EDMI Microsystems and Microelectronics

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

Lecture #5

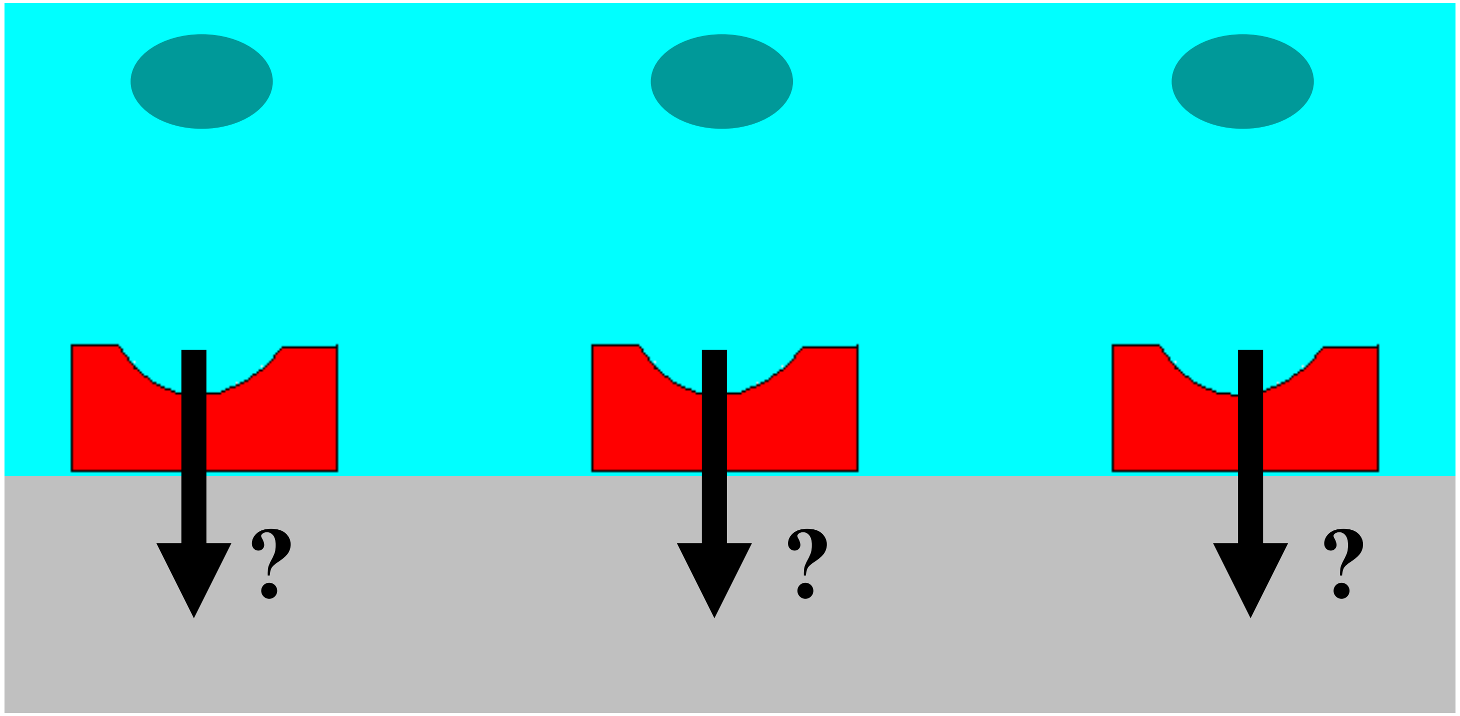
Amperometric Biosensors (with Oxidases and P450)

Lecture Outline

(Book Bio/CMOS: Chapter' paragraphs § 8.2 & 8.5-8)

- 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

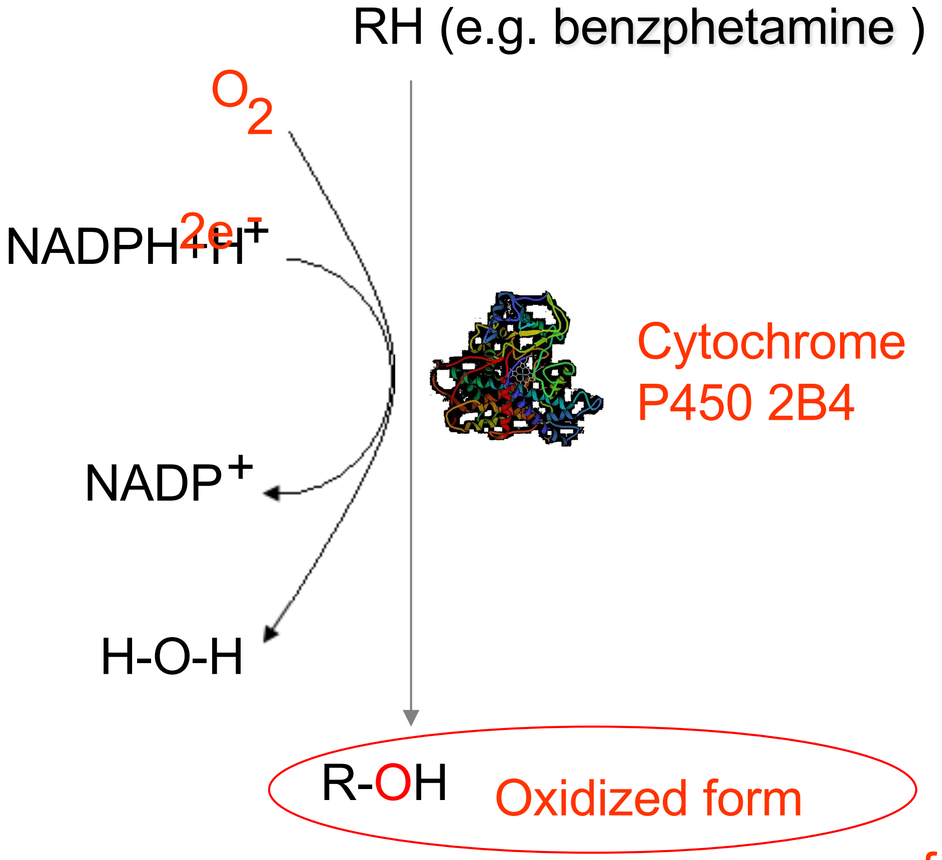
The typical redox involving a cytochrome P450 is as follows:

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

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:

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

P450 Cytochromes working Principle



RH (e.g. benzphetamine)

O_2

NADPH+H$^+$

2e$^-$

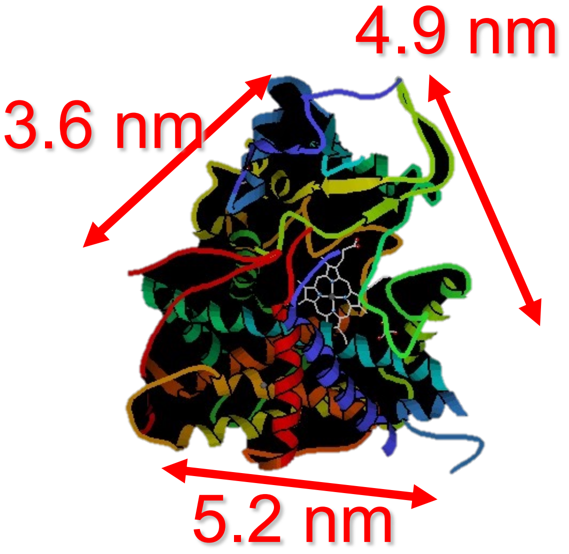
NADP$^+$

H-O-H

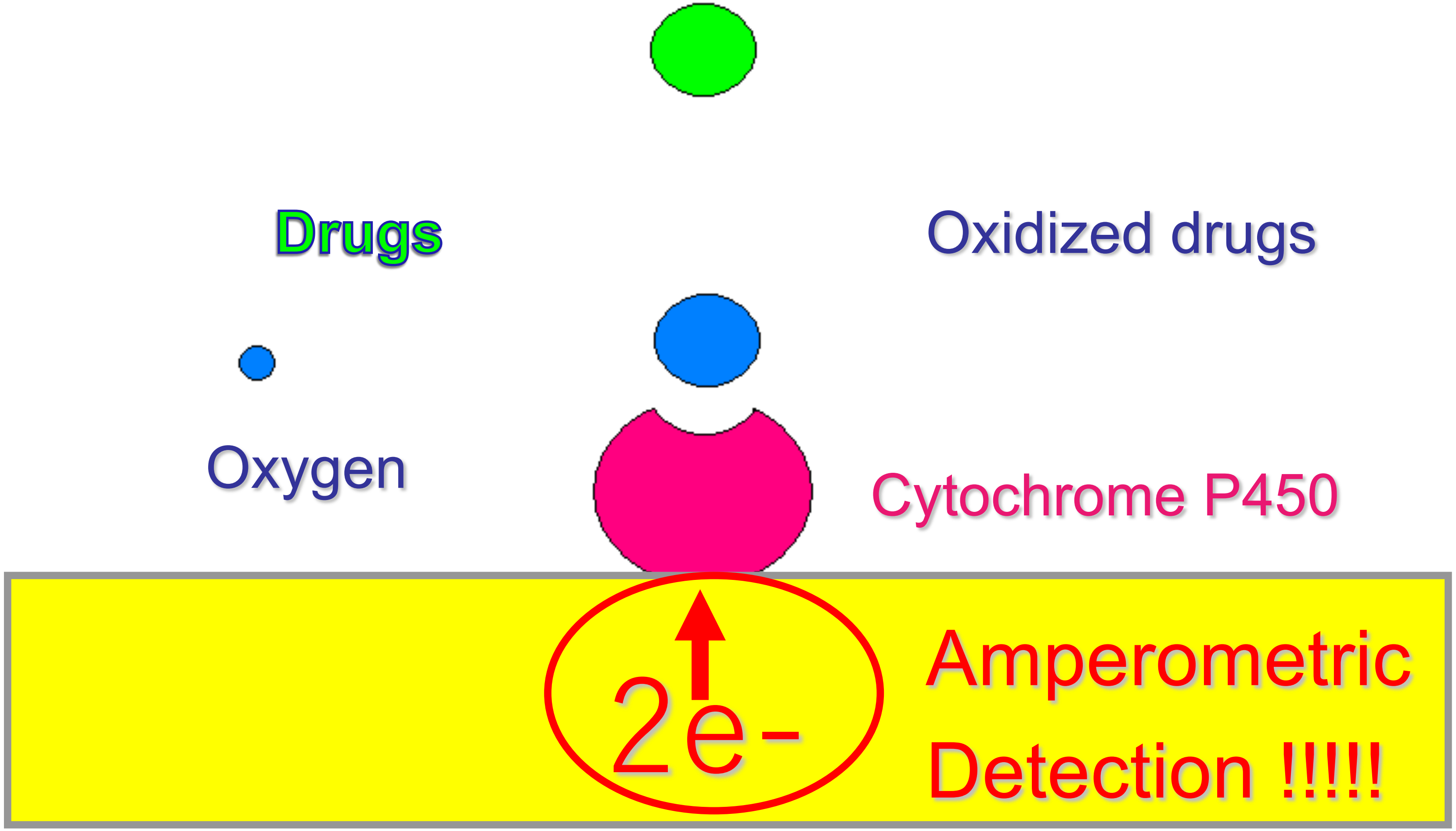
Cytochrome
P450 2B4

R-OH Oxidized form

more soluble
then
faster secreted



P450-based Detection



Drugs

Oxidized drugs

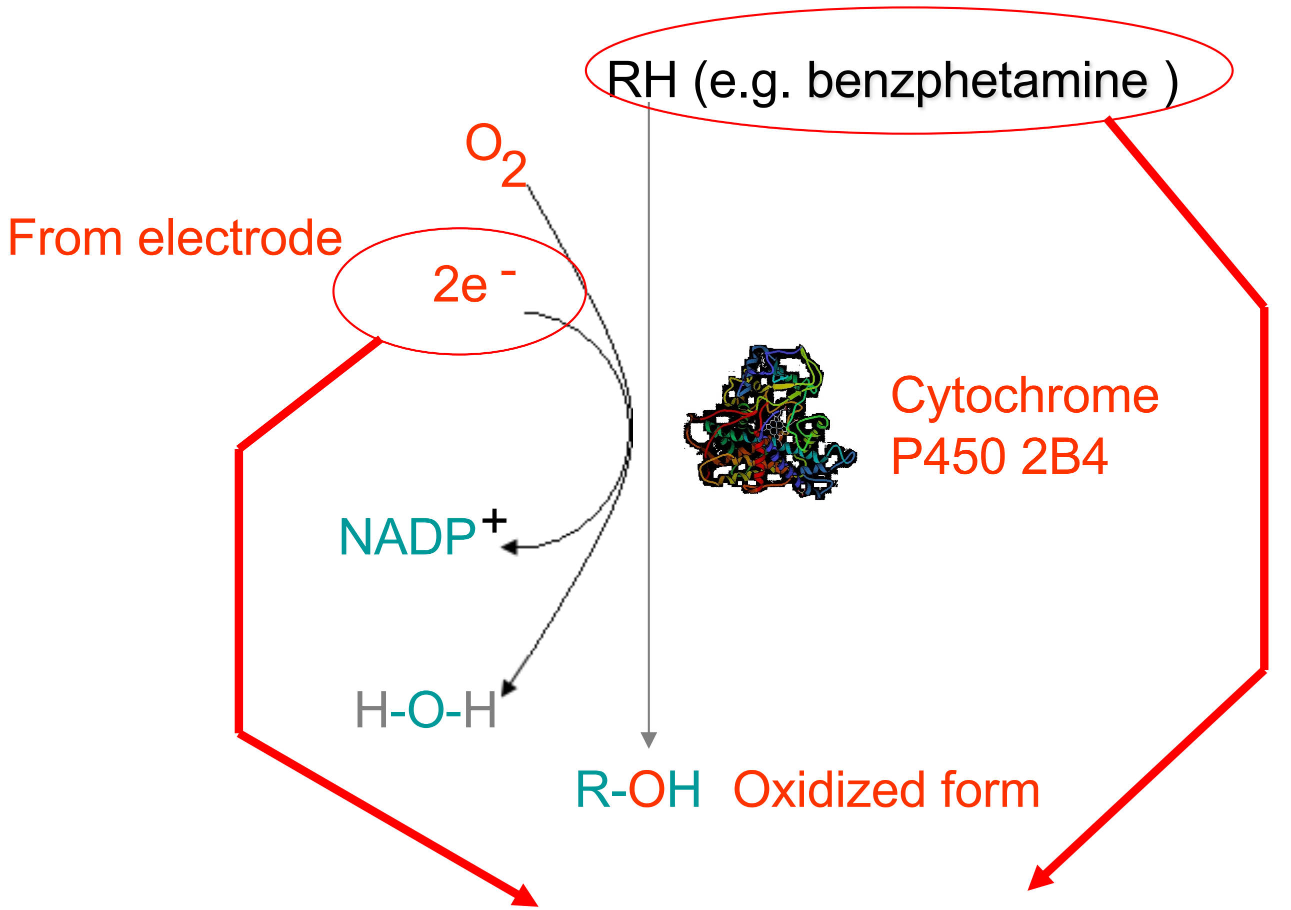
Oxygen

Cytochrome P450

2e-

Amperometric Detection !!!!!

P450 based Detection



RH (e.g. benzphetamine)

O_2

From electrode

$2e^-$

Cytochrome P450 2B4

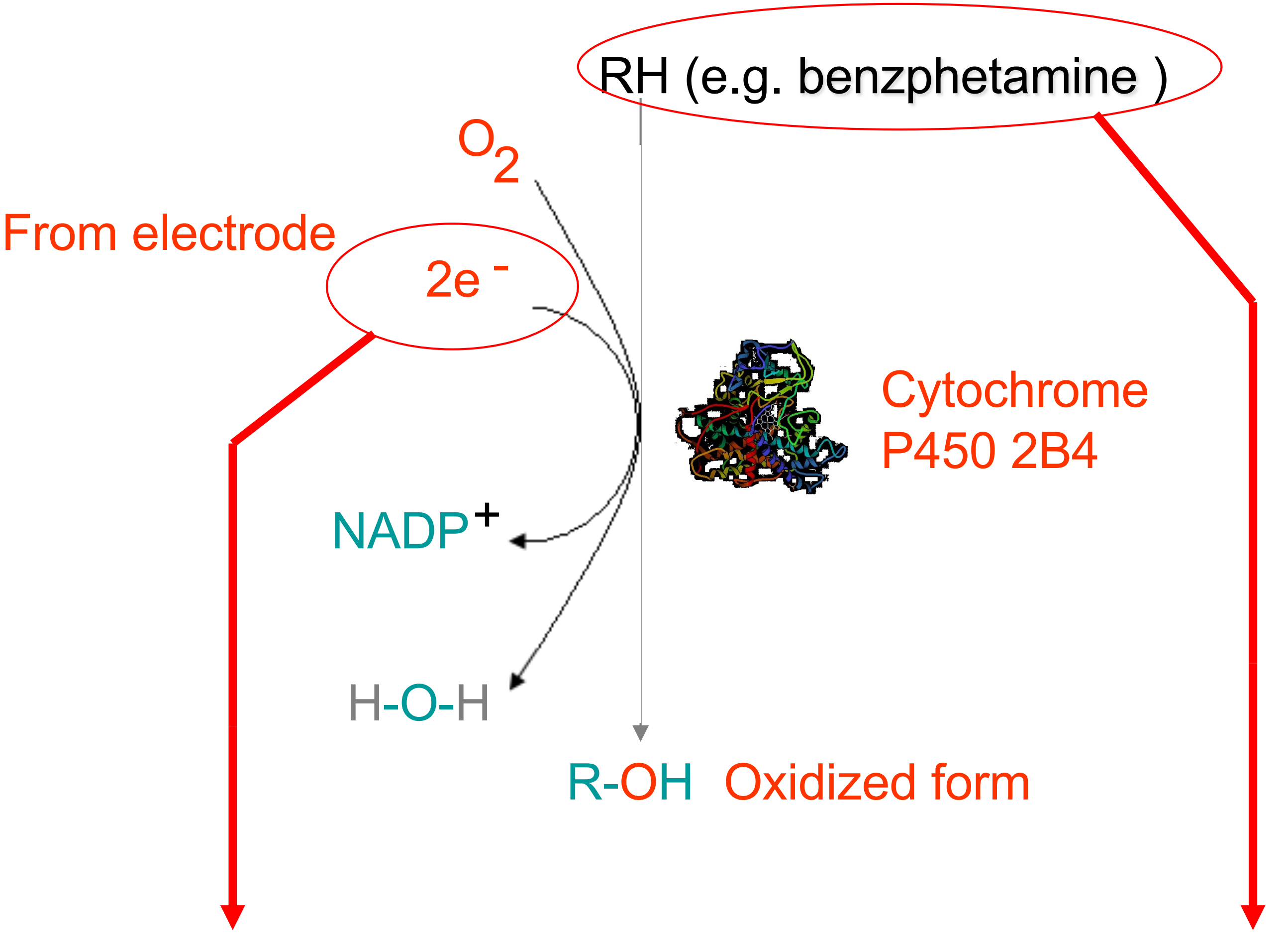
$NADP^+$

H-O-H

R-OH Oxidized form

What' s about the electro-CHEMICAL properties?

P450 based Detection



RH (e.g. benzphetamine)

O_2

From electrode

$2e^-$

Cytochrome
P450 2B4

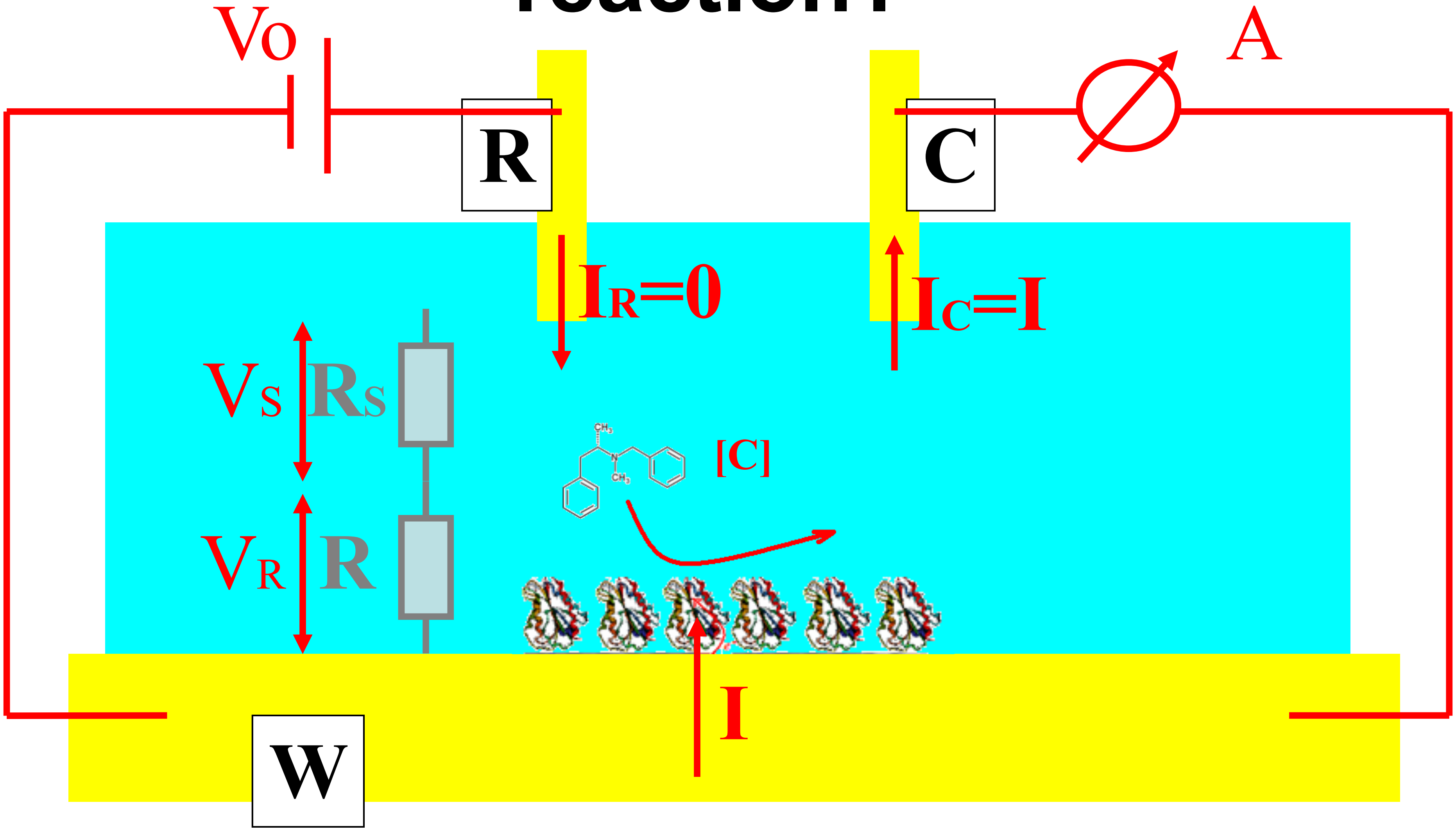
$NADP^+$

H-O-H

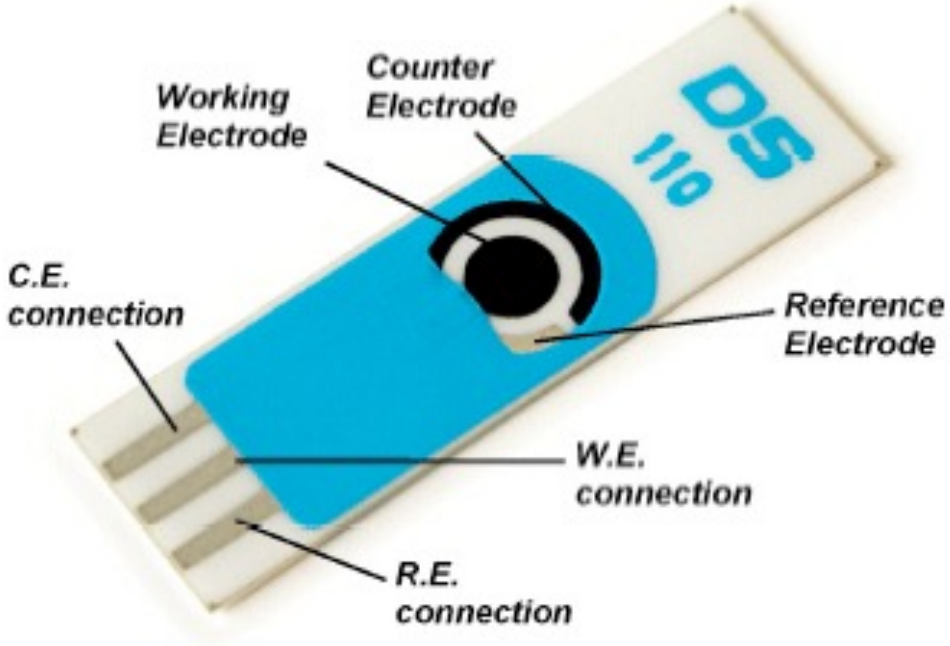
R-OH Oxidized form

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

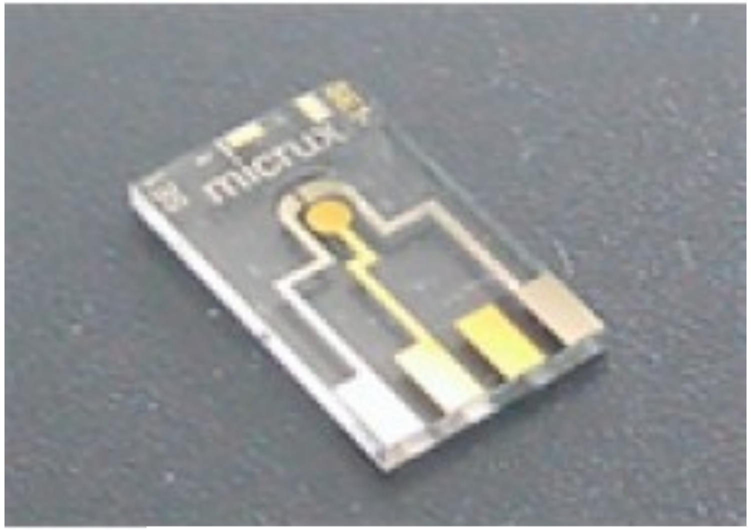
How to measure a redox reaction?

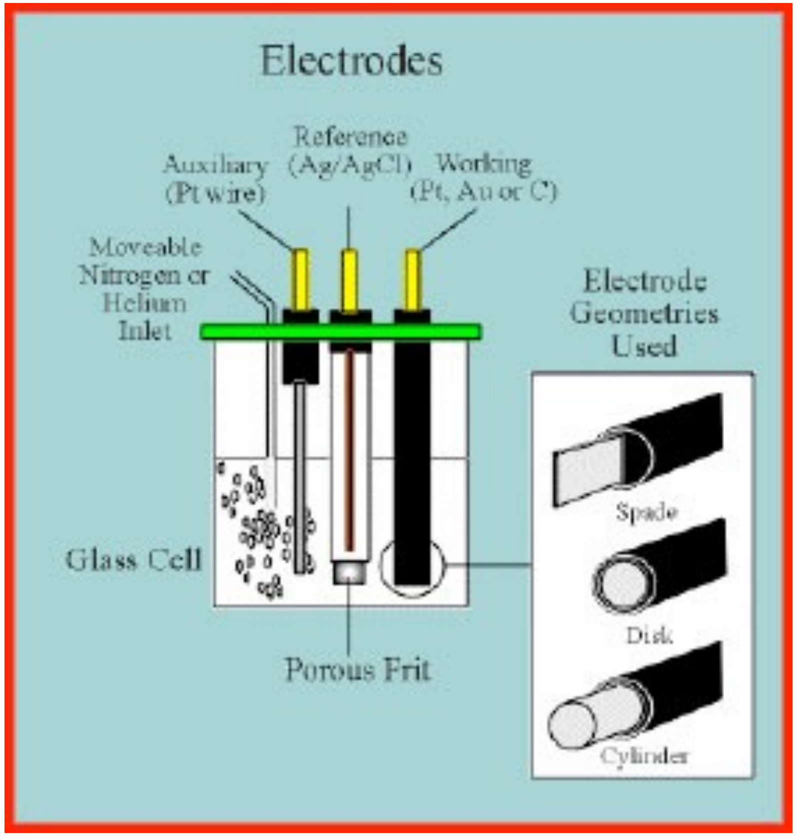


The three-electrode Electrochemical cell

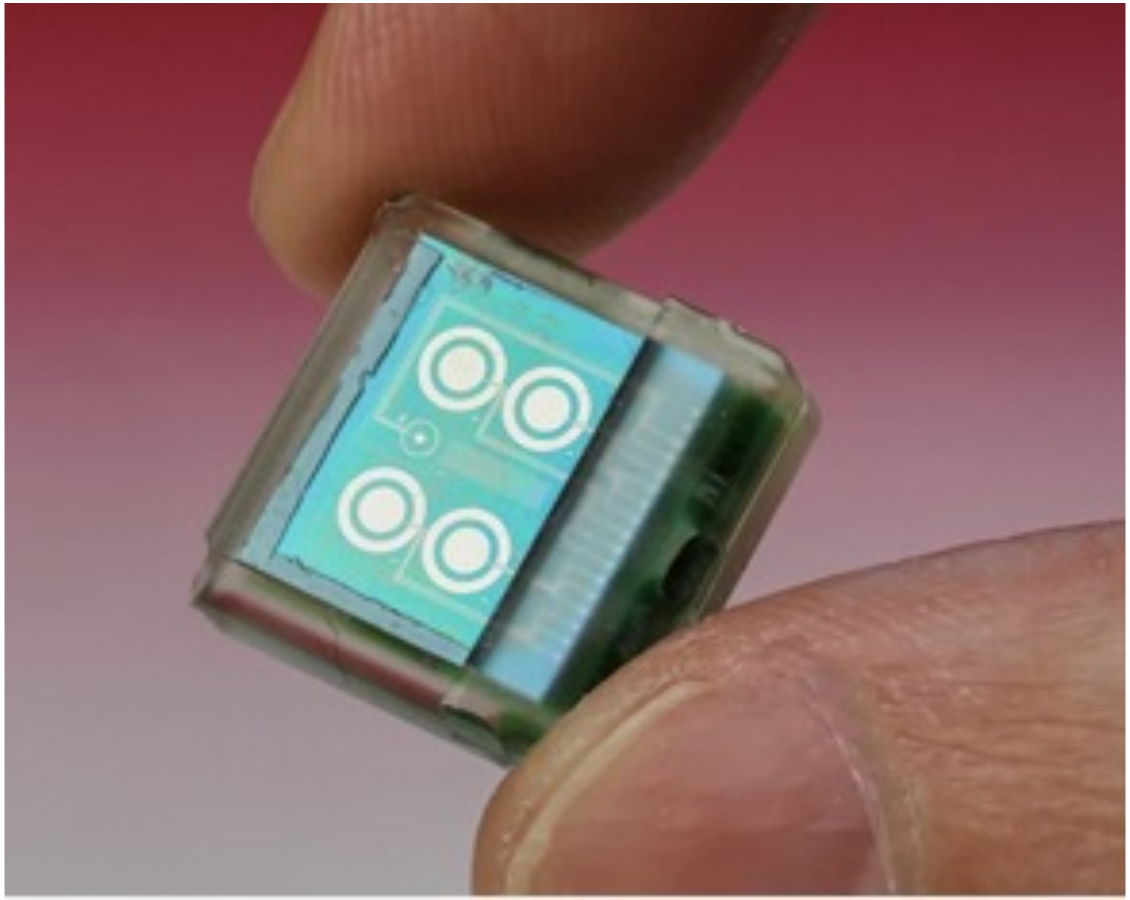


Different kinds of three-electrode Electrochemical-cell

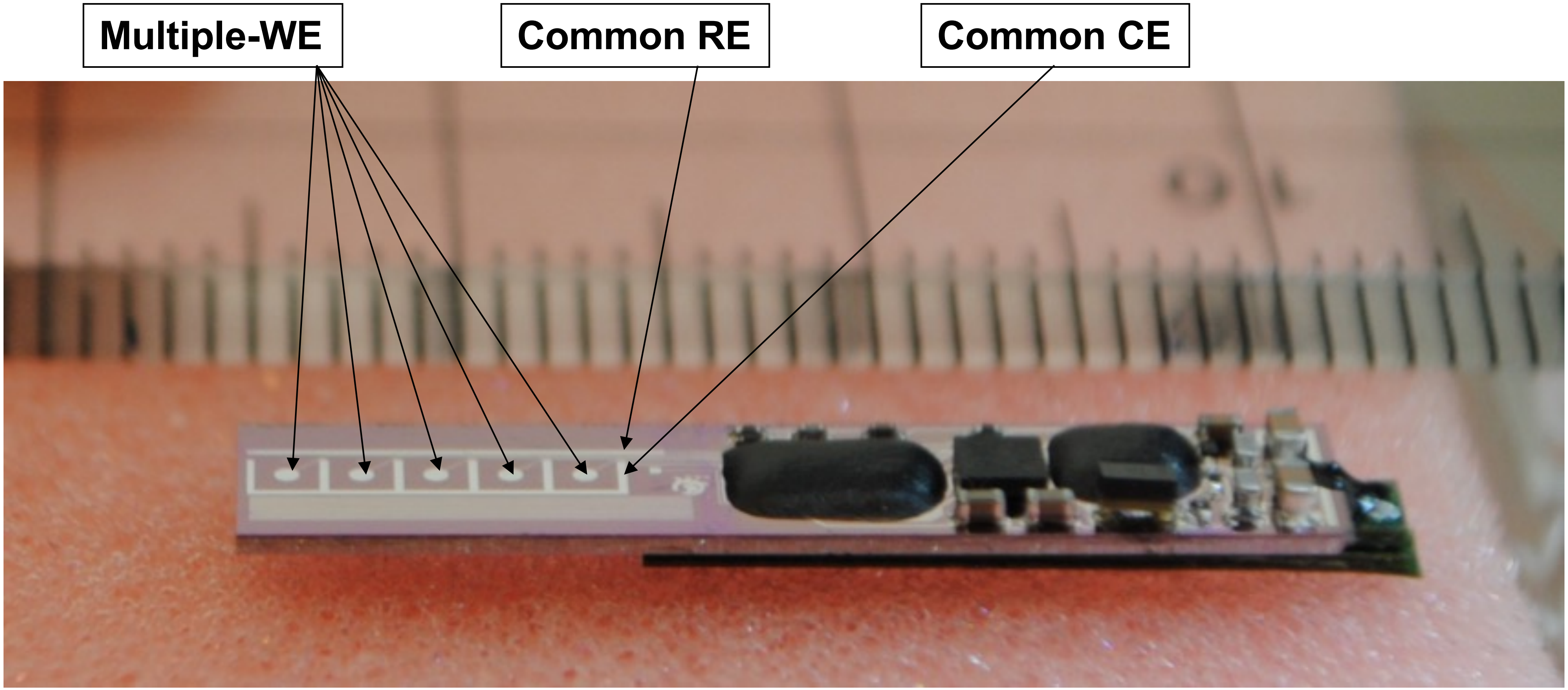




The three-electrode Electrochemical cells



Electrochemical cells with multiple-electrodes

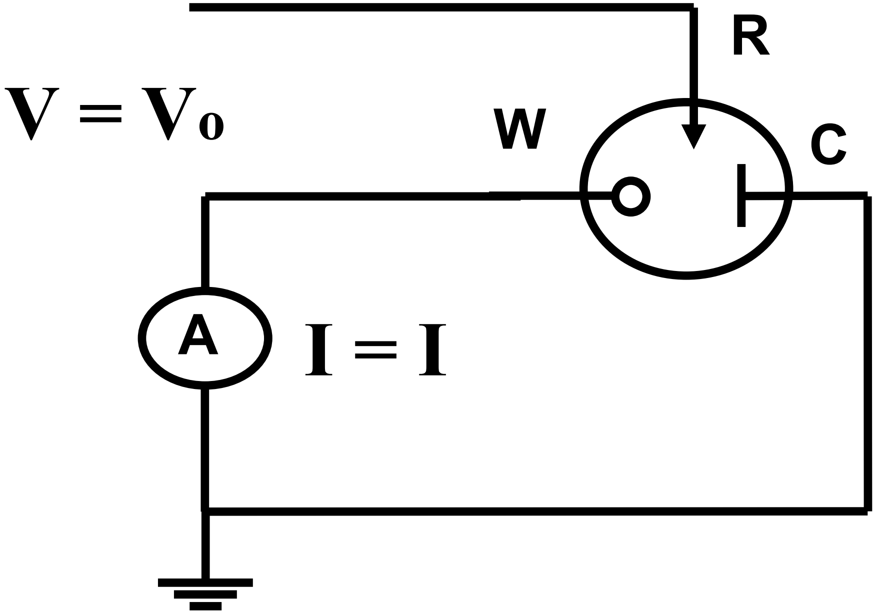


Multiple-WE

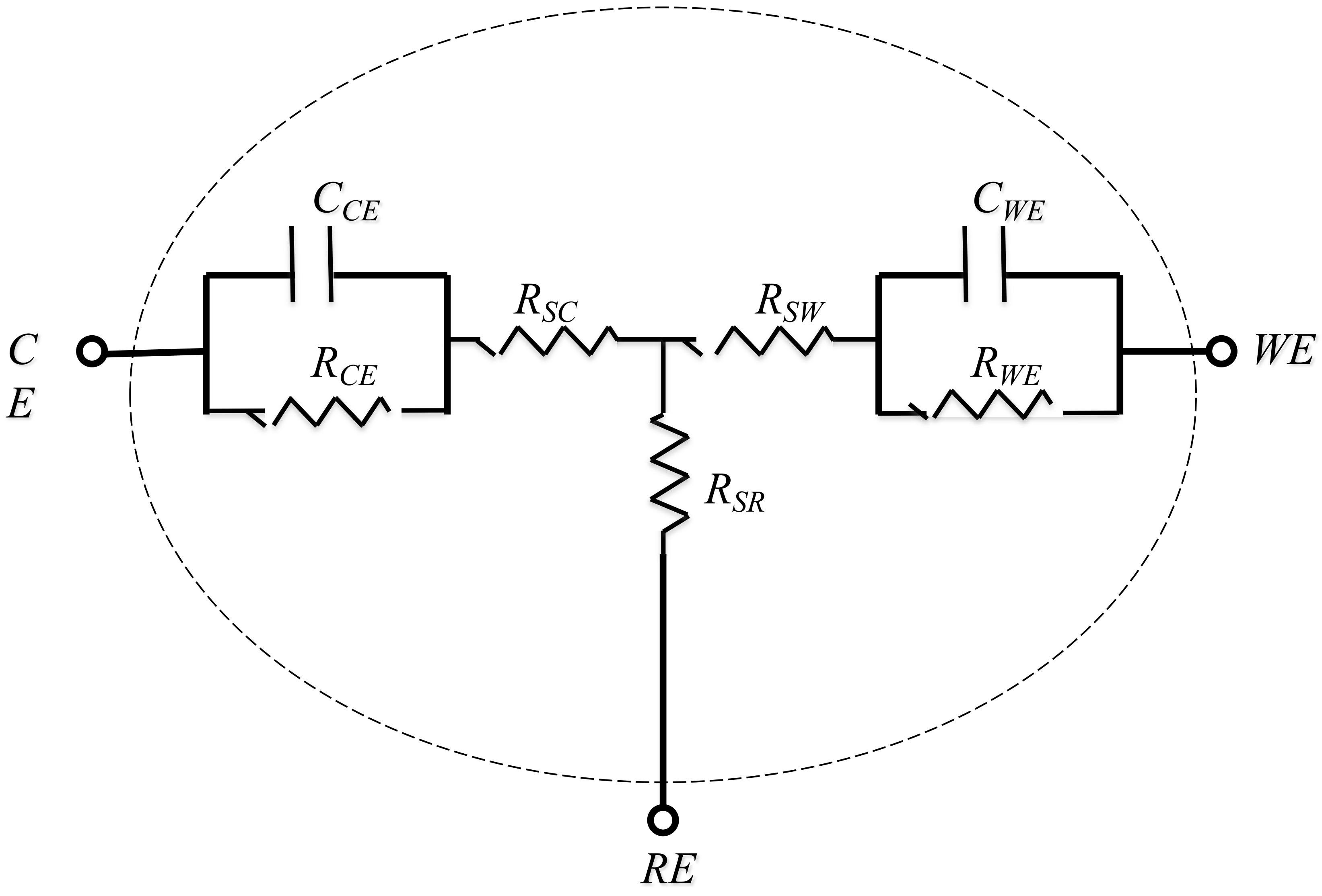
Common RE

Common CE

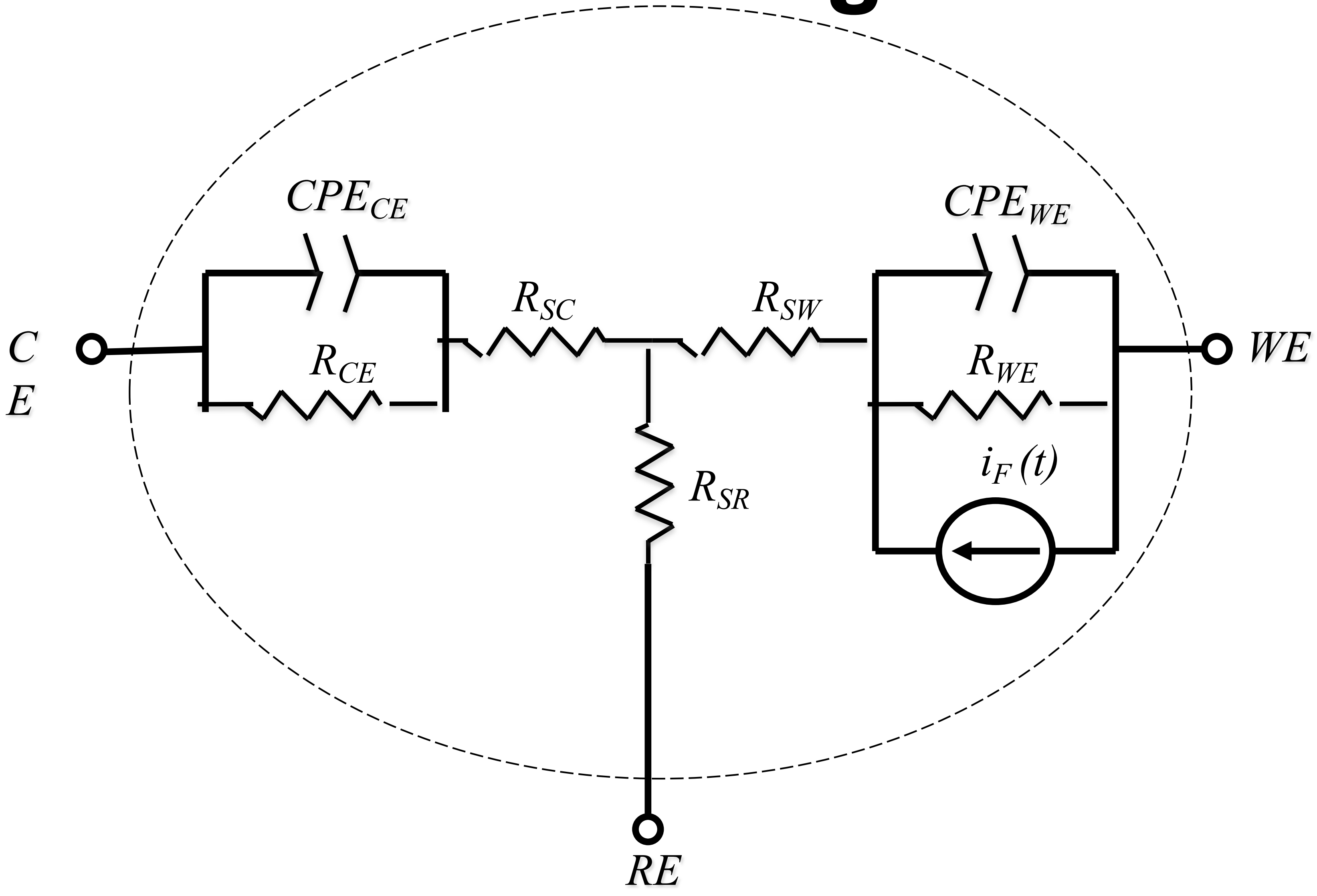
Detection Constrains



Equivalent circuit



Equivalent circuit with Faradaic current-generator



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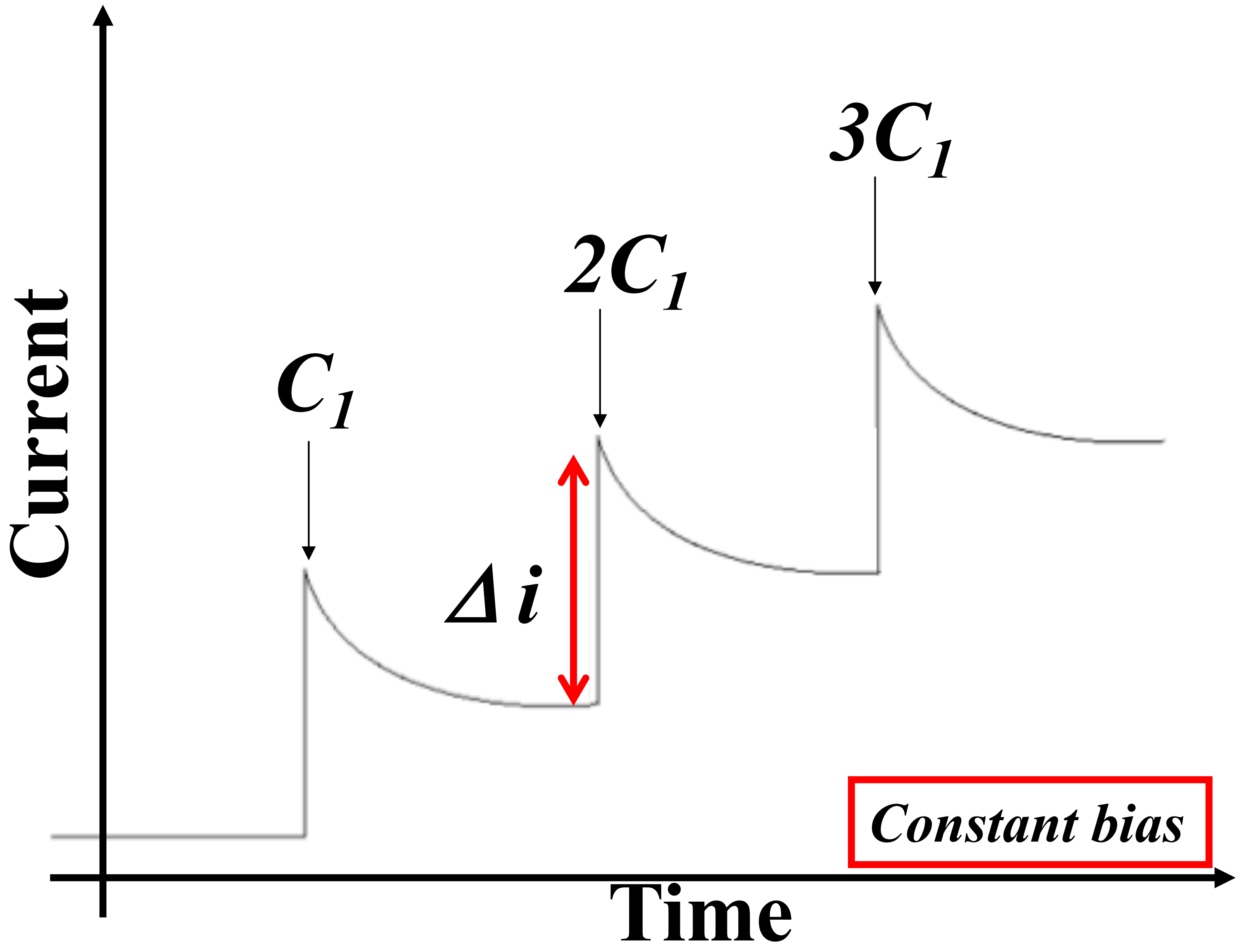
Faradaic currents from Crono-Amperometry



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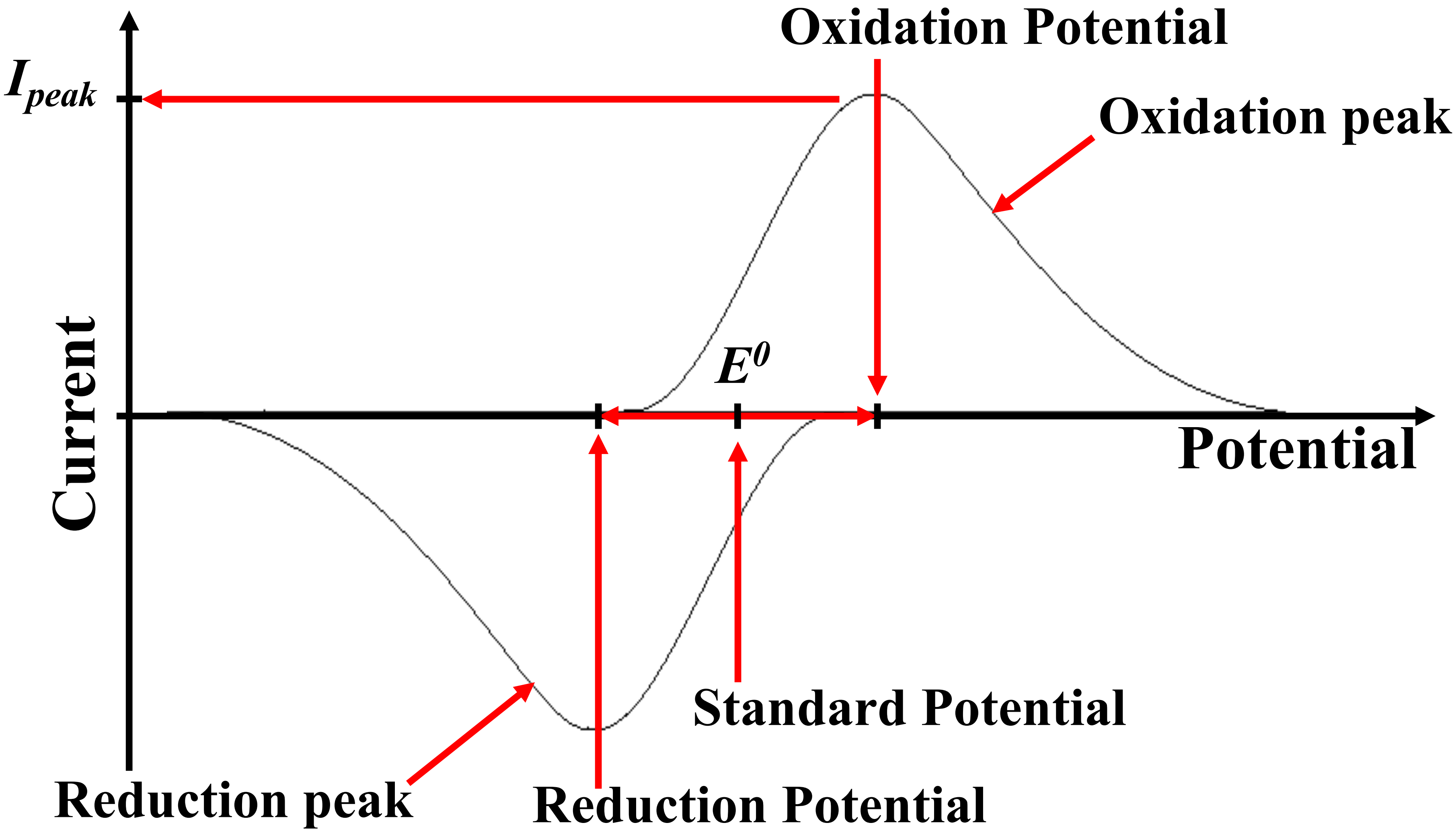
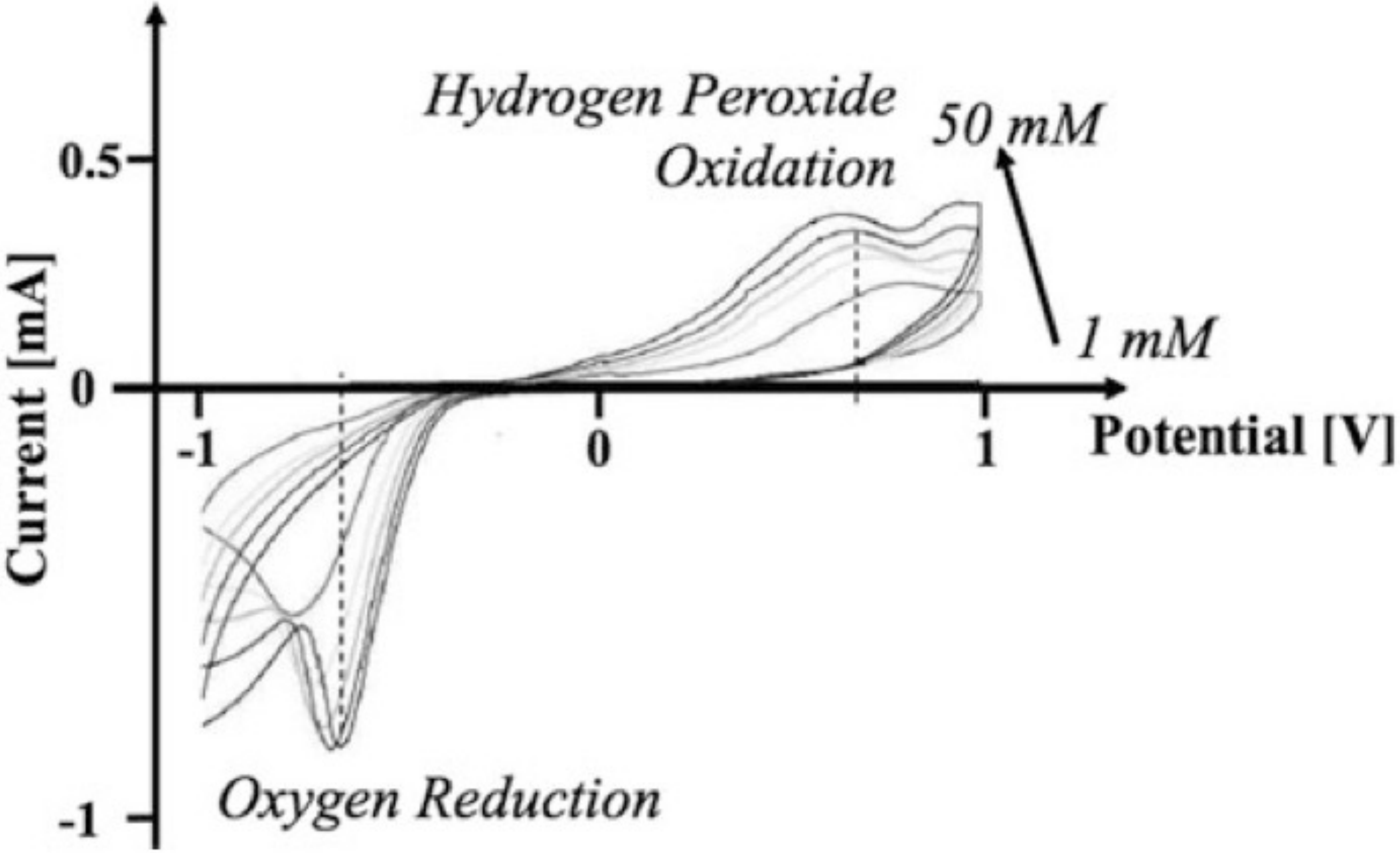
Faradaic currents from Cyclic Voltammetry



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:

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

The produced Oxygen can be further reduced :

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

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

$$F(s) = L_s[f(t)] = \widehat{f}(s) = \int_0^{+\infty} f(t)e^{-st}dt$$

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

$$L_s\left[af(t) + bg(t)\right] = aL_s[f(t)] + bL_s[g(t)] = a\widehat{f}(s) + b\widehat{g}(s)$$

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

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

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):

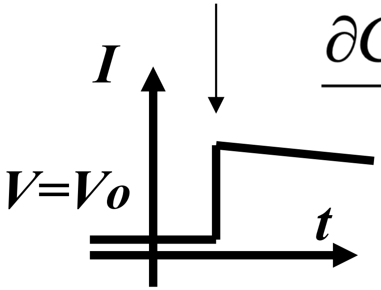
$$j_m \cong -D\frac{\partial C(x,t)}{\partial x}$$

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

$$\begin{cases} \boxed{\begin{array}{l} C(x,0) = C_o \\ \lim\limits_{x\to\infty} C(x,t) = C_o \end{array}} \\ C(0,t) = 0, t \to \infty \end{cases} \rightarrow \quad C(x,0) = C(x\to\infty,t) = C_0$$

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

The Cottrell Equation

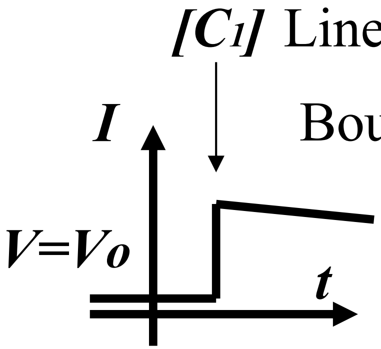
$$\frac{\partial^2 \widehat{C}(x,s)}{\partial x^2} - \frac{s}{D}\widehat{C}(x,s) = -\frac{C_0}{D}$$

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

$$\boxed{\begin{array}{l} \lim\limits_{x\to\infty} C(x,t) = C_0 \\ L_s[t^n] = \dfrac{n!}{s^{n+1}} \end{array}} \longrightarrow \lim_{x\to\infty} \widehat{C}(x,s) = \frac{C_0}{s}$$

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

The Cottrell Equation



Linear diffusion equation

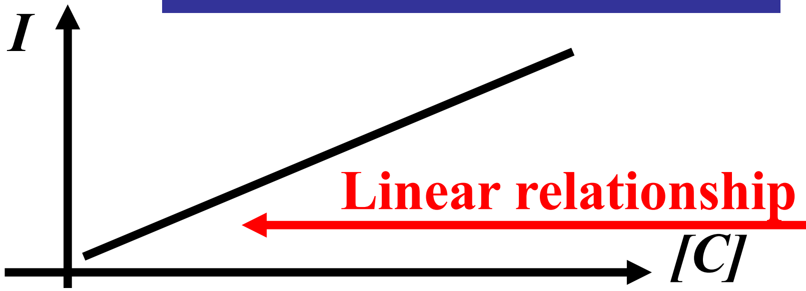
Boundary conditions $\hat{C}_0(x,s) = \frac{C_0}{s} + A(s)e^{-\sqrt{s/D}x}$

By definition

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

$$\frac{\hat{i}(s)}{nFA} = D\frac{\partial \hat{C}(x,s)}{\partial x}\bigg|_{x=0}$$

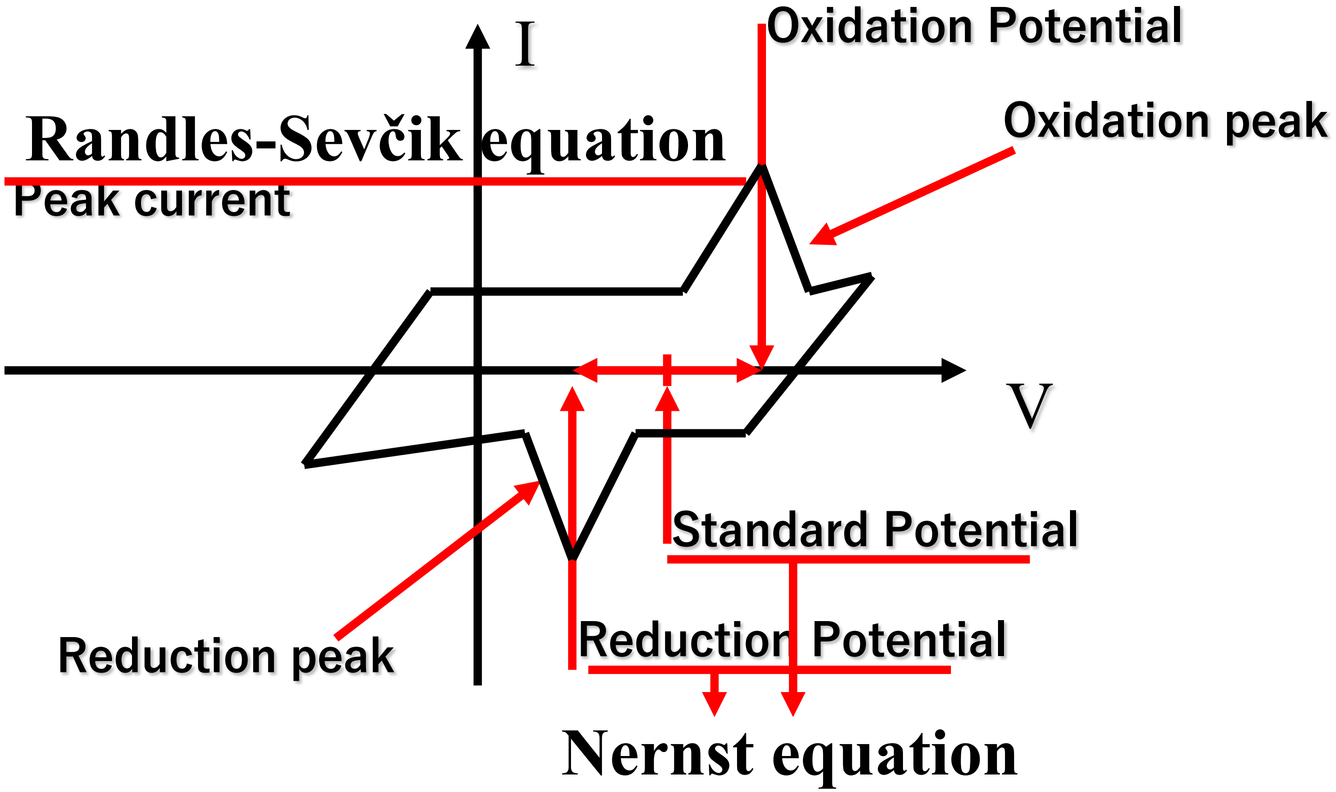
$$\hat{i}(s) = nFA\frac{\sqrt{D}C_0(x,s)}{\sqrt{s}}$$



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

Cottrell equation

Redox reactions from Voltammetry



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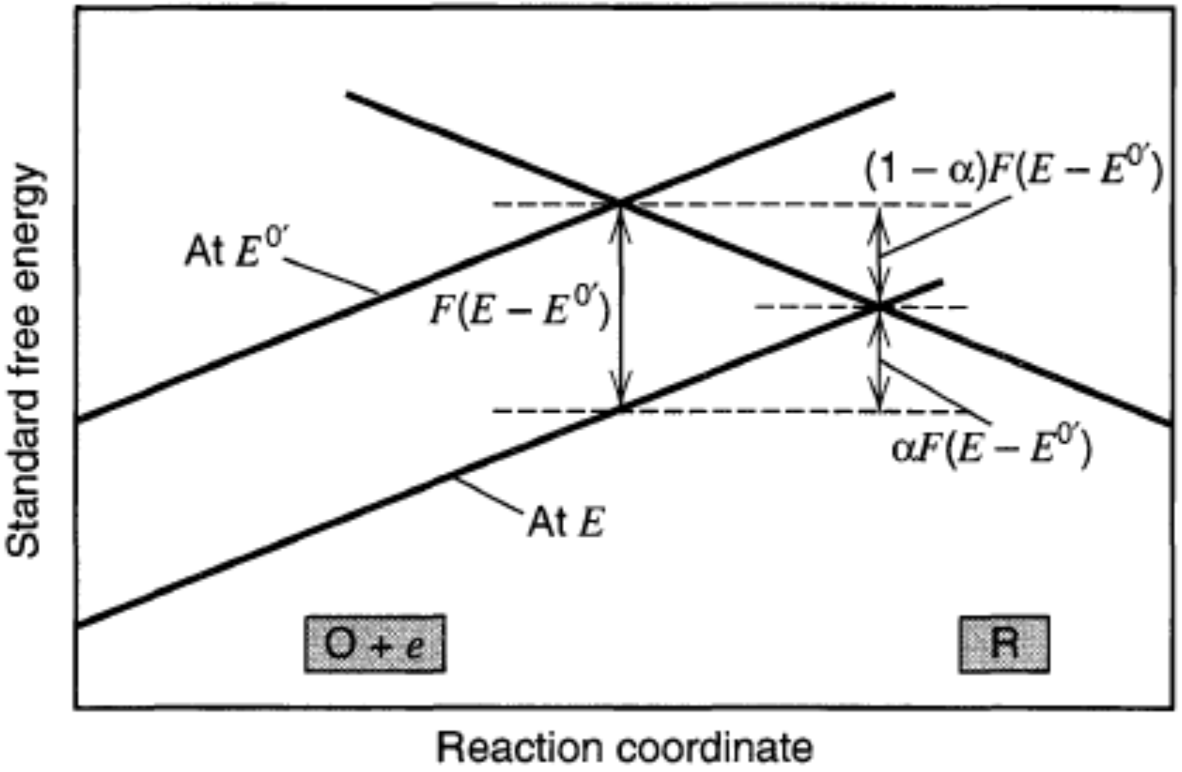
Redox Reactions



Nernst Equation

Redox Reaction

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

Equilibrium Constants

$$\left\{\begin{array}{l} k_c = k_c^0 e^{-\frac{\Delta G_c}{RT}} = k_c^0 e^{-\frac{\Delta G_c^0 + \alpha F(E-E^0)}{RT}} \\ k_a = k_a^0 e^{-\frac{\Delta G_a}{RT}} = k_a^0 e^{-\frac{\Delta G_a^0 - (1-\alpha)F(E-E^0)}{RT}} \end{array}\right. \begin{array}{l} = \\ = \end{array} \boxed{\begin{array}{l} k_c^0 e^{-\frac{\Delta G_c^0}{RT}} \\ k_a^0 e^{-\frac{\Delta G_a^0}{RT}} \end{array}} \begin{array}{l} e^{-\frac{\alpha F(E-E^0)}{RT}} \\ e^{\frac{(\;-\alpha)F(E-E^0)}{RT}} \end{array}$$

@ Equilibrium:

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

Nernst Equation

Redox Reaction

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

$$\begin{cases} k_c = k^0 e^{-\frac{\alpha F(E-E^0)}{RT}} \\ k_a = k^0 e^{\frac{(1-\alpha)F(E-E^0)}{RT}} \end{cases}$$

The current from the redox is

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

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

@ Equilibrium:

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

Nernst Equation

@ Equilibrium:

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

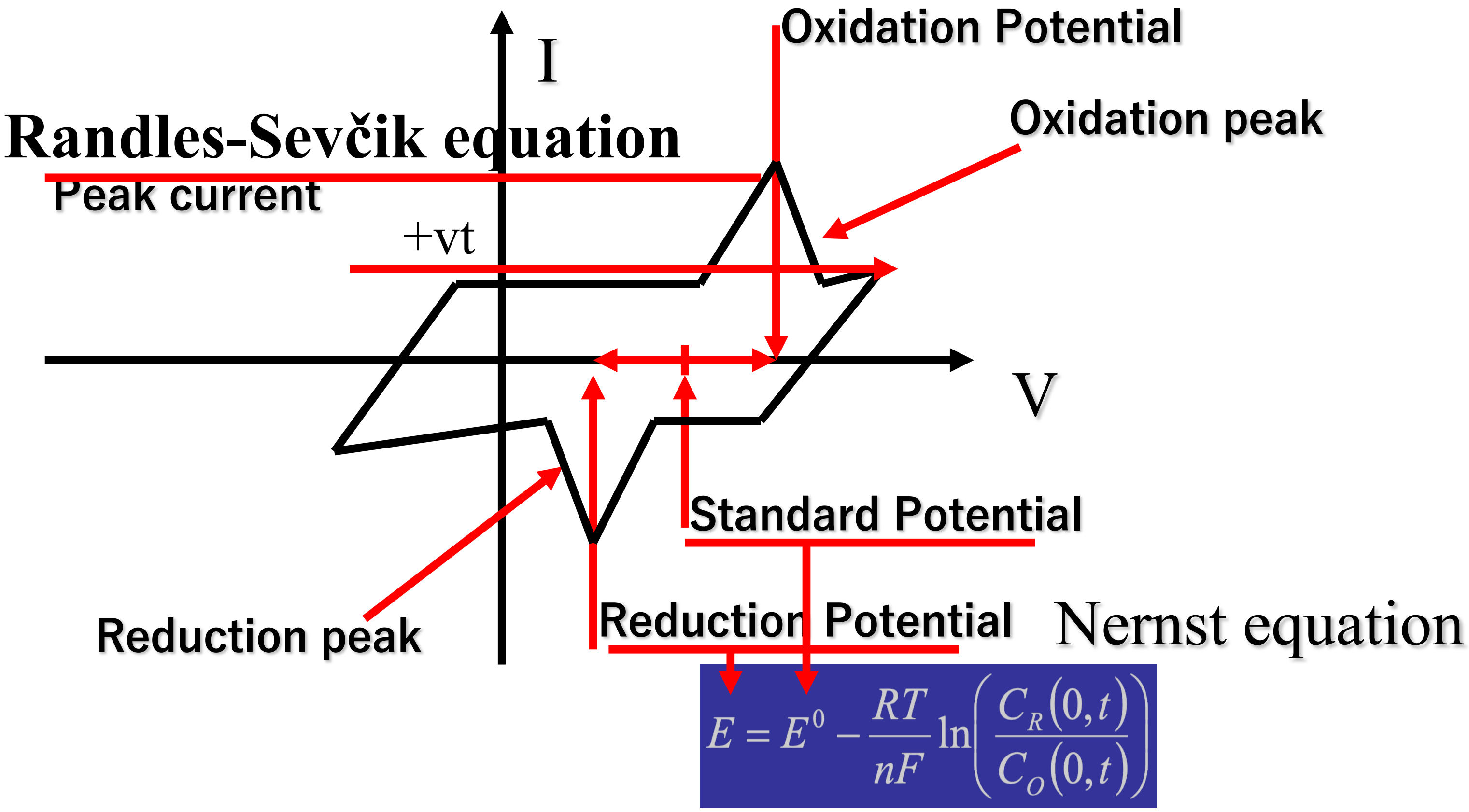
$$i = 0 \Rightarrow \frac{C_O(0,t)}{C_R(0,t)} = e^{\frac{F(E-E^0)}{RT}} \Rightarrow \frac{F(E-E^0)}{RT} = \ln\left[\frac{C_O(0,t)}{C_R(0,t)}\right]$$

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

Nernst equation

If *n* electrons are involved!

Redox reactions from Voltammetry



Oxidation Potential

Oxidation peak

Randles-Sevčik equation

Peak current

Standard Potential

Reduction peak

Reduction Potential

Nernst equation

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

Randles-Sevčik Equation

Voltage Sweep

$$E = E_i + \upsilon t$$

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

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

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

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

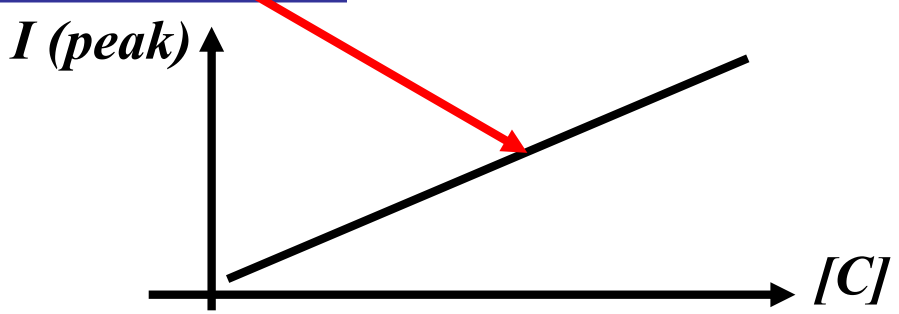
Randles-Sevčik Equation

Voltage Sweep

$$E = E_i + \upsilon t$$

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

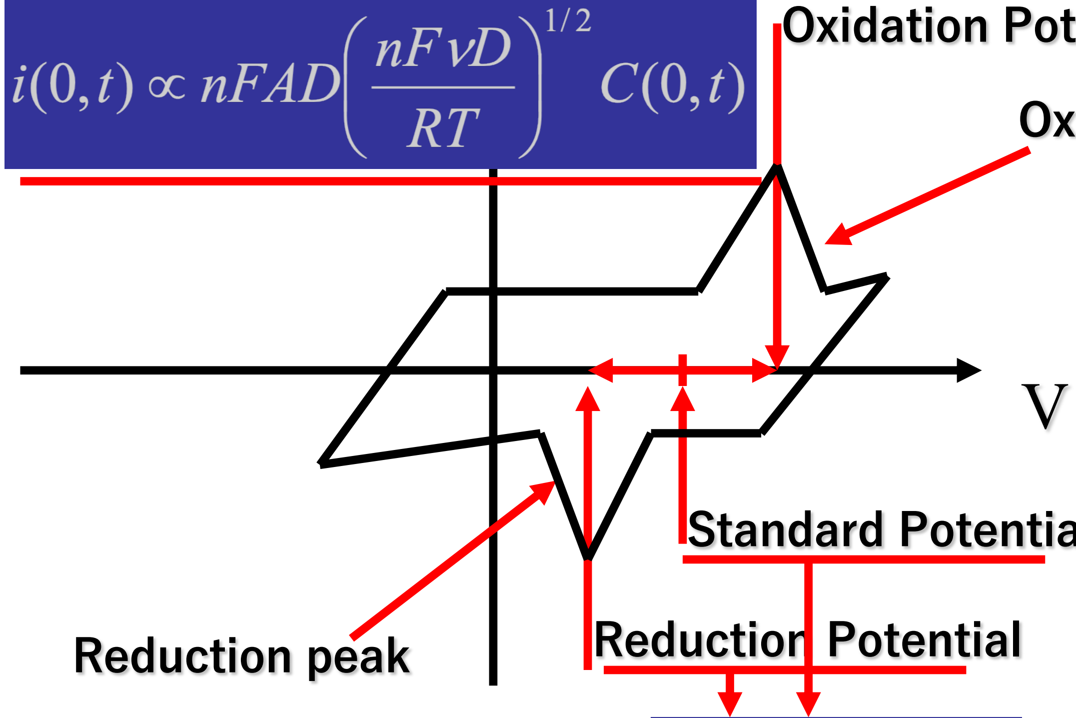
$$i(t) = nFAD\sqrt{\frac{nFDv}{RT}}\,C(0,t)$$



Redox reactions from Voltammetry

Randles-Sevčik equation for the Peak current

$$i(0,t) \propto nFAD\left(\frac{nF\nu D}{RT}\right)^{1/2} C(0,t)$$



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

Nernst equation