DNA Sequencing with Sinusoidal Voltammetry

**MODEL 1   Voltammetric Instrumentation**

1. Polarography is one type of voltammetric technique that provides a background for understanding cyclic voltammetry. In a polarographic experiment there are three electrodes: a counter electrode, a reference electrode and a working electrode. Explain what happens between each pair of electrodes.
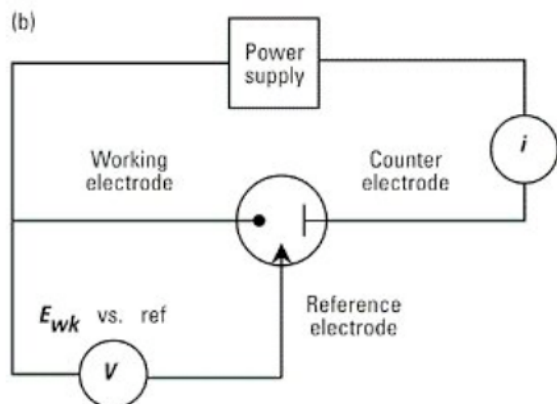

2. A typical DC polarogram is provided below. What are the units for the y-axis? In polarography, the working electrode is made of Hg and drops a new drop every t_d seconds. What causes the "sawtooth" pattern? Why is a new drop needed?
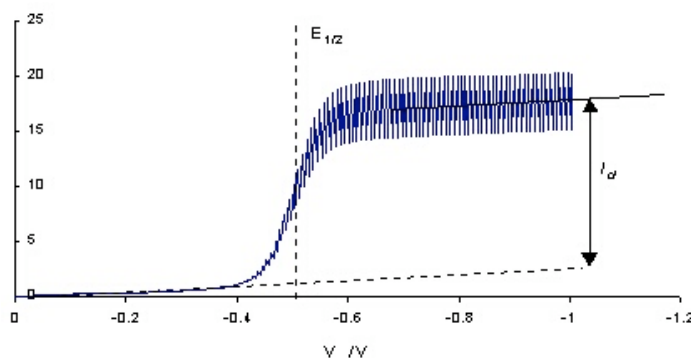
3. The analyte concentration can be quantified in a polarographic experiment by the Ilkovich equation below which relates the diffusion current (i_d) to the diffusion coefficient of the analyte in the medium (D), the number of electrons transferred per mole of analyte (n), the mass flow rate of Hg through the capillary (m), the drop lifetime (t), the analyte concentration (c) and k, a constant of proportionality dependent on the units used. Explain the reason for each term.

$$i_d = knD^{1/2}m^{2/3}t^{1/6}c$$

4. In a polarographic experiment, there is a charging current and a faradaic current. What is the origin of each? The polarographic solution is usually viscous, highly charged and not stirred. Suggest why.
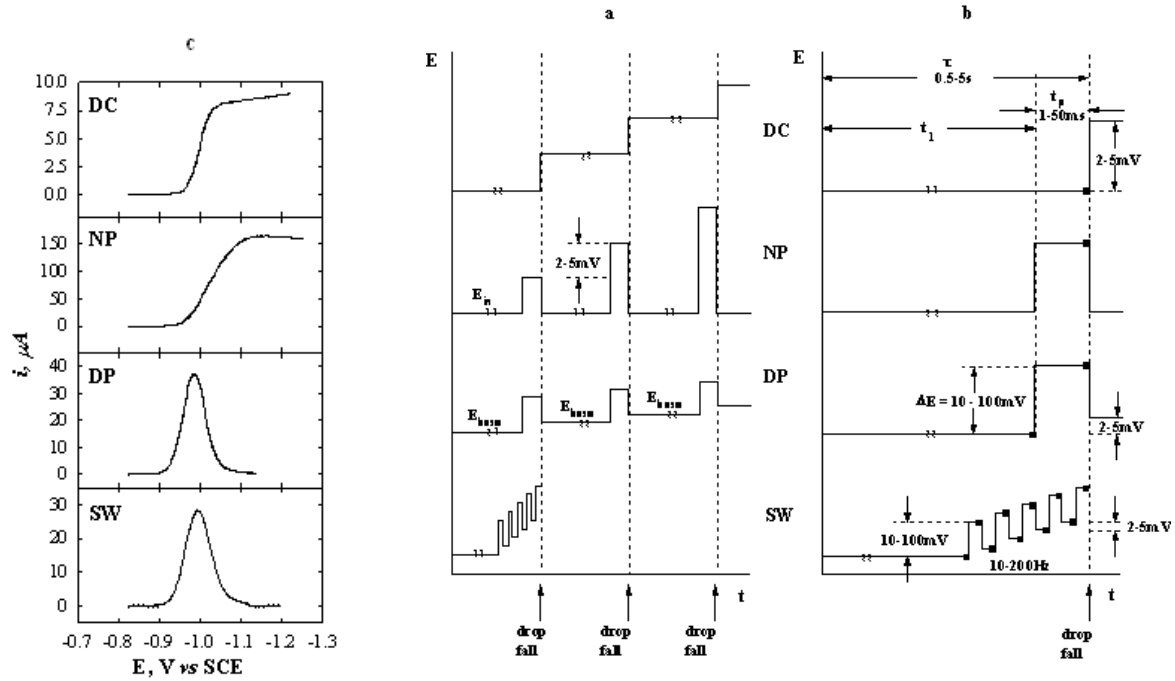

5. The diagrams above show the polarogams and their associated voltage steps with time and the current sampling (small dark rectangles). The DC and the NP techniques both give a curve that looks like a titration. Using electrons analogous to protons, draw an alpha diagram for the reaction: $M^{2+}(aq) + e- \rightarrow M^{+}(aq)$ and show which species are predominant at each region in the diagram. What is the electrochemical analog for pK_a?

6. What is E° for the system above? Does it change based on technique? Explain.

7. Suggest how the polarogram in Q2 would look in the NP mode as opposed to the DC mode. Why?

8. The DP and SW techniques give a derivative of the NP and DC curves. Looking at the pulse sequence, suggest how.

MODEL 2   **Cyclic Voltammetry**   The diagram shows a typical CV for an electrochemical reversible reaction at the working electrode ($FeIII(CN)_6^{3-}/FeII(CN)_6^{4-}$ couple).
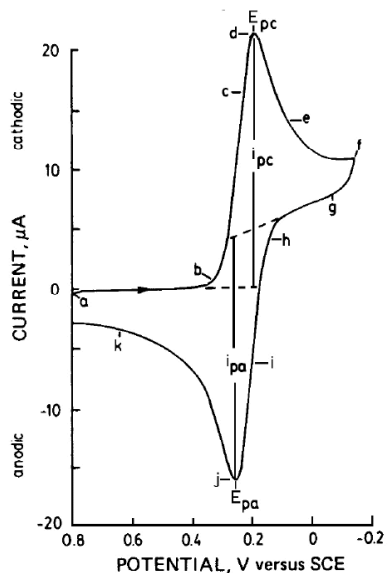
1. The CV scan proceeds in the direction from a – k. Draw a picture above of the *applied* voltage profile as a function of time from a-k.

2. Is there any electrolysis at point a?

3. What is staring to happen at point b?

4. What species concentration is substantially diminished at point d?

5. From point *d* to *e* why does the current decay?

6. What happens at point f (the switching potential)?

7. What reaction predominates between *f* and *h?*

8. What species is predominant ($\alpha \sim 1$) at point j?

9. As the scan rate increases the shape from f-k usually looks much different. How would it look and why would it look that way? The equation for the peak current i_p, in CV is below where v = scan rate.

$$i_p = 2.69x10^5 n^{3/2}AD^{1/2}Cv^{1/2}$$

MODEL 3   Sinusoidal Cyclic Voltammetry
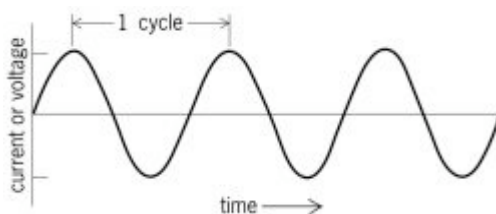
1. This sine wave is drawn in the time domain. Plot the current in the frequency (x-axis) domain.

2. Draw a superimposed sine wave with double the frequency and the same amplitude. Draw the sum of the two waves. Plot the sum of the two waves in the frequency domain.

3. The authors describe SCV in several prior papers. How does each quote inform us about how the technique is implemented?

"…when large amplitude sine waves are used (instead of triangle waves), the peak current is no longer linearly related to the amplitude of the superimposed sine wave and as the amplitude increases so does distribution of the faradaic signal into the higher harmonics."

"The current response to this sinusoidal excitation is monitored in the frequency domain instead of the time domain. The background charging current is primarily a phase-shifted sine wave with a major component at the fundamental frequency. In contrast, the faradaic current has a lot of components in the higher harmonics due to the nonlinearity associated with the current due to oxidation (or reduction)."

"The second harmonic frequency is monitored using a conventional lock-in amplifier in a continuous scan cyclic voltammetric experiment. The background charging current is predominantly composed of odd harmonic frequencies, while the analyte faradaic signal is composed of both odd and even harmonic frequencies. By locking-in on the second harmonic frequency one can isolate the faradaic current from the charging current in the frequency domain."

"The acquisition software for the SV experiment collects four sinusoidal cycles, containing 512 points, in a single scan and in real time performs a fast Fourier transform (FFT). The scans are digitally sampled at 128 times the excitation frequency, and about 200 scans are collected in a single experiment. The collection of a large number of points, on the order of 102,400, increases the resolution of the lower frequency components. Usually, only the first 10 harmonics of the frequency domain, consisting of a magnitude and phase angle for each, are saved."

MODEL 4   Cyclic Voltammetry of Electrochemical Primer Labels

(A) $E_{1/2}$= 39 mV
(B) $E_{1/2}$= 73 mV
(C) $E_{1/2}$= 202 mV
(D) $E_{1/2}$= 268 mV
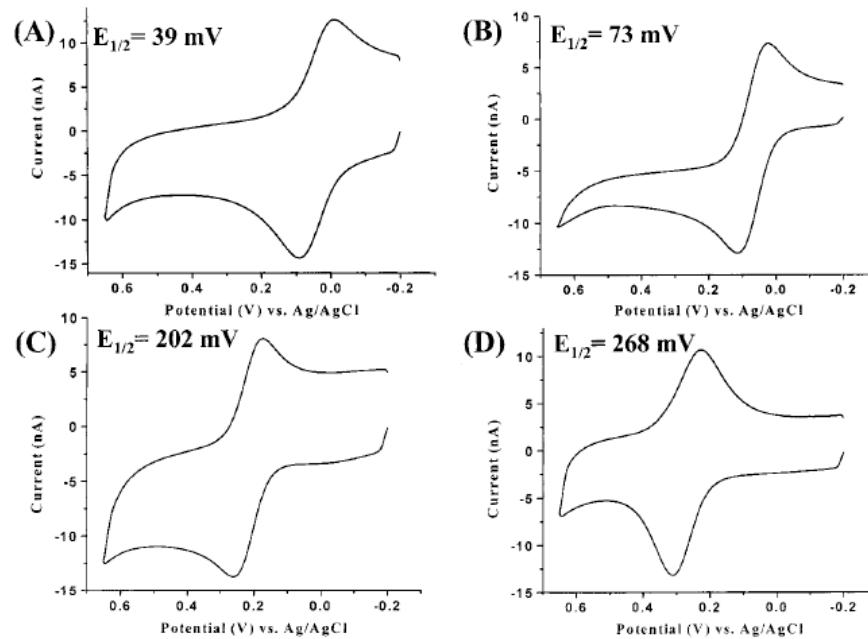
1. The authors state that the ΔE_{peak} for each tagged primer is close to 60 mV, which indicates reversible Nernstian behavior. What do they mean? What other evidence is there for reversibility?

2. Explain how the following diagram was generated and how it allows the authors to obtain all four signals required for DNA labeling.
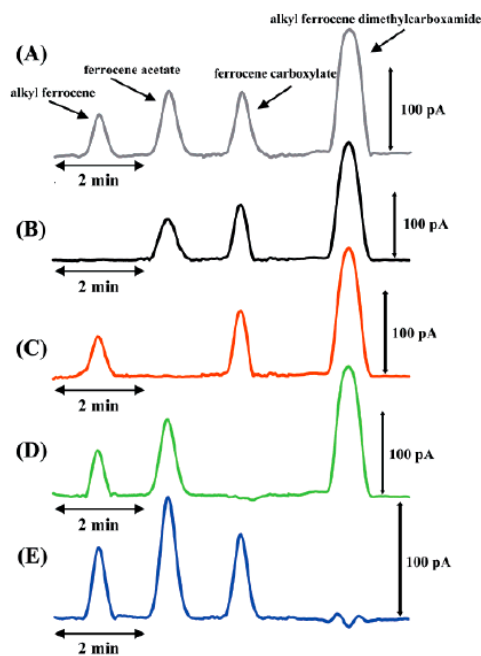
Figure legend: (A) Phase-common response: the time domain signal at the third harmonic (33 Hz) was selected to show all of the four tagged T3 primers locked at a common phase angle of 160°. (B-E) Phase nulled response for each tagged T3 primer: (B) AF tag, the time domain response is locked at 168°, which is exactly +90° out of phase with the optimum phase angle for the AF tag; (C) FA tag, the time domain response is locked at 139°, which is +90° out of phase with the FA tag; (D) FC tag, the time domain response is locked at 83°, which is -90° out of phase with the FC tag; (E) AFD tag, the time domain response is locked at 41°, which is -90° out of phase with the AFD tag.

3. What are the main advantages and disadvantages of using electrochemical labels for DNA sequencing?

Appendix: Further ideas to help in understanding SCV
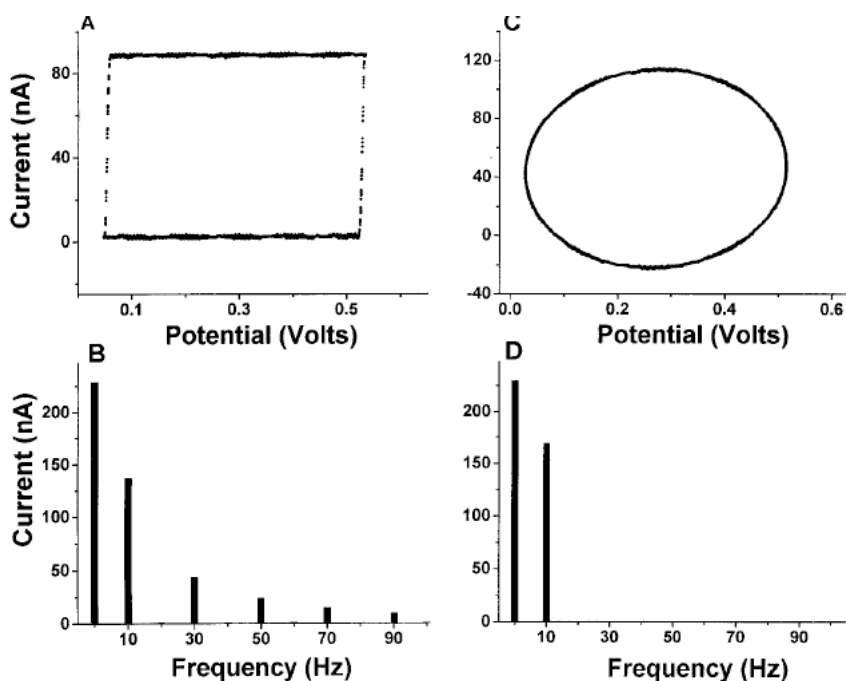
Figure 1. Comparison of charging currents in sinusoidal voltammetry with those in cyclic voltammetry. (A) Background voltammogram in cyclic voltammetry. (B) Frequency domain response of the cyclic voltammogram. (C) Background voltammogram in sinusoidal voltammetry. (D) Frequency domain response of the sinusoidal voltammogram. The dummy electrochemical system consists of a resistor (0.56 kΩ) and a capacitor (0.2 μF) in series. This combination was used to simulate an electrochemical cell with only charging current components. Excitation waveform: 10 Hz{Triangle wave for A and sine wave for C}, 0.5 V_{p-p}, +0.3 V dc offset.

…the faradaic current is distributed over several different harmonic frequencies, which are generated due to nonlinear effects of diffusion-limited mass transfer. … the background charging current in a cyclic voltammetry experiment is composed of primarily odd harmonic frequencies, while the faradaic current is composed of both odd and even harmonic frequencies.

The use of a sinusoidal wave as an excitation waveform instead of a triangle wave as used previously should aid in even higher discrimination between the faradaic and the background charging current. AC voltammetry utilizes a small-amplitude sine wave which is added to a potential ramp to modulate the current output. Typically, a modulation potential amplitude up to 20 mV is used; higher amplitudes are not used to specifically avoid contributions from higher order harmonics. Therefore, small-amplitude modulations (typically 10 mV) are used to minimize nonlinear effects and enhance resolution. This may be thought of as a limiting case for sinusoidal voltammetry as all of the information is intentionally contained in only the lowest order harmonics. …a large-amplitude sinusoid is necessary to regenerate the electrode surface and to induce a nonlinear faradaic response that will shift the faradaic signal to higher frequencies. When the background consists primarily of charging current, the frequency spectrum of the background current generated by a sine wave excitation potential is concentrated at the fundamental harmonic, as compared to the current response to a triangle wave, which has components at all odd harmonics (figure above). Consequently, not only the even harmonics (e.g., second) but also the higher odd harmonics (e.g., third, fifth etc.) can be utilized for detection when a sine wave is used as an excitation potential waveform to achieve the best possible sensitivity and selectivity for a measurement.
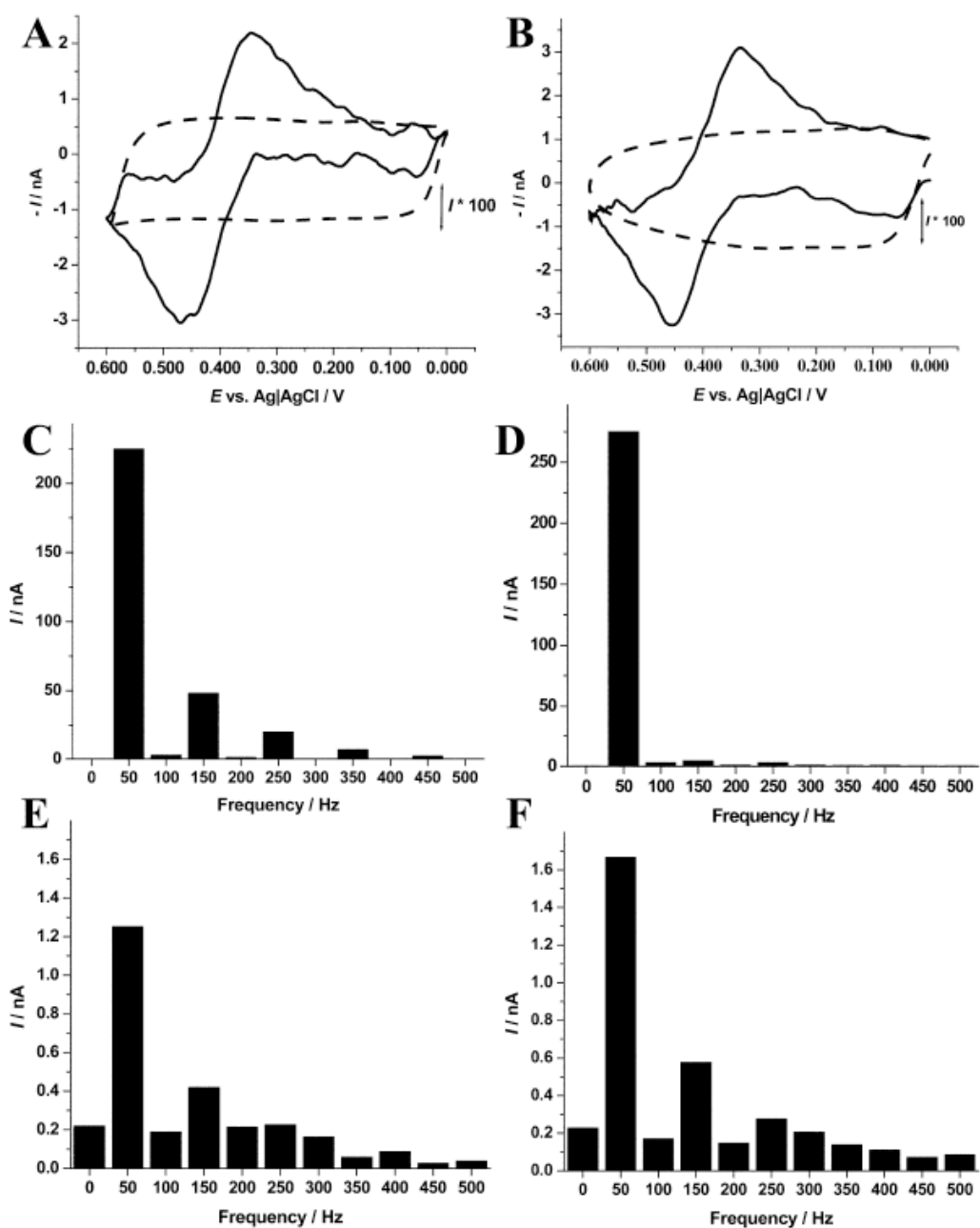
Fig. 1. Comparison of cyclic and sinusoidal voltammetric responses. A 10 μM solution of FCA in 0.1 M phosphate buffer with 0.15 mM of sodium perchlorate added was injected into the FIA system. The scan frequency used was 50 Hz (60 V s^{-1}) and 0–600 mV vs. Ag | AgCl was applied to a 25 μm polished gold disk microelectrode. (A) Cyclic voltammograms for the background (dotted trace, current attenuated by a factor of 100) and faradaic current (solid trace). (B) The sinusoidal voltammograms of the background (dotted trace, current attenuated by a factor of 100) and faradaic current (solid trace). (C) The frequency spectrum of the background response using a triangular wave excitation. (D) The frequency spectrum of the background response using a sine wave excitation. (E) The background subtracted frequency spectrum for 10 μM FCA excited with a triangular wave. (F) The frequency spectrum for 10 μM FCA using a sine wave excitation waveform.

Authors explanation of the diagram above: The faradaic current response obtained using either a triangular or sine wave contains similar frequency elements. The similarity between these two waveforms is illustrated in Figs 1A and B, where the traditional time domain response (I /E curve) at a 25 mm gold electrode is shown. In panel A of Fig. 1, the conventional cyclic voltammogram is shown for the charging current (dotted line) and the background subtracted faradaic response (solid line) of 10 mM FCA. The background current is about two orders of magnitude larger than the faradaic current and is thus scaled by a factor of 100 on the graph. The same experimental parameters used for the CV experiment in Fig. 1A are used in Fig. 1B, with the exception that a sine wave is used to sweep through the potential window. The potential sweep rate (V/s), called the scan rate in CV, is equivalent to the frequency of the sinusoid (Hz), defined here as the scan frequency. In addition to the different excitation waveforms, the charging current shape is different for CV and SV (Fig. 1A and B dotted trace). In CV the charging current is rectangular in shape resulting from the sharp transition at E_{switch}, while in SV it has a more rounded oval shape due to the smooth transition in potential.

The most striking difference between CV and SV illustrated in Fig. 1 is the background power spectrum. The background power spectrum (primarily contributions from charging current associated with scanning the gold electrode) of a triangular wave excitation contains signal at the fundamental and odd harmonics (Fig. 1C). The sharp transition at the top of the excitation waveform (E_{switch}) is responsible for background power distribution to the odd harmonics. The distribution is limited to the odd harmonics because the triangle is symmetrical within the applied potential window. The use of a smooth transitioned sine wave as the excitation waveform essentially eliminates background contributions at higher harmonics (Fig. 1D). The background subtracted faradaic current for a 10 mM injection of FCA is non-linear and a large part of its signal intensity is distributed to the higher harmonics, as is shown in the frequency domain spectra for both CV (Fig. 1E) and SV (Fig. 1F). However, the lack of background in all of the higher harmonics using a sine wave excitation affords greater flexibility in utilizing all possible harmonics.