The purpose of my experiments was to study the current response of myoglobin solutions at carbon nanofiber (CNF) electrodes. This research was stimulated by cyclic voltammetry (CV), particularly to research the response of the carbon nanofiber electrode to varying Mb concentrations that had been oxidized to Fe(II) with potassium ferricyanide, and dissolved in Tris buffer (pH 6.5). Through these experiments, small but significant peaks were found in the CVs that corresponded to myoglobin redox activity. This discovered by comparing Mb CVs to control CVs containing pH 6.5 Tris buffer. The two types of CVs were found to be almost identical except for peaks that most likely indicate myoglobin redox activity. This hypothesis was found to be true as the peaks would diminish if the solution tested had less myoglobin and increase if more myoglobin was added. To attain numerous concentrations of myoglobin, a UV spectrophotometer was used to calculate the present concentration and what mass must be added to obtain a new concentration. By evaluating these different concentrations of myoglobin with CNFs, a limit of detection at which the peaks no longer appear is found. It can be concluded from these experiments that myoglobin’s redox reactions can be detected in the presence of carbon nanofiber electrodes.

**Abstract**

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**Procedure**

- **Protein Purification and Preparation**
  - Triton buffer with pH of 6.5
  - 0.05g of Triply-allylamine(poly(2-(4-VA)HEMA)) was dissolved to 0.1 ml using nanopure H2O.
  - Adjusted the pH to 6.5 using concentrated sulfuric acid (H2SO4)
  - Added potassium dichromate (K2Cr2O7) and sodium nitrite (NaNO2) to CNFs
  - Oxidizing iron atom in myoglobin from Fe(II) to Fe(III)
  - 40 mg of myoglobin was dissolved in 0.4 mL of buffer
  - A few crystals of potassium ferricyanide (K3Fe(CN)6) were added
  - The solution was allowed at 4°C for 24 hours
  - Using ultraviolet-visible (UV-Vis) spectrophotometer, measurements were taken with Mb(II) at 434 and 410 nm
  - Mb(II) was reduced to Mb(III) by adding a few crystals of sodium hydroxide (NaOH) and also measured
  - Mb concentration was determined by measuring absorption difference between Fe(III) and Fe(II) at 434 nm
  - A molar absorptivity of 114,000 M^-1 cm^-1 was used in Beer’s law calculations
  - Mb solutions were further diluted to working concentrations using Triton buffer

- **CNF Electrode Preparation**
  - Wetting an electrode
  - Acrylic was applied to upper stem and 2.2 mm square on CNF
  - CNF was attached to copper clamp in electrochemical cell
  - Electrodes were cycled with a cyclic potential sweep from -0.55 to 0.45 V at 100 mV/s
  - CNFs show great redox chemistry at many electrode surfaces, including CNFs. Therefore, Mb is a possible biosensor component that may be diluted using a variety of electroanalytical techniques.

- **Calculations**
  - The electrochemical cell was purged with either N2 or Ar for 10-15 min. prior to CV
  - A blank of internal gas was maintained in the cell throughout all measurements
  - **Cyclic voltammetry (CV)** is one of the most effective electroanalytical techniques because it is both sensitive and easy to use. A CV is derived from current (i) as a function of potential (V). The potential of the working electrode is scanned at a constant rate (scan rate). The anodic peak potential (Epa) and the cathodic peak potential (Epc) are the points at which the current is maximum. The anodic peak current (Ipa) and the cathodic peak current (Ipc) are the currents at which the potential is maximum. The redox reactions (rns) appear in CV as peaks which may be further analyzed.

- **Carbon nanofibers (CNFs) at left are a type of incomparable material with many attractive features, such as small size, large surface area and high chemical stability. These features make CNFs ideal for electrochemical biosensing schemes. The Stevenson group specializes in the synthesis and detailed study of CNFs electrodes assembled in-house.**

**Results and Discussion**

- **Average redox potential: -0.232 V**
  - All CVs yielded a reaction reversibility other than 1, and are thus categorized as quasi-reversible
  - They differ in their peak-to-peak separation for one electron transfer, 55 mV, but it is expected that Mb rns have such a separation
  - Changes between Fe(II) and Fe(III)

- **Limit of detection**
  - Point of concentration at which myoglobin can no longer be substantially identified using CV analytis
  - Found to be around 100 µM

- **Anodic peaks were found to be unstable, possibly because in the cathodic sweep, Fe(III) is reduced to Fe(II), and this may bind oxygen and produce disorderly CVs**

- **Surface Coverage of Cathodic peaks was directly proportional to Mb solution concentration**
  - These values indicate that Mb was covering almost all of the electrode surface and showing good redox chemistry

**Conclusion**

**Acknowledgements**

**References**