EDMI Microsystems and Microelectronics

MICRO-614: Electrochemical Nano-Bio-Sensing and Bio/CMOS interfaces

Lecture #5

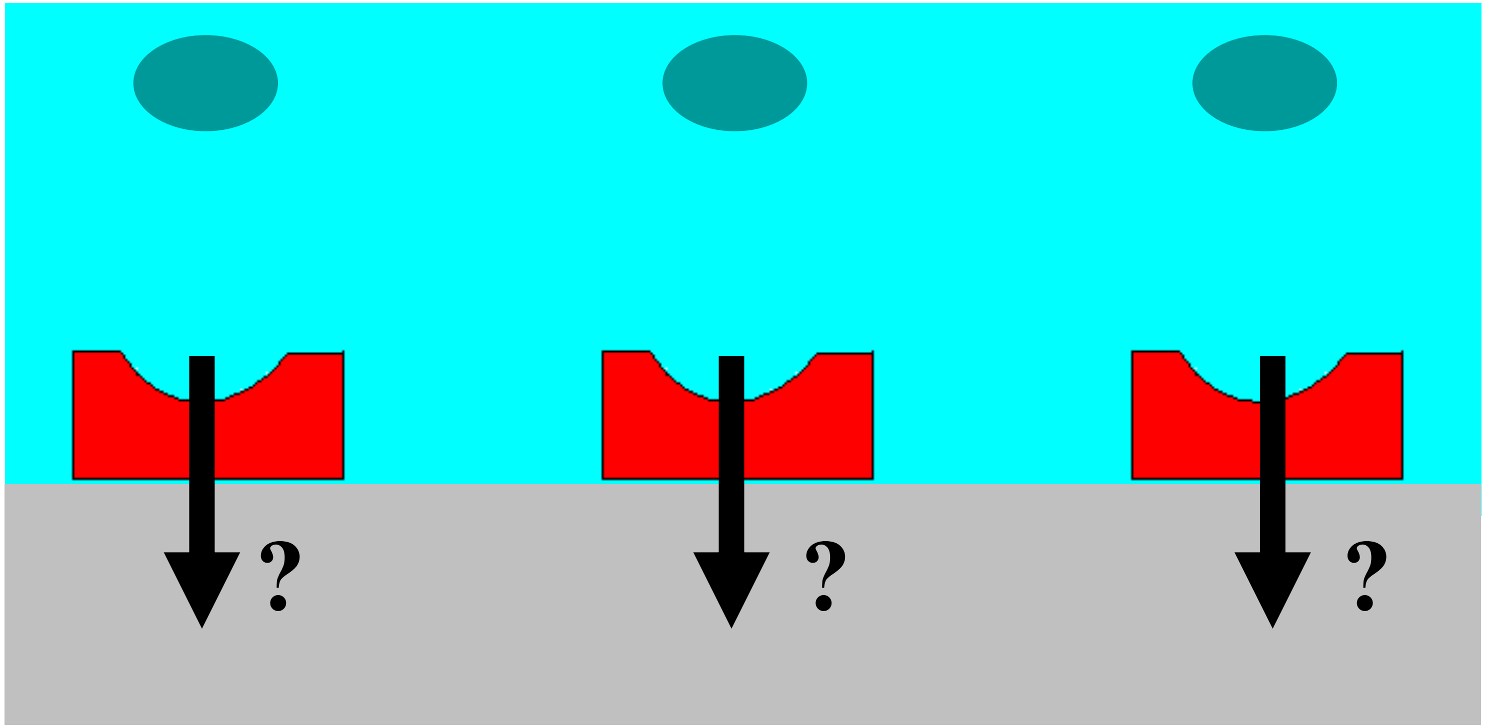
Amperometric Biosensors (with Oxidases and P450)

Lecture Outline

(Book Bio/CMOS: Chapter' paragraph § 5.2 & 10.2)

- P450 based principle of detection
- Electrochemical interfaces with enzymes
- Faradaic currents at the interface
- Electrochemical cells and equivalent circuits

CMOS/Sample interface



The interface between the CMOS circuit and the bio-sample needs to be deeply investigated and organized

Redox with P450

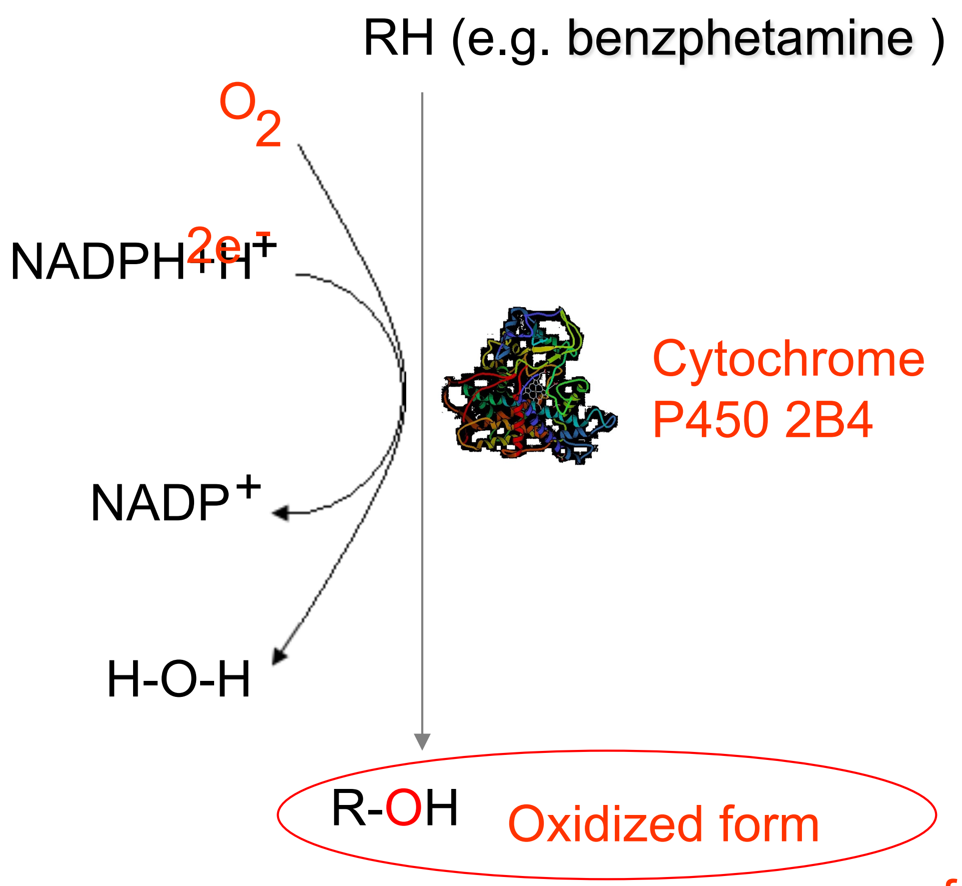
The typical redox involving a cytochrome P450 is as follows:

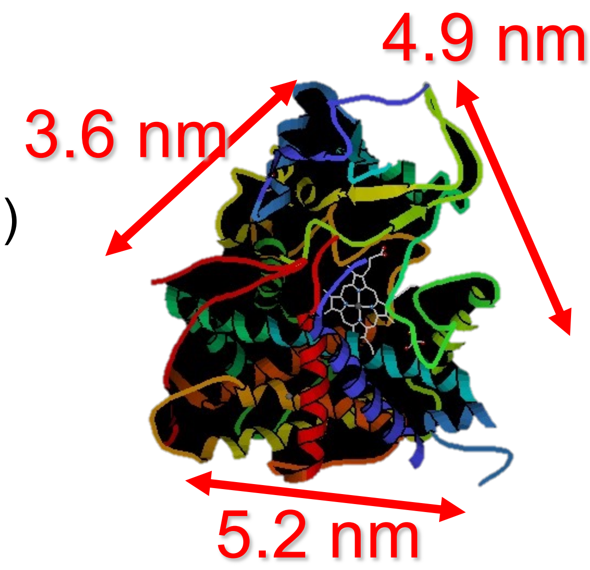
$$RH + O_2 + NADPH + H^+ \xrightarrow{P450} ROH + NADP^+ + H_2O$$

The coenzyme NADPH is mainly providing the need for two electrons required by the drug transformation. Without NADPH, the reaction occurs in water solution using hydrogen ions by water but need two extra electrons:

$$RH + O_2 + 2H^+ + 2e^- \xrightarrow{P450} ROH + H_2O$$

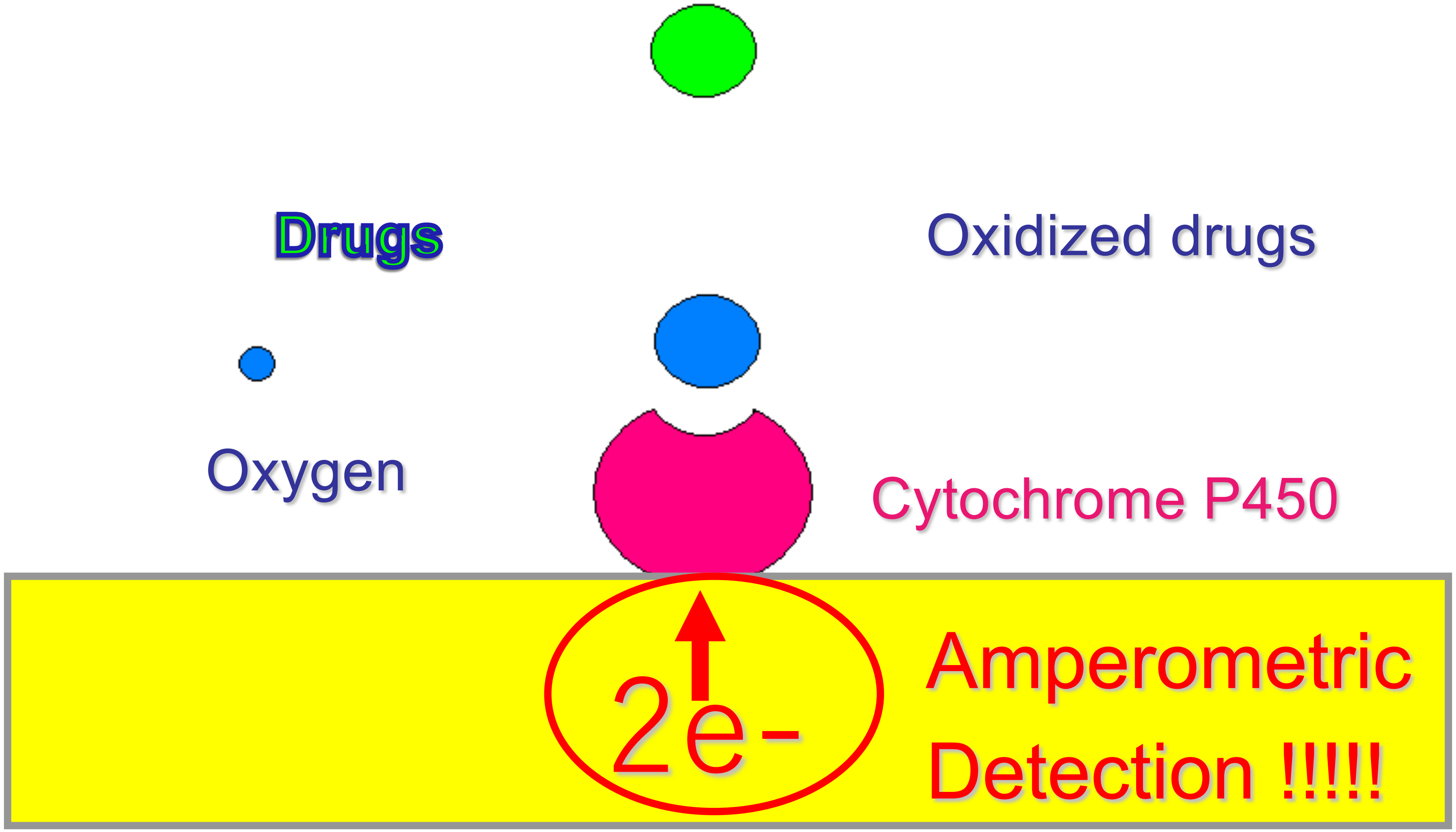
P450 Cytochromes working Principle



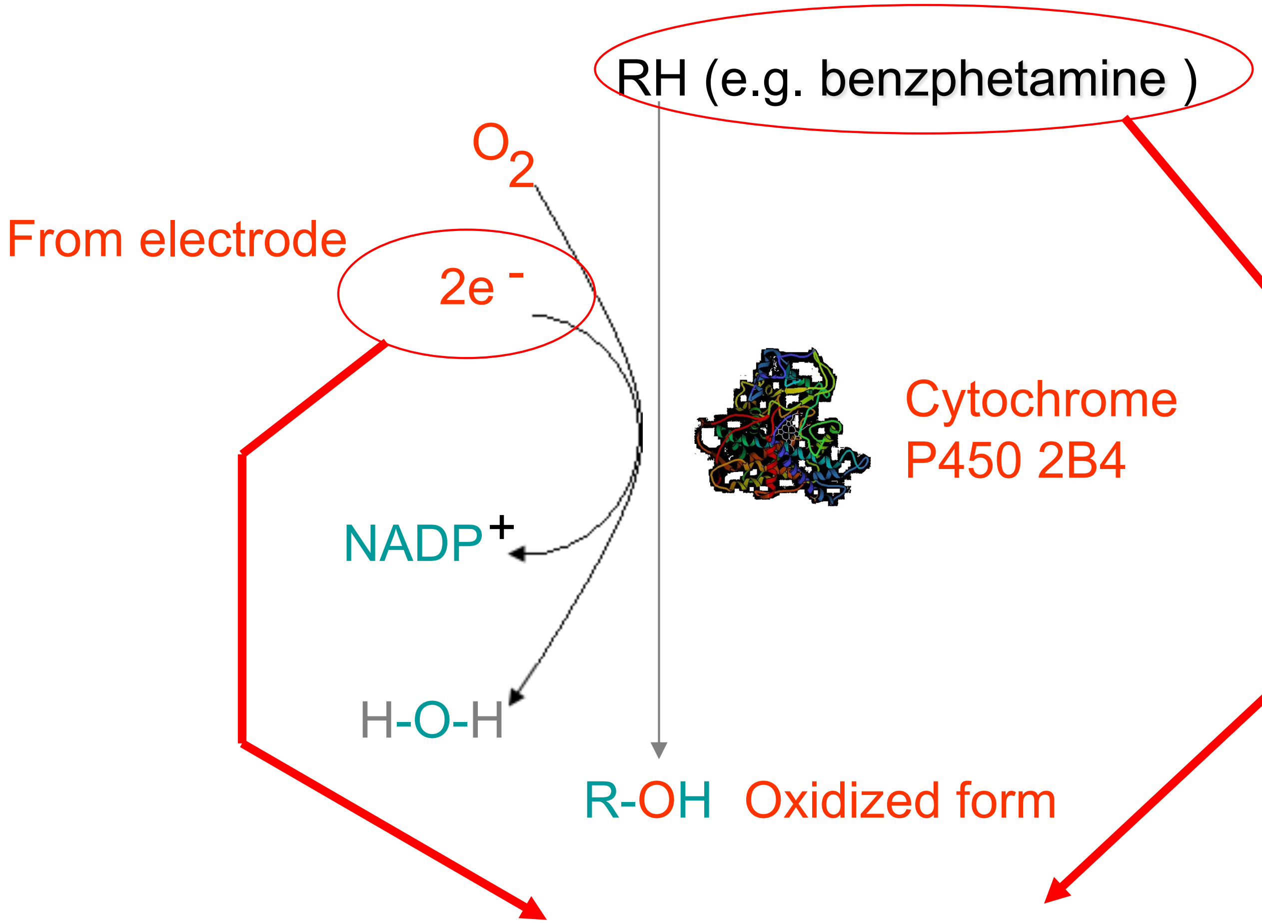


more soluble
then
faster secreted

P450-based Detection

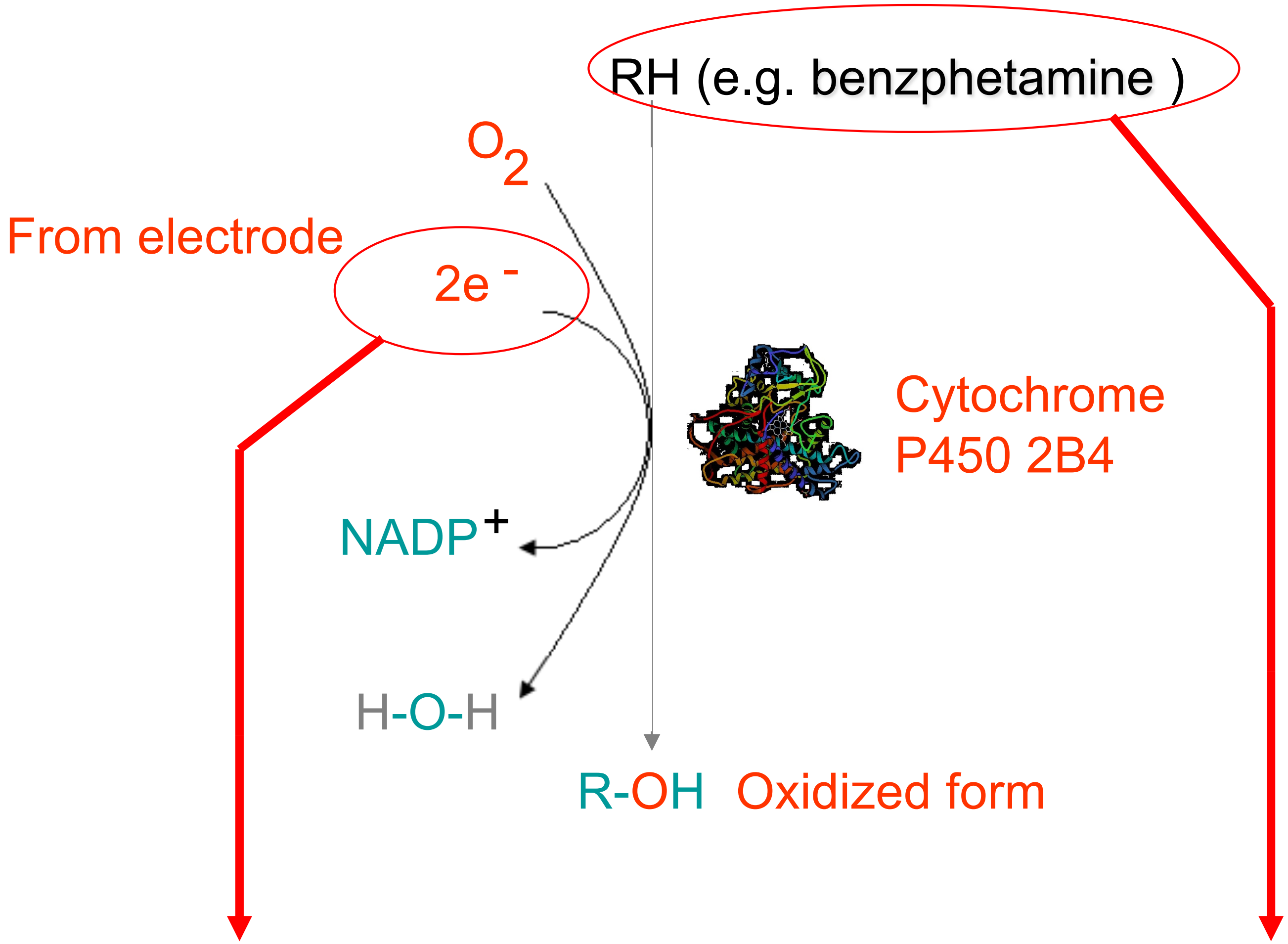


P450 based Detection



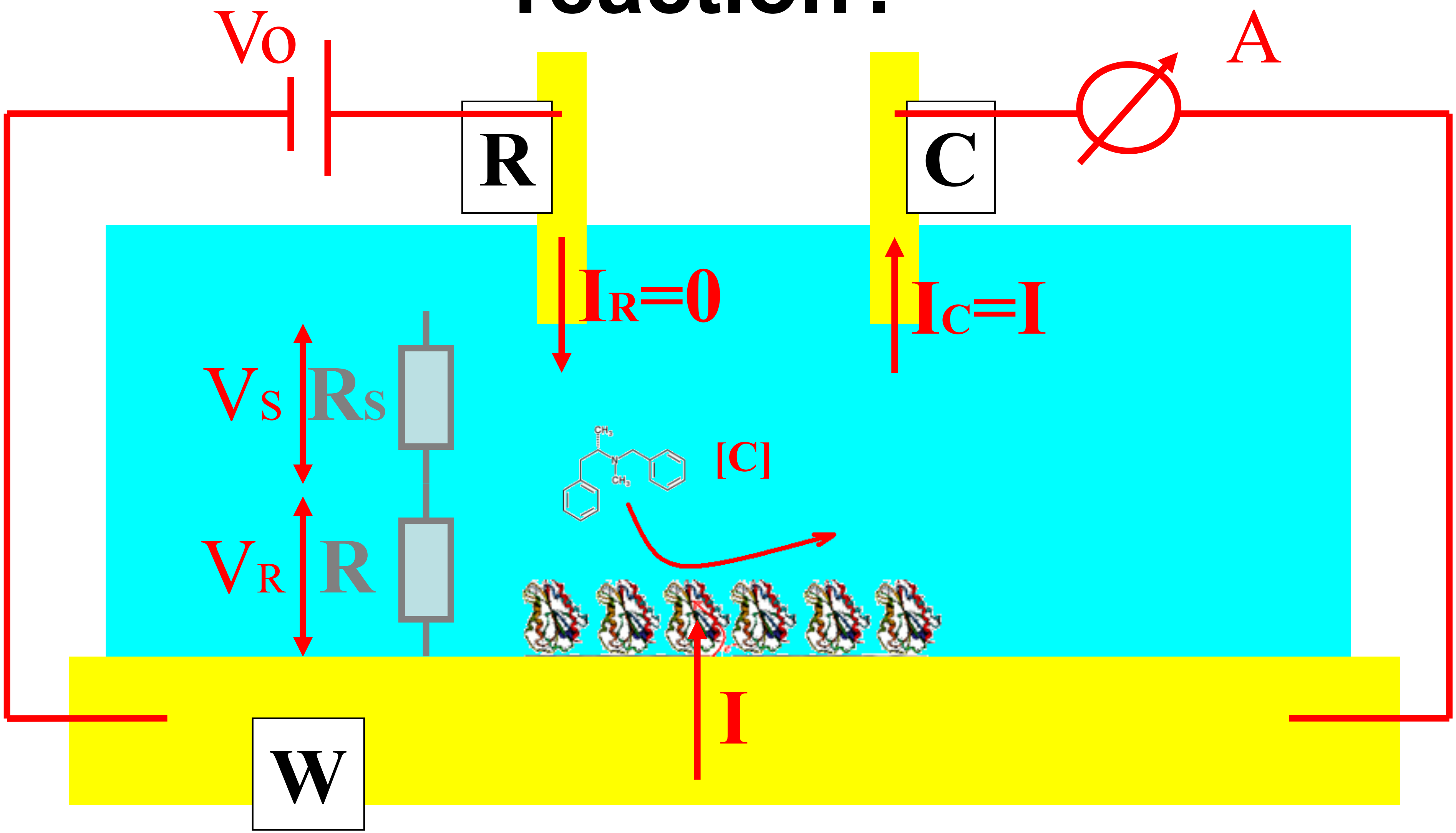
What’s about the electro-CHEMICAL properties?

P450 based Detection

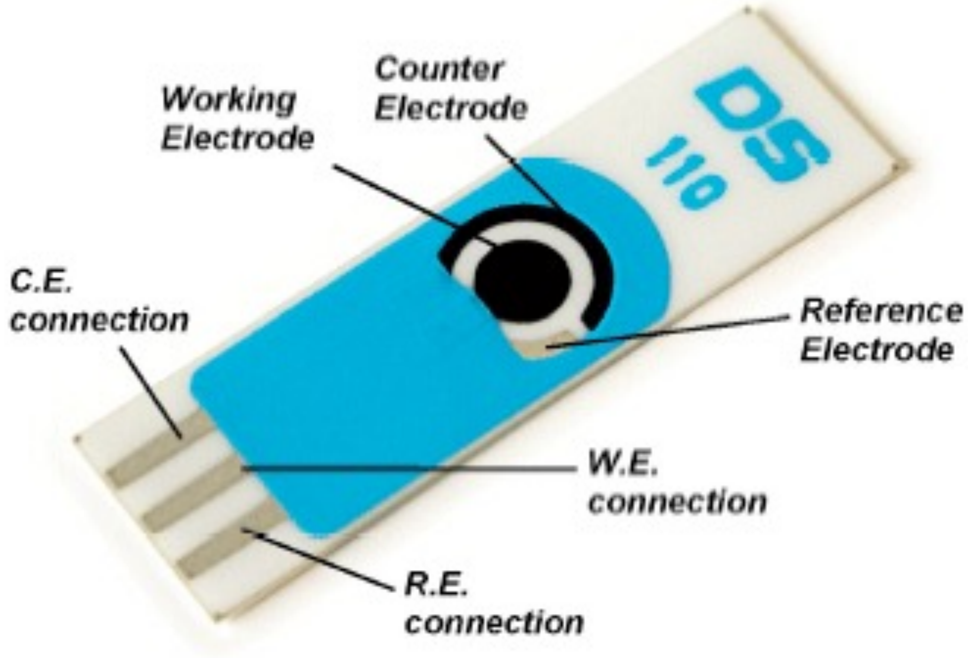


Current versus the redox species amount [concentration] upon the time

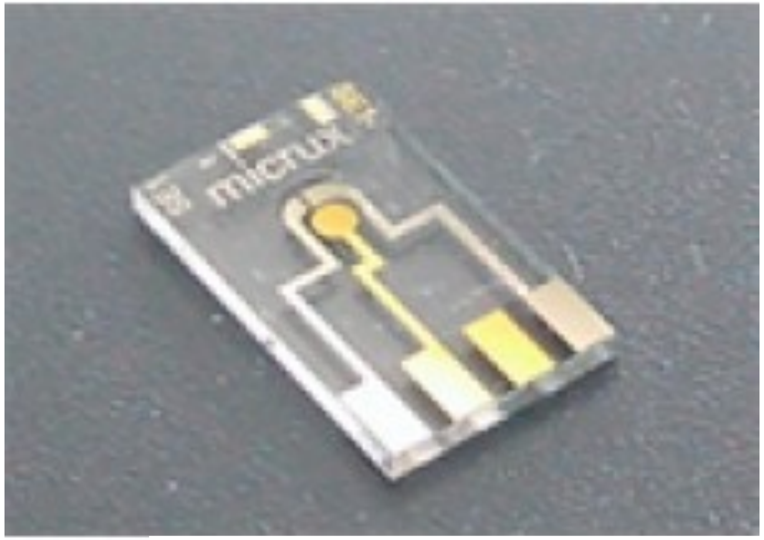
How to measure a redox reaction?

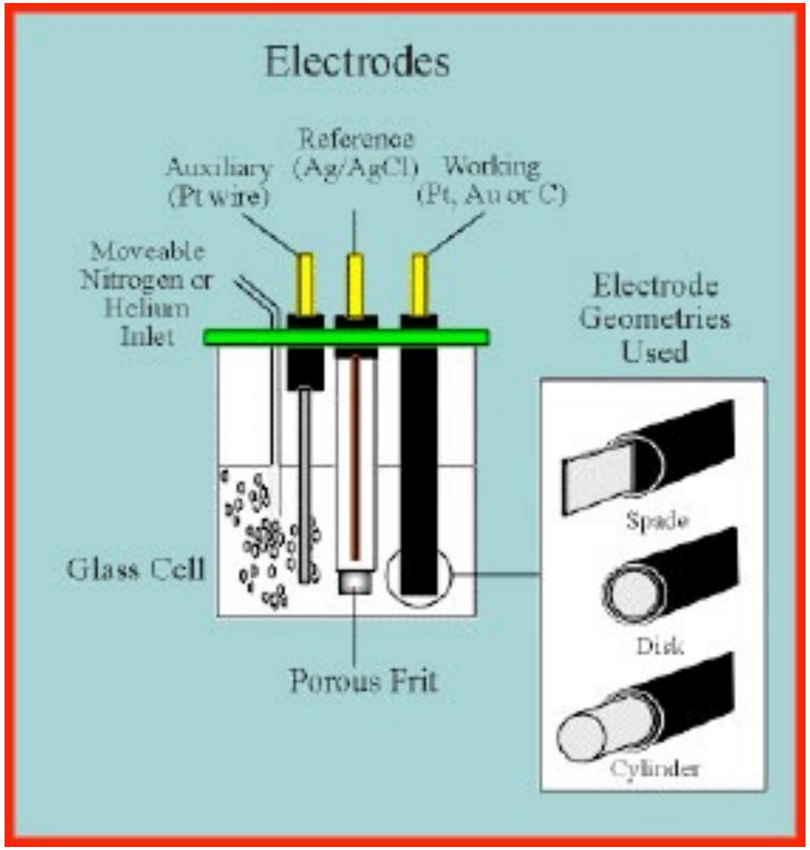


The three-electrode Electrochemical cell

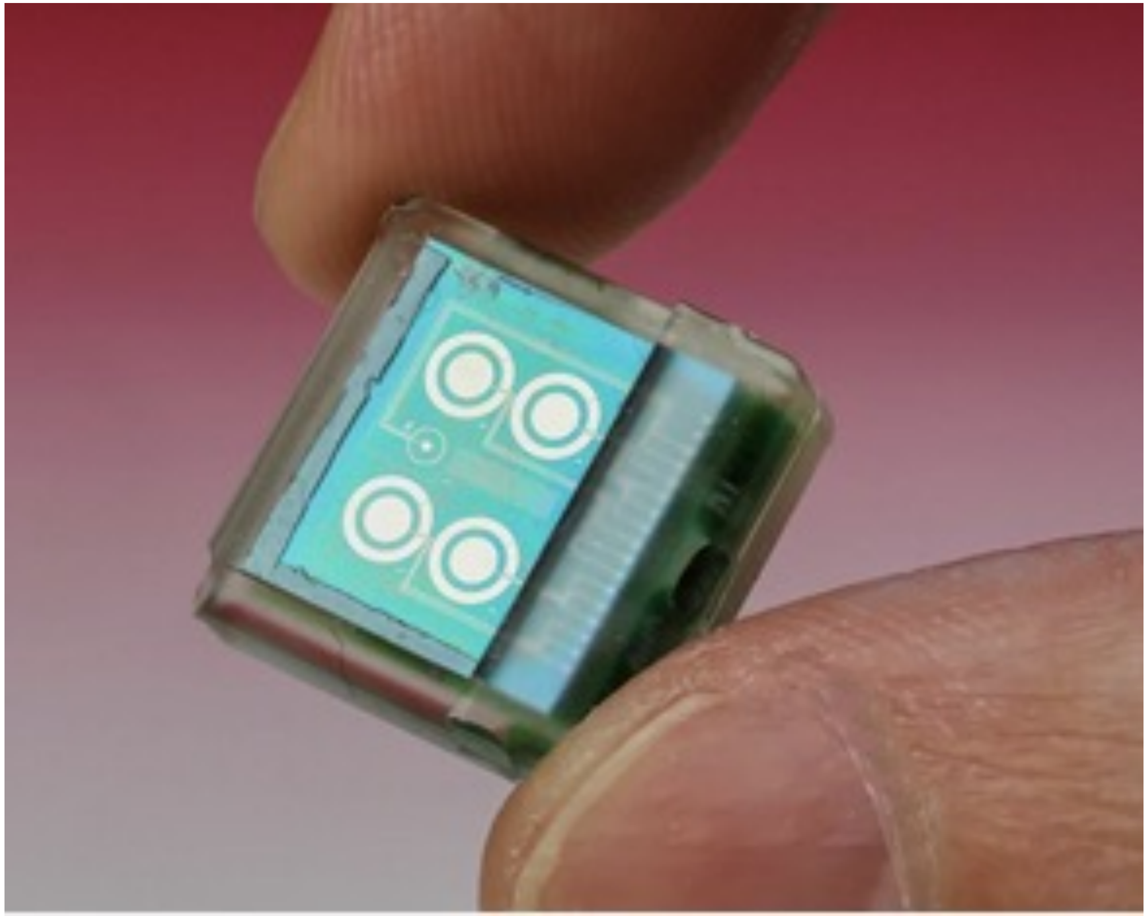


Different kinds of three-electrode Electrochemical-cell

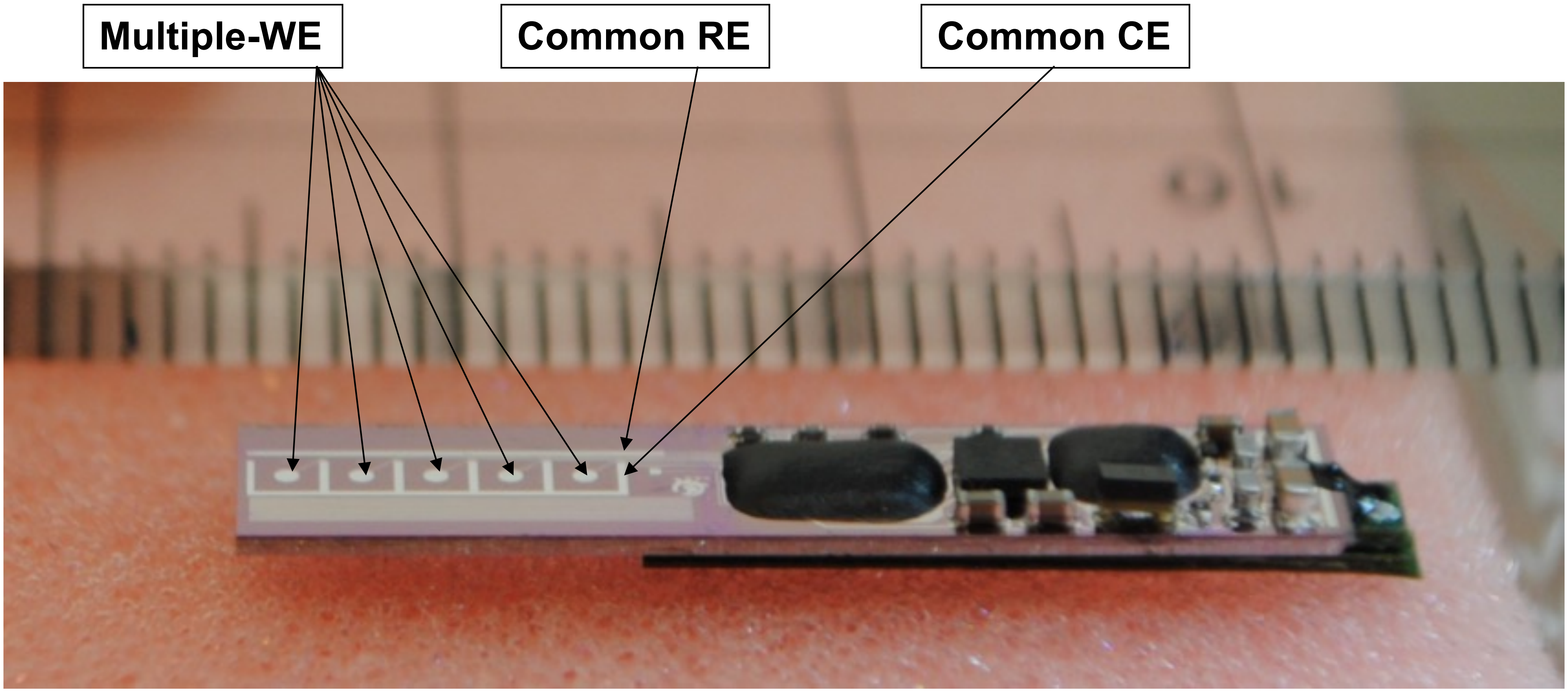




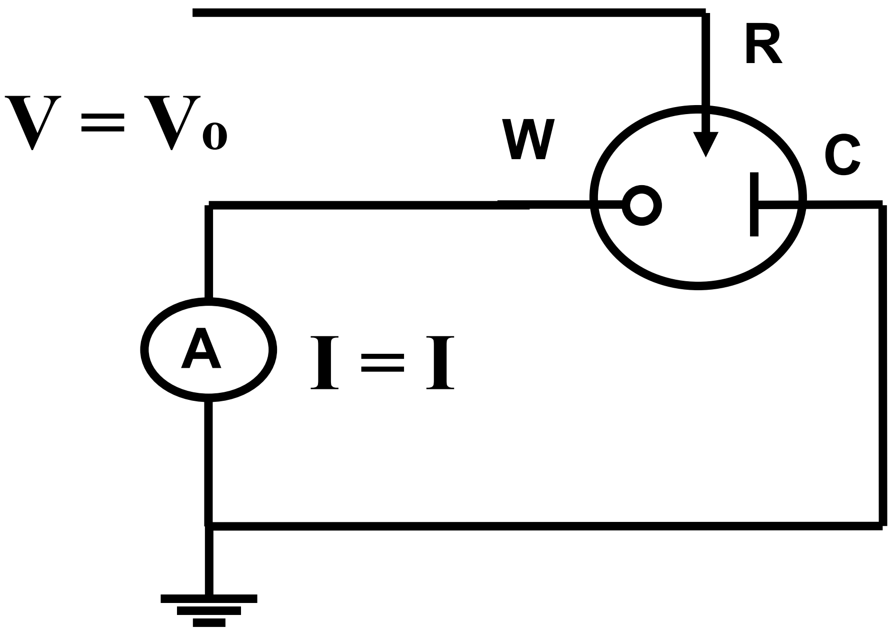
The three-electrode Electrochemical cells



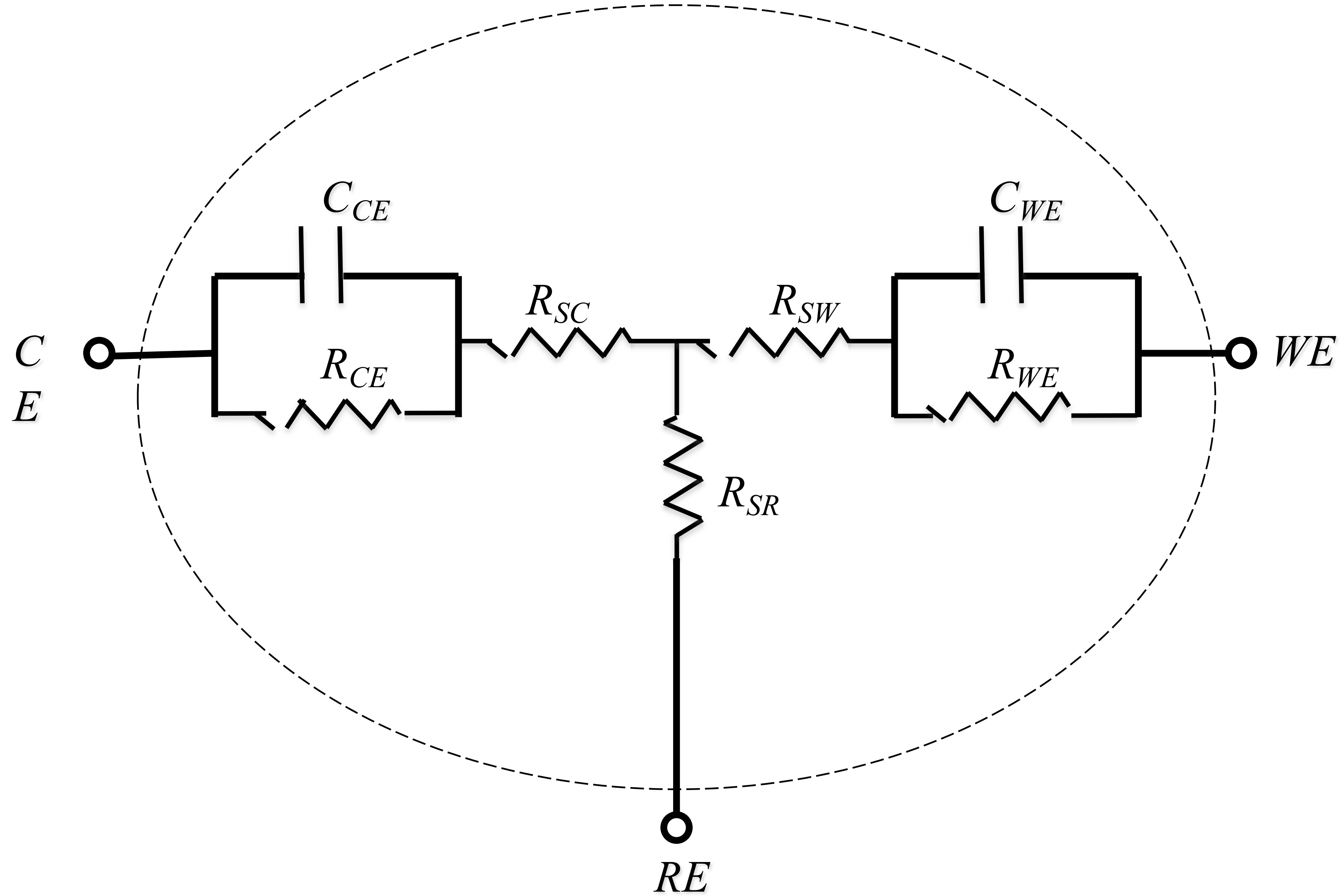
Electrochemical cells with multiple-electrodes



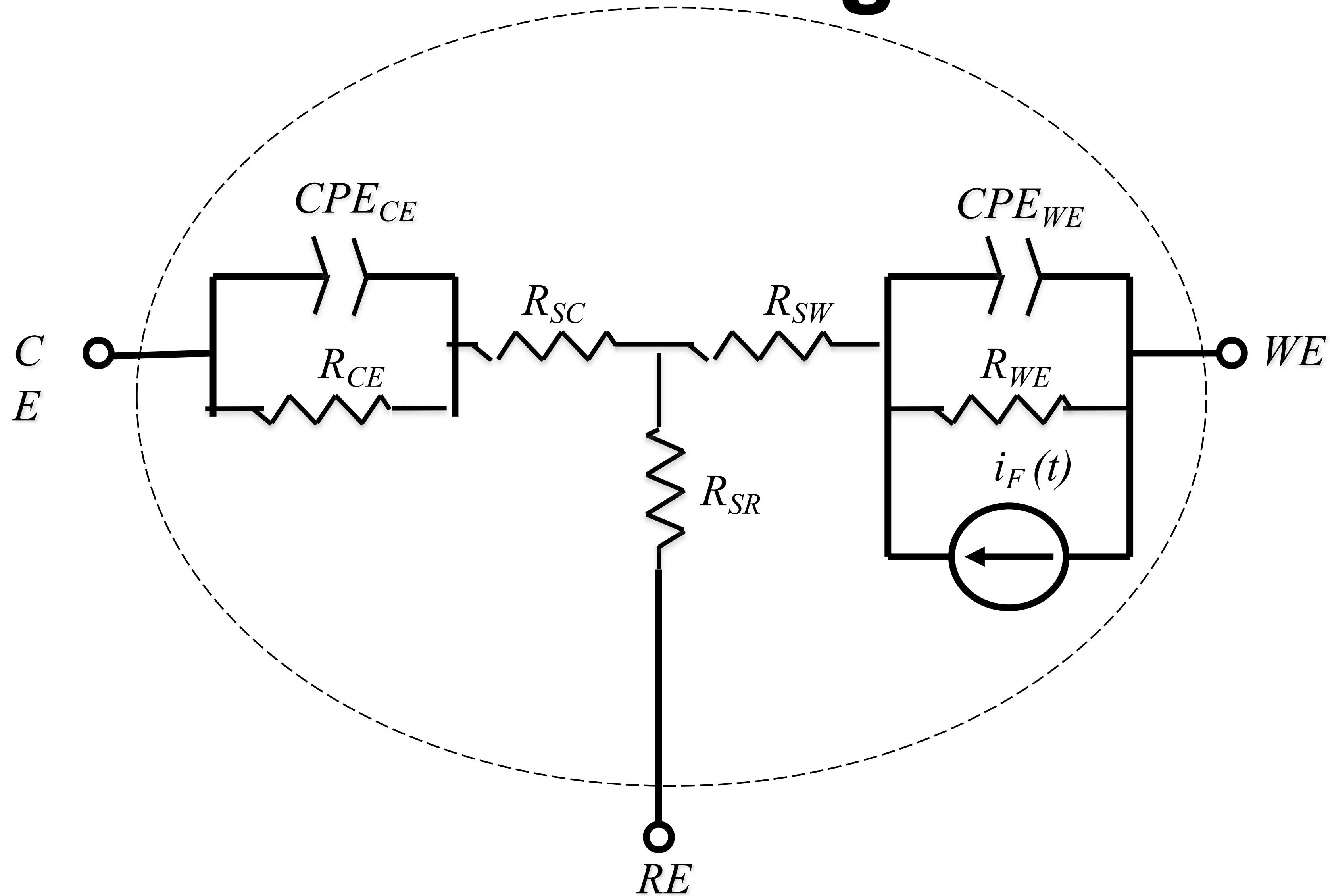
Detection Constrains



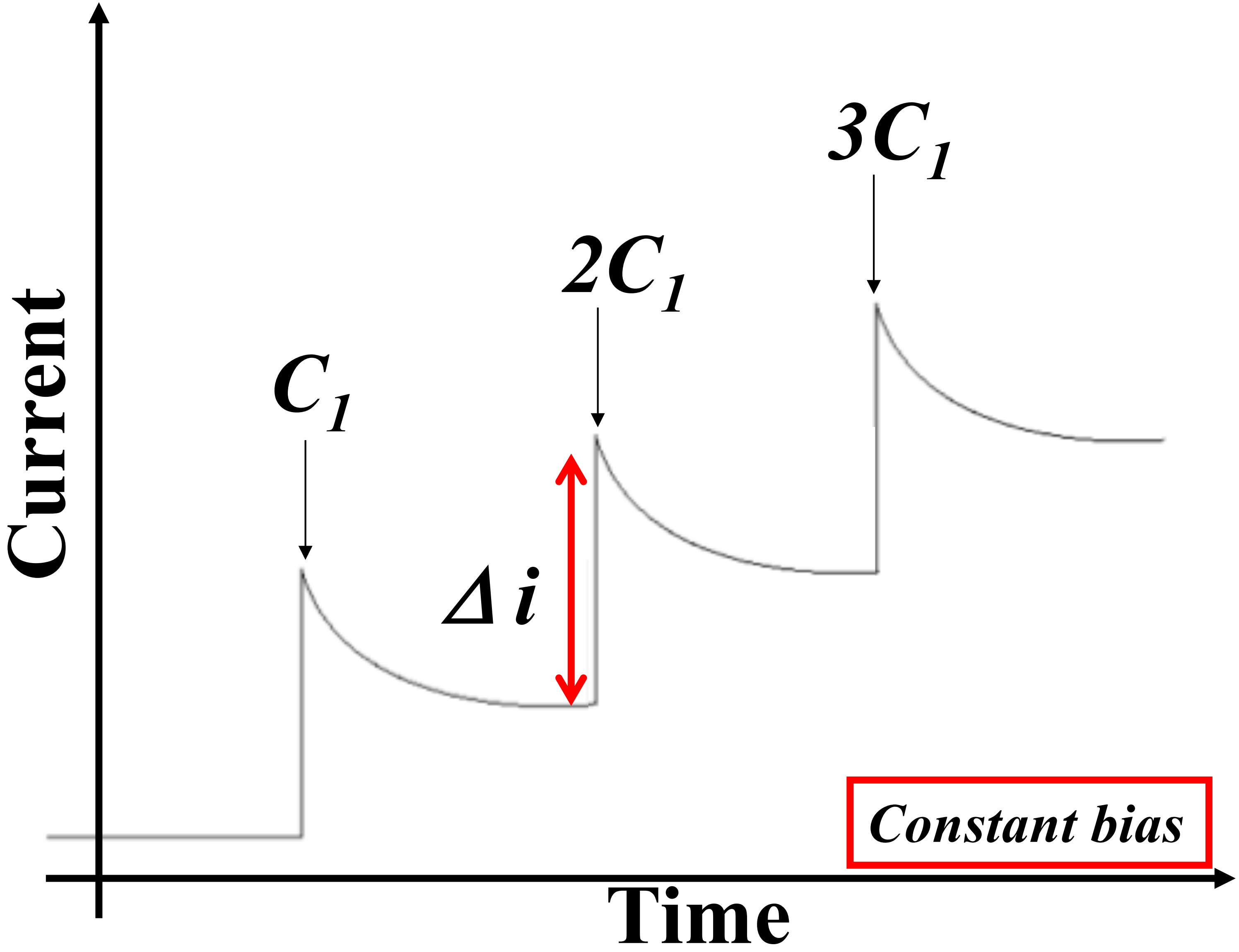
Equivalent circuit



Equivalent circuit with Faradaic current-generator

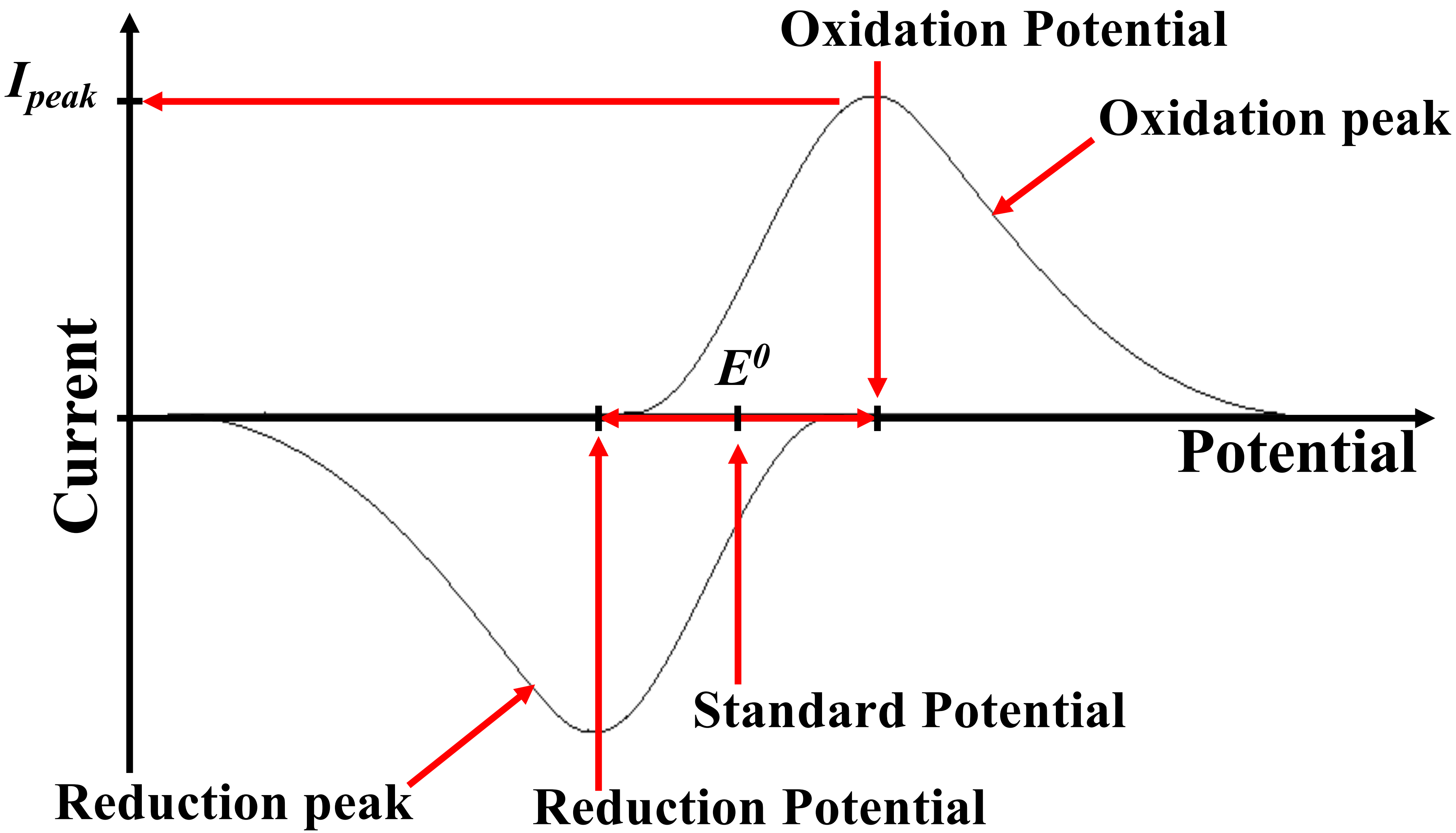


Faradaic currents from Crono-Amperometry

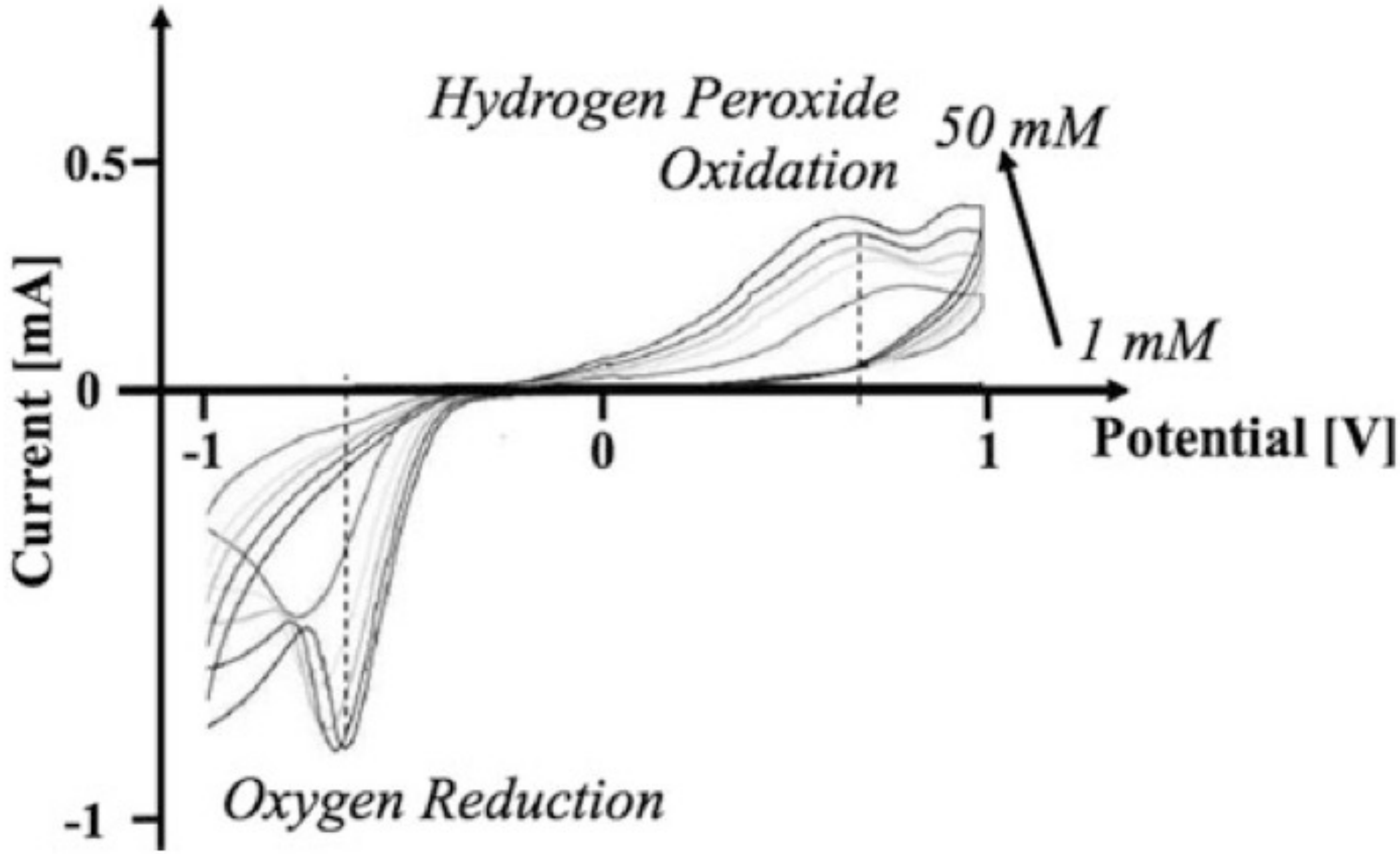


S.Carrara ©

Faradaic currents from Cyclic Voltammetry



CV with Hydrogen Peroxide



O+ reduction and H2O2 oxidation observed by potential sweeping

Redox with Hydrogen Peroxide

The hydrogen peroxide provides two possible redox reactions. An oxidation:

$$H_2O_2 \xrightarrow{+650\ mV} O_2 + 2H^+ + 2e^-$$

The produced Oxygen can be further reduced :

$$O_2 + 4H^+ + 4\ e^- \xrightarrow{-700\ mV} 2H_2O$$

Relevant Redox Reactions Equations?

$$V_{I_{MAX}} = f([C])$$

Nernst equation

$$I = f([C],V)\Big|_{\frac{dV}{dt}\neq 0}$$

Randles-Sevčik equation

$$I = f([C],t)\Big|_{V=Const}$$

Cottrell equation

To derive electrochemical Equations we need of the Laplace's Transforms

$$\widehat{f}(s) = L[f(t)] = \int_0^{\infty} f(t) e^{-st} dt \qquad L_s[t^n] = \frac{n!}{s^{n+1}}$$

$$L[af(t) + bg(t)] = a\widehat{f}(s) + b\widehat{g}(s)$$

$$L_s\left[\frac{\partial f(t)}{\partial t}\right] = s\widehat{f}(s) - f(0)$$

$$L_s\left[\frac{\partial^2 f(t)}{\partial t^2}\right] = s^2 \widehat{f}(s) - s\, f(0) - \left[\frac{\partial f(t)}{\partial t}\right]_{t=0}$$

Fick's Laws

The mass flow also has a direction driven by the gradient of concentration (defined by means of the vector differential operator):

$$\vec{j}_m = -D\,\vec{\nabla}\,C\left(\vec{x},t\right)$$

In non-vector form (by rotating the x-axis in the direction of the maximum flux and neglecting the variations on y- and z-axes):

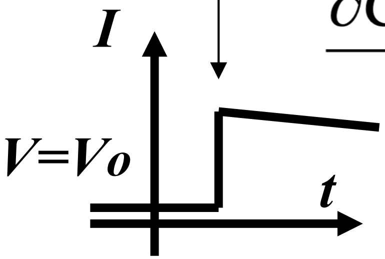
$$j_m \cong -D\,\frac{\partial C(x,t)}{\partial x}$$

The accumulation rate is provided by the mass flux through a fluidic volume:

$$\frac{\partial C(x,t)}{\partial t} = -\frac{\partial j_m}{\partial x} \longrightarrow \frac{\partial C(x,t)}{\partial t} = D\,\frac{\partial^2 C(x,t)}{\partial x^2}$$

The Cottrell Equation

[C1] Linear diffusion equation



$$\frac{\partial C(x,t)}{\partial t} = D\frac{\partial^2 C(x,t)}{\partial x^2}$$

$$L_s\left[\frac{\partial f(t)}{\partial t}\right] = s\hat{f}(s) - f(0)$$

$$s\hat{C}(s,t) - C(x,0) = D\frac{\partial^2 \hat{C}(s,t)}{\partial x^2}$$

Boundary conditions

$$\begin{cases} C(x,0) = C_0 \\ \lim_{x\to\infty} C(x,t) = C_0 \\ \lim_{t\to\infty} C(0,t) = 0 \end{cases}$$

$$C(x,0) = C(x \to \infty, t) = C_0$$

$$\frac{\partial^2 \hat{C}(x,s)}{\partial x^2} - \frac{s}{D}\hat{C}(x,s) = -\frac{C_0}{D}$$

The Cottrell Equation

$$\frac{\partial^2 \widehat{C}(x,s)}{\partial x^2} - \frac{s}{D}\widehat{C}(x,s) = -\frac{C_0}{D}$$

$$\widehat{C}(x,s) = \frac{C_0}{s} + A(s)e^{-\sqrt{s/D}x} + B(s)e^{s\sqrt{s/D}x}$$

$$\lim_{t \to \infty} C(0,t) = 0$$

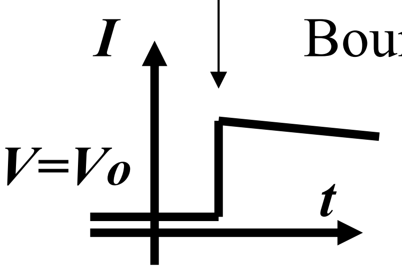
$$L_s[t^n] = \frac{n!}{s^{n+1}}$$

$$\lim_{x \to \infty} \widehat{C}(x,s) = \frac{C_0}{s}$$

$$B(s) = 0, \textit{while } \widehat{C}(x.s) = \frac{C_0}{s} + A(s)e^{-\sqrt{s/D}x}$$

The Cottrell Equation

[C1] Linear diffusion equation

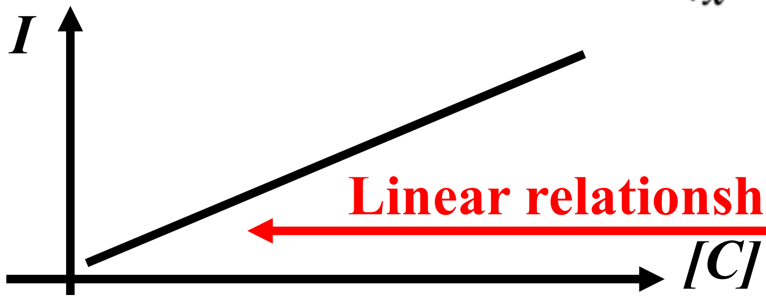


Boundary conditions $$\hat{C}_0(x,s) = \frac{C_0}{s} + A(s)e^{-\sqrt{s/D}x}$$

By definition

$$j(x,t) = \frac{i(t)}{A} = nF\left[\frac{\partial C(x,t)}{\partial x}\right]_{x=0}$$

$$\frac{\hat{i}(s)}{A} = nF \left.\frac{\partial \hat{C}(x,s)}{\partial x}\right|_{x=0}, \quad \hat{i}(s) = nFA\frac{\sqrt{D}C_0(x,s)}{\sqrt{s}}$$

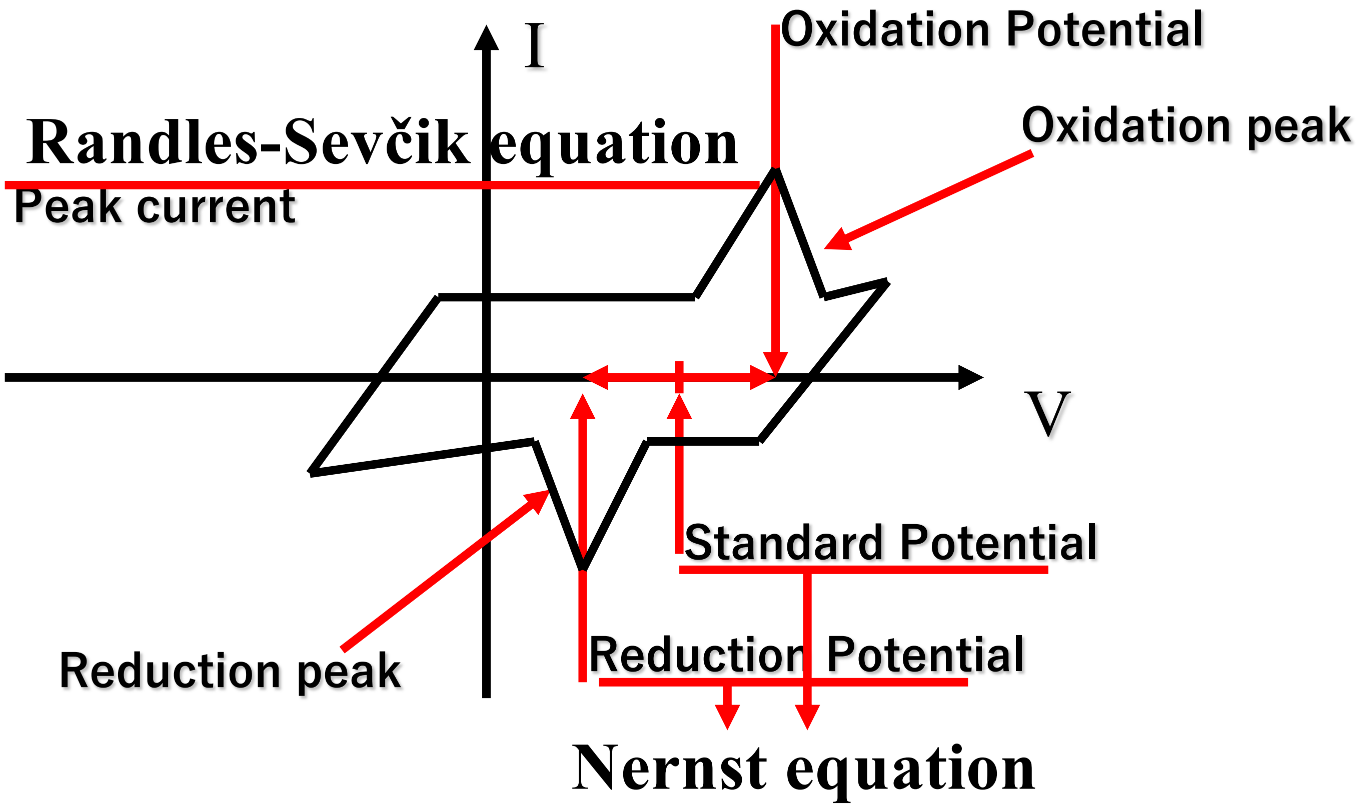


Linear relationship

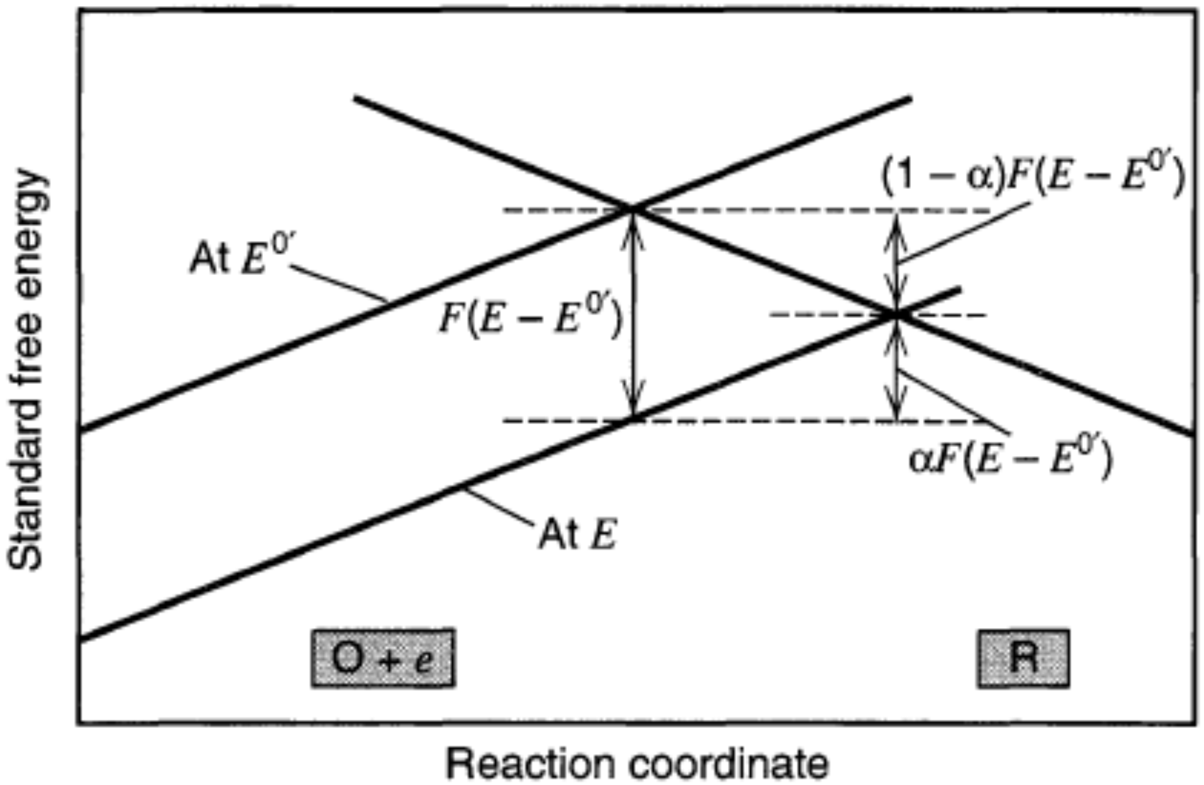
$$i(0,t) = \frac{nFA\sqrt{D}C(0,t)}{\sqrt{\pi t}}$$

Cottrell equation

Redox reactions from Voltammetry



Redox Reactions



Nernst Equation

Redox Reaction

$$O + e \underset{K_a}{\overset{K_c}{\Leftrightarrow}} R$$

Equilibrium Constants

$$\begin{cases} k_c = k_c^0 e^{-\frac{\Delta G_c}{RT}} = k_c^0 e^{-\frac{\Delta G_c^0 + \alpha n F(E - E^0)}{RT}} = k_c^0 e^{-\frac{\Delta G_c^0}{RT}} e^{-\frac{\alpha F(E-E^0)}{RT}} \\ k_a = k_a^0 e^{-\frac{\Delta G_a}{RT}} = k_a^0 e^{-\frac{\Delta G_a^0 - (1-\alpha) n F(E - E^0)}{RT}} = k_a^0 e^{-\frac{\Delta G_a^0}{RT}} e^{\frac{(1-\alpha)F(E-E^0)}{RT}} \end{cases}$$

@ Equilibrium:

$$E = 0; \alpha = 0.5; k_c = k_a \Rightarrow k_c^0 e^{-\frac{\Delta G_c^0}{RT}} = k_a^0 e^{-\frac{\Delta G_a^0}{RT}} \equiv k^0$$

Nernst Equation

Redox Reaction

$$O + e \underset{K_a}{\overset{K_c}{\Leftrightarrow}} R \qquad \begin{cases} k_c = k^0 e^{-\frac{\alpha nF(E-E^0)}{RT}} \\ k_a = k^0 e^{\frac{(1-\alpha)nF(E-E^0)}{RT}} \end{cases}.$$

The current from the redox is

$$i = i_c - i_a = nFA\left[k_c C_O(0,t) - k_a C_R(0,t)\right]$$

$$i = nFAk^0\left[C_O(0,t)e^{-\frac{\alpha nF(E-E^0)}{RT}} - C_R(0,t)e^{\frac{(1-\alpha)nF(E-E^0)}{RT}}\right]$$

@ Equilibrium:

$$i = 0 \Rightarrow C_O(0,t)e^{-\frac{\alpha nF(E-E^0)}{RT}} = C_R(0,t)e^{\frac{(1-\alpha)nF(E-E^0)}{RT}}$$

Nernst Equation

@ Equilibrium:

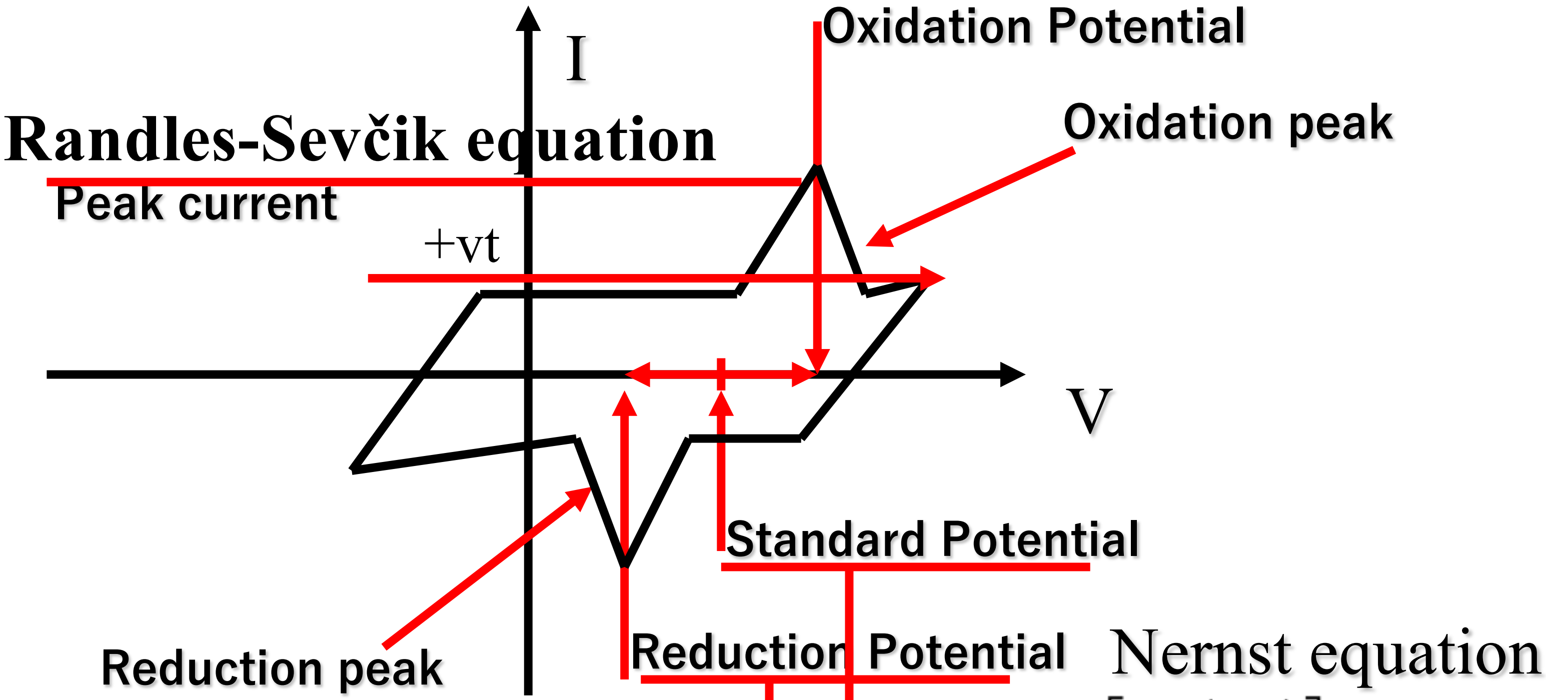
$$i = 0 \Rightarrow C_O(0,t)e^{-\frac{\alpha nF(E-E^0)}{RT}} = C_R(0,t)e^{\frac{(1-\alpha)nF(E-E^0)}{RT}}$$

$$\frac{C_O(0,t)}{C_R(0,t)} = e^{\frac{nF(E-E^0)}{RT}} \Rightarrow \frac{nF(E-E^0)}{RT} = \ln\left[\frac{C_O(0,t)}{C_R(0,t)}\right]$$

$$E = E^0 + \frac{RT}{nF}\ln\left[\frac{C_O(0,t)}{C_R(0,t)}\right]$$ Nernst equation

If n electrons are involved!

Redox reactions from Voltammetry



$$E = E^0 + \frac{RT}{nF} \ln\left[\frac{C_O(0,t)}{C_R(0,t)}\right]$$

Randles-Sevčik Equation

Voltage Sweep

$$E = E_i + \upsilon t$$

$$\frac{C_O(0,t)}{C_R(0,t)} = e^{\frac{F\left(E_{\text{initial}} + vt - E^0\right)}{RT}}$$

$$\widehat{C}_0(x,s) = \frac{C_0}{s} + A(s)e^{-\sqrt{s/D}x}$$

$$j(x,t) = \frac{i(t)}{A} = nFD\left[\frac{\partial C(x,t)}{\partial x}\right]_{x=0} \Rightarrow i(t) = nFAD\left[\frac{\partial C(x,t)}{\partial x}\right]_{x=0}$$

$$\left[\frac{\partial C(x,t)}{\partial x}\right]_{i=i_{\text{peak}}} \propto \sqrt{\frac{nFDv}{RT}}C(0,t). \Rightarrow i_{\text{peak}}(t) \propto nFA\sqrt{\frac{nFDv}{RT}}C(0,t).$$

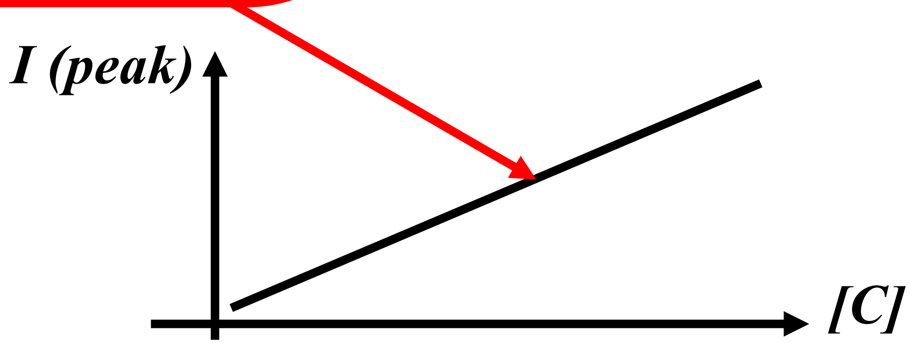
Randles-Sevčik Equation

$$\frac{C_O(0,t)}{C_R(0,t)} = e^{\frac{F\left(E_{\text{initial}}+\nu t - E^0\right)}{RT}}$$

Voltage Sweep

$$E = E_i + \upsilon t$$

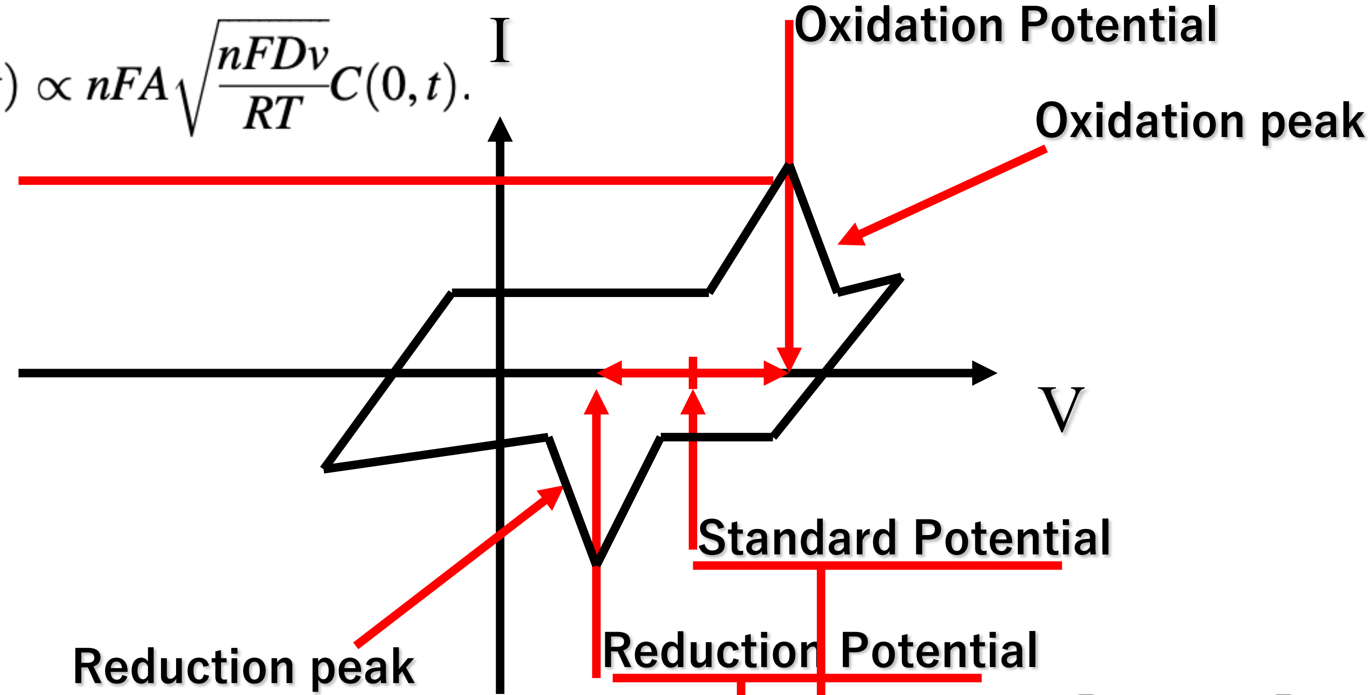
$$i_{\text{peak}}(t) \propto nFA\sqrt{\frac{nFD\nu}{RT}}\,C(0,t)$$



Redox reactions from Voltammetry

Randles-Sevčik equation for the Peak current

$$i_{\text{peak}}(t) \propto nFA\sqrt{\frac{nFDv}{RT}}C(0,t).$$



$$E = E^0 + \frac{RT}{nF}\ln\left[\frac{C_\text{O}(0,t)}{C_\text{R}(0,t)}\right].$$

Nernst equation