]. These

tuberculosis sensors have been the focus of my research for the better part of three years. Dr. Mohanty's group has been able to manufacture three-electrode electrochemical systems with these TiO_2 sensors, and much of my work has been in developing effective sensor and equipment casings for distribution to clinical centers like Mulago Hospital in Kampala, Uganda. Researchers in Uganda have now collected data from these electrochemical experiments using breath from over a hundred patients with varying medical histories. With this data, we hope to find a correlation between certain results in cyclic voltammetry and amperometry tests and positive tuberculosis diagnoses. However, while cyclic voltammetry tests have proven promising, their data are particularly difficult to analyze. This is the focus of my current research.

BACKGROUND

Cyclic voltammetry (CV) is an analytical method used with potentiostats for finding the oxidation and reduction peaks of certain electrochemical systems. In our case, we hope to find reduction peaks that are unique to methyl nicotinate suspended in air. Typically, CV results are distinctly "duck-like": they possess sharp points at each end of the voltage sweep and have rounded curves at the voltages of oxidation and reduction. There are two curves in each CV test: a forward sweep and a backward sweep, which reach different currents due to electrochemical hysteresis (see **Figure 1**).[6] Our CV data, because it is in the gas phase, typically does not have this shape and can have inconsistent forward- and back-scan shapes (see **Figure 2**). Our reductive scan (or forward) scan also tends to be above the oxidative (or backward) scan. These differences make it especially difficult to analyze and interpret, necessitating the development of a custom analysis software.

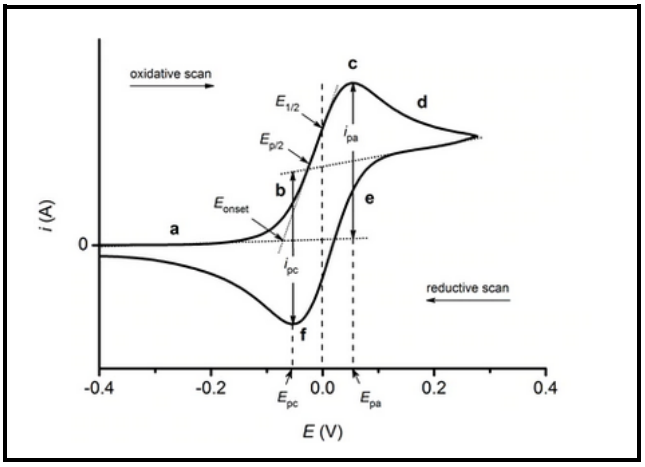

Figure 1 - Typical CV curve, retrieved from (5)

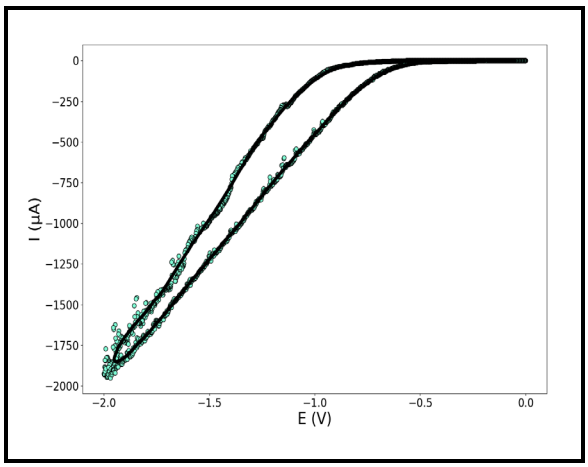

Figure 2 – Sample of our CV data

METHODS

In order to compare CV data to one another, we chose a series of characteristics to be pulled from each data set. These are as follows: the drop-off point, which is the intersection between the initial current and the maximum slope of the forward scan, analogous to E_{onset}[6]; the current at -2.0V; and the area between the forward and backward scan.

Using python, I was able to develop a program that automatically extracts these characteristics from given data sets. It was quite straightforward to pull out the final current value, but the drop-

off point and area required more personalized algorithms. To find the drop-off point, I initially used the python package SciPy's in-built numerical methods to find the point of maximum slope (PoMS) of the forward scan. I then defined a line with this maximum slope originating at the PoMS and found the intersection between this line and the initial value for the current. This method proved successful for cleaner data sets but struggled with noisier sets. Even after taking a rolling average of the data to minimize the effect of noise, it still gave results inconsistent with manual drop-off point measurements. Instead of using this method, I devised a method whereby I defined the point at which the current dropped below 20% of the minimum value as the drop-off percent point (DOPP). Like the PoMS, I defined the intersection between the derivative at the DOP and the initial current as the drop-off point. This method proved more consistent between automated runs and better reflected manual drop-off point assignments. To find the area between the points, I used the trapezoidal rule with absolute value sub-areas. This prevented the overall area from decreasing if the forward and backward scans intersected, which they can often do in low-current-range CV experiments. I tested my methods by applying them in the analysis of 100 CV test results.

RESULTS

Figure 3 shows three histograms, one for each of the three key CV characteristics, compiled from the results of 100 CV test analyses. **Figure 4** shows examples of four different data patterns we have observed from CV tests.

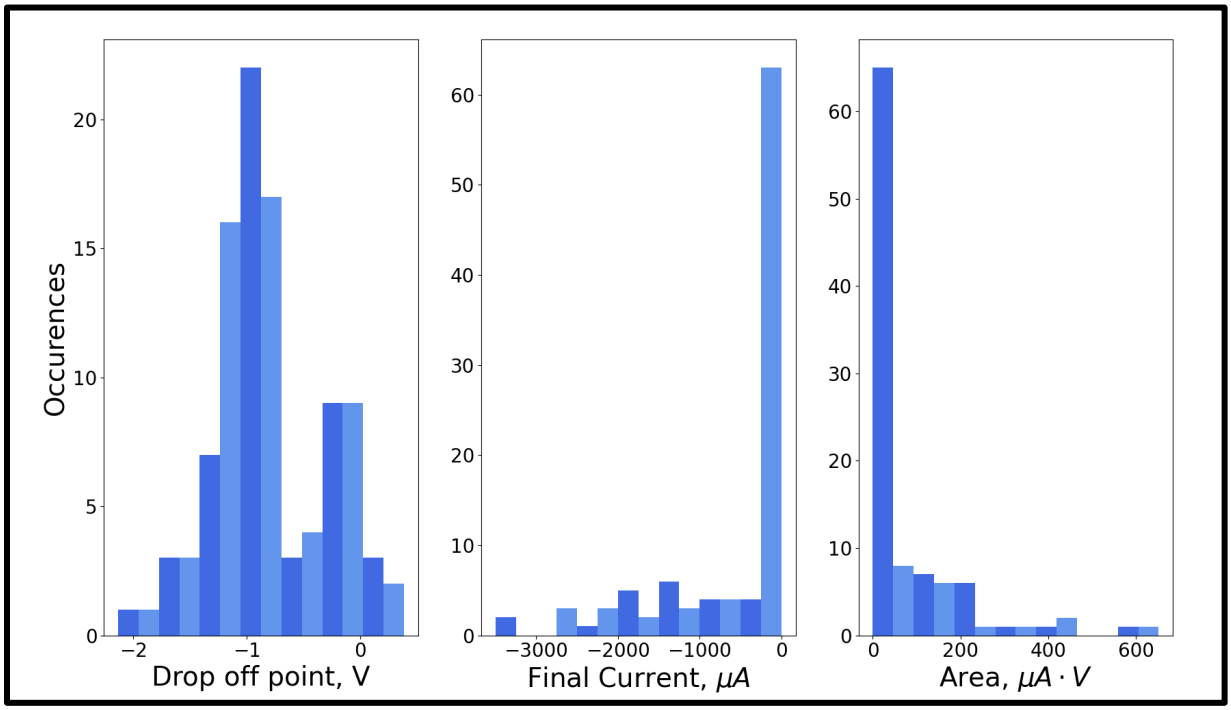

Figure 3 – Histograms for each characteristic and containing data from all 100 CV tests.

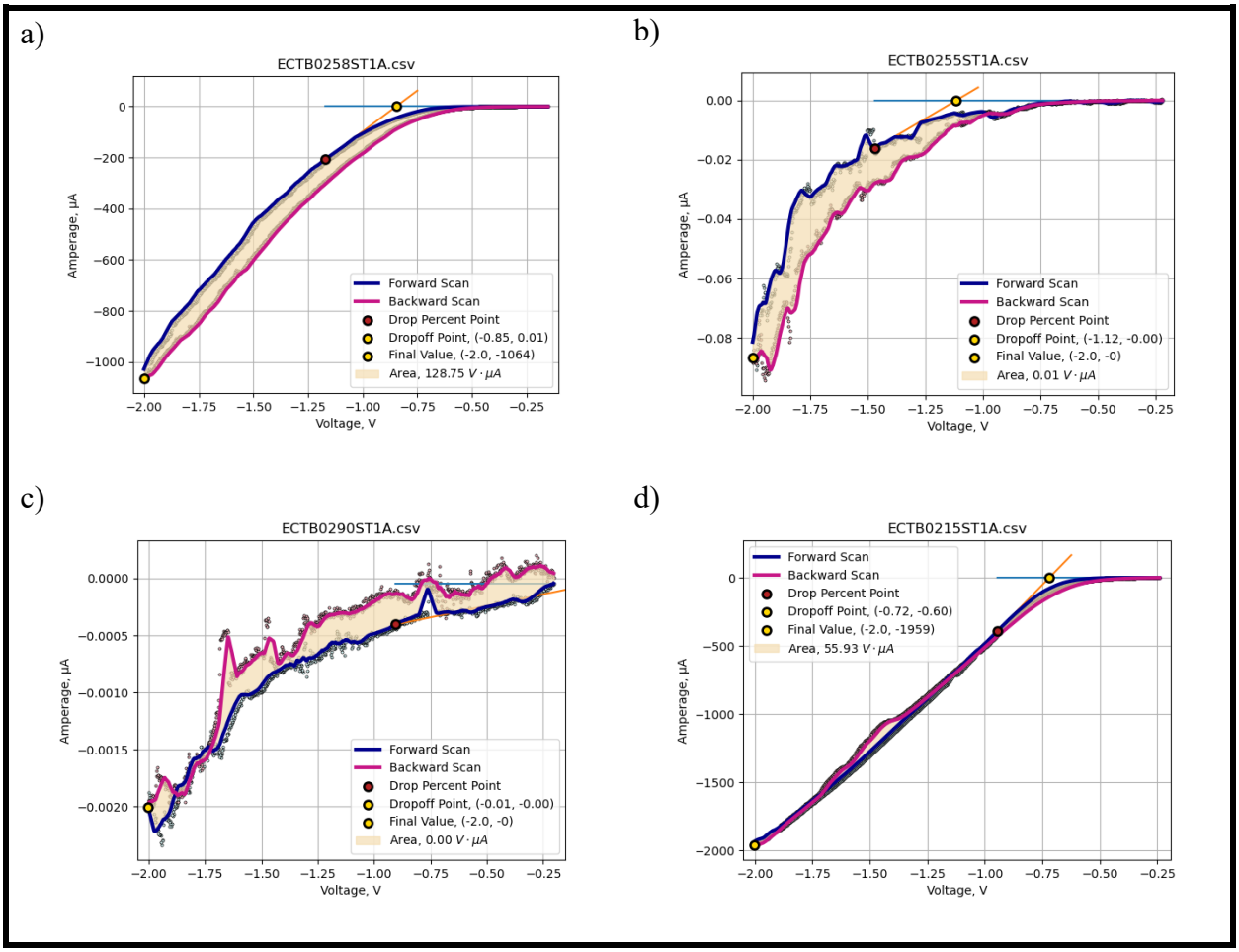

Figure 4 – Four sets of CV test data alongside analysis lines and results.

DISCUSSION AND CONCLUSION

When analyzing the CV test data, I noticed that most sets fell into one of four categories. The useable data sets looked much like **Figure 4a**, with distinct, smooth forward and back scans. My algorithm was designed for these data sets in mind, and it is not surprising that their results lined up best with manual calculations. The other three categories, then, contain various deviations from ideal data behavior.

The first deviation category, with an example in **Figure 4b**, includes data sets with significant amounts of noise, making calculating the drop-off point nearly impossible. Sometimes, these data sets can have intersecting forward and back scans, which is not ideal for CV experiments. The second deviation category (**Figure 4c**) includes data which span over very small current ranges. The final deviation category (**Figure 4d**) includes data which span over expected current ranges, but which show little difference between the forward and back scans.

Almost all these abnormalities are believed to be a result of imperfections in the sensors or errors made while conducting the CV tests. Since the data from these tests cannot elucidate any information about the ability of the sensors, it would be useful to remove them for the sake of statistical analysis while marking them as sensor failures for the sake of success-failure

estimations. The importance of this exclusion is highlighted by **Figure 3**, which shows a vast majority of data sets having near-zero area and/or final current. By identifying these categories of abnormalities, I hope to implement filtering functions which can isolate data sets by their symptoms.

The first filtering functions will be straightforward: I can use simple search functions to isolate data sets with near-zero area and/or final current. The hardest data sets to filter will be those with lots of noise. These data sets may not necessarily have key characteristics that seem out-of-place at first glance, which may make these sets hard to isolate. I plan to circumvent this problem by implementing and algorithm that estimates the first derivative of the graph and searches for rapid variations. From this, I hope to generate an index that can be used to sort data by quality and presence of noise. Then, the program user can manually decide an appropriate index cutoff and perform analyses with a certain degree of acceptable noise.

I have packaged this software and made it accessible to Dr. Mohanty's research team for application in automated CV analysis. This will be very useful as we move to make breath-based VOC sensors more accessible, versatile, and accurate. As we improve our methods of collection and experimentation, I plan also to update this software as necessary.

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