Master in Electrical and Electronics Engineering

EE-517: Bio-Nano-Chip Design

Lecture #4

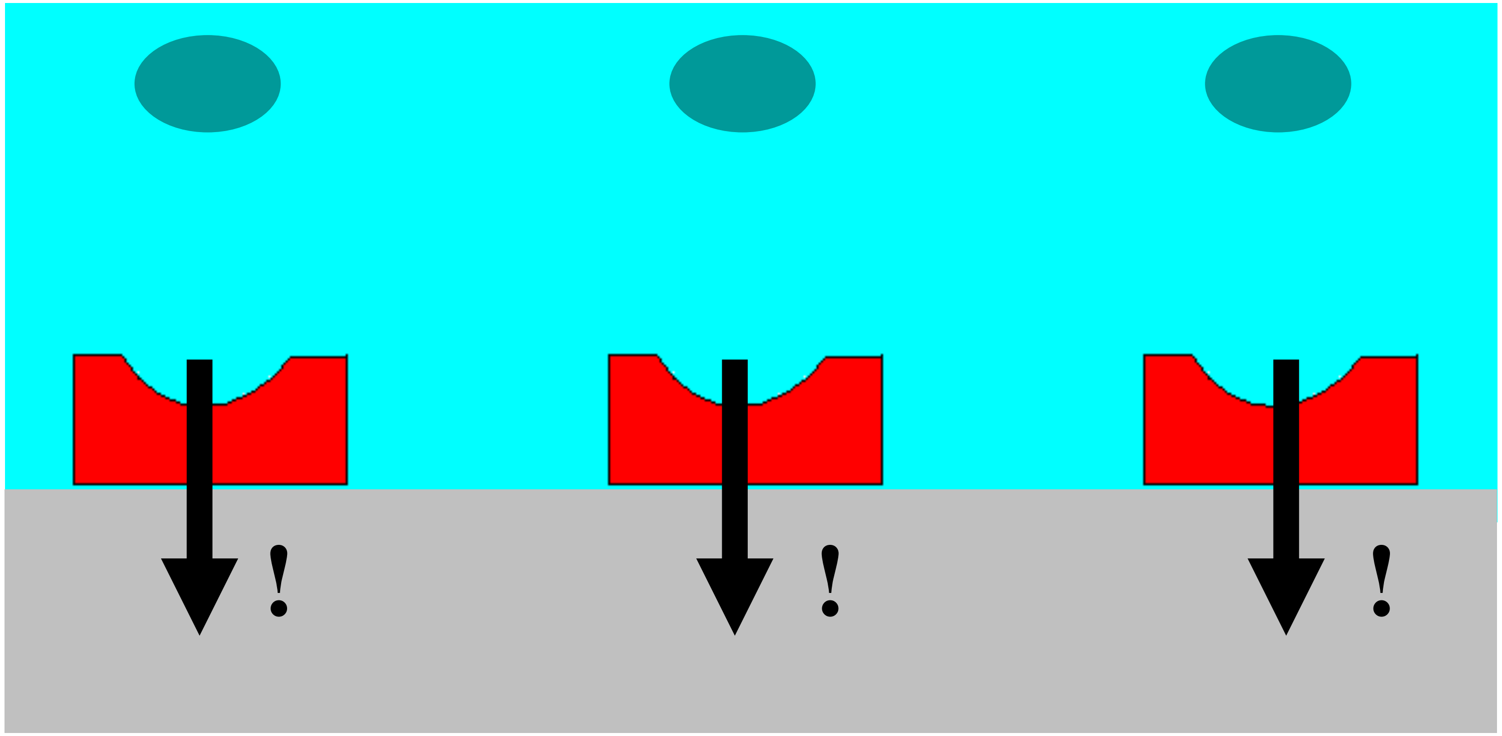
Probe Detection Principles (Faradaic Processes)

Lecture Outline

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

- Electrochemical interfaces with enzymes
- Faradaic currents at the interface
- Electrochemical cells and equivalent circuits
- Calibration curve, Sensitivity, and LOD

CMOS/Sample interface



We may exploit some catalyzed reactions with transfer of electrons for detection aims at our Bio/CMOS interfaces

Q1

Recall:
What is a catalyst?

A. A molecule accelerating chemical reactions
B. A molecule accelerating only biochemical reactions
C. A biomolecule accelerating chemical reactions
D. A molecule accelerating only redox reactions

Q2

Recall:
What is an enzyme?

A. An organic molecule
B. A protein
C. A DNA sequence
D. A catalyst
E. A biological catalyst

Q3

What is a Redox Enzyme?

A. An enzyme that accelerates only Oxidations

B. An enzyme that accelerates only Reductions

C. An enzyme that involves transfer of electrons between chemical species

D. An enzyme that catalyzes oxidation-reduction reactions

Enzymes’ based detection

In the case of some enzymes, we can easily exploit the redox reactions involving their substrates to the aim of electrochemical direct detection: e.g., that’s the case of oxidases and cytochromes.

Redox with oxidases

The typical redox involving an oxidase is as follows:

$$XOD/FAD + X \rightarrow XOD/FADH_2 + X_p$$

The FAD (Flavin Adenine Dinucleotide) is a functional part of the protein that gains a hydrogen molecule after the reaction. Therefore, the oxidase is not yet ready for another transformation because the FAD has gained the H_2. To return to its initial state, the enzyme needs to release that hydrogen molecule:

$$XOD/FADH_2 + O_2 \longrightarrow XOD/FAD + H_2O_2$$

Redox with oxidases

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

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

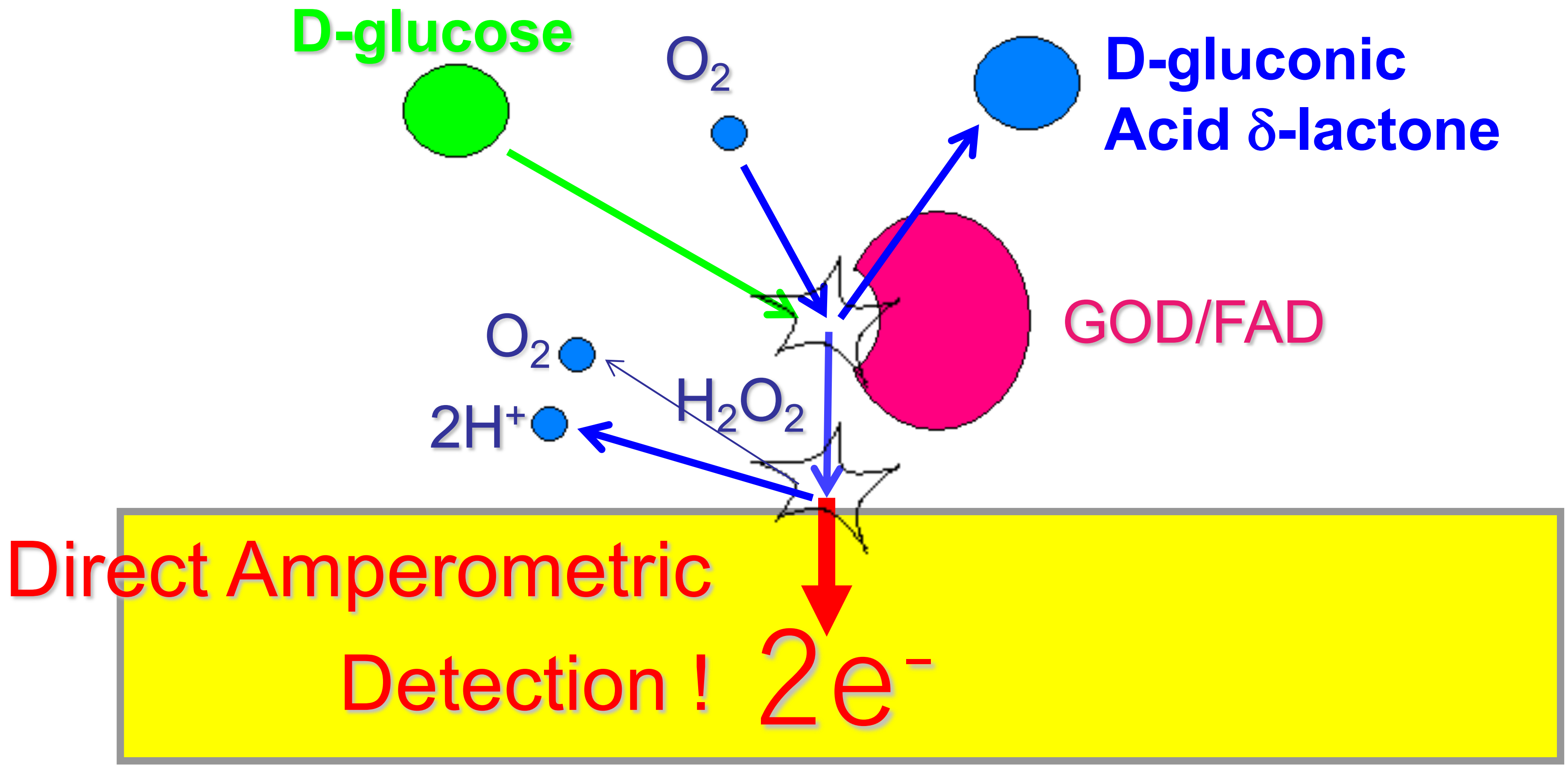
And a reduction:

$$H_2O_2 + 2H^+ + 2e^- \xrightarrow{+1540mV} 2H_2O.$$

A third redox is provided by the oxygen reduction:

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

Oxidases based detection

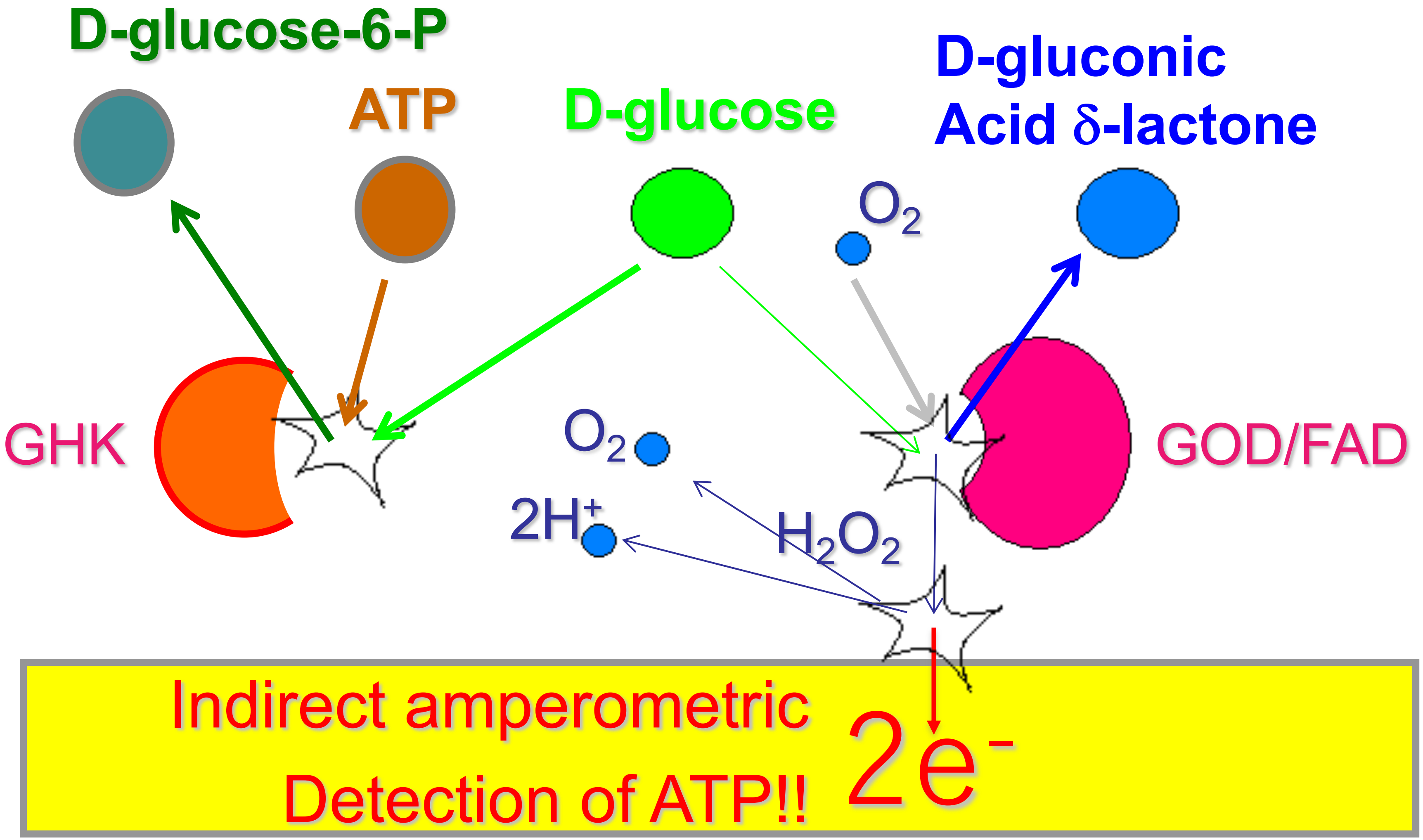


Q4

All the enzyme transfer electrons among species?

A. Yes, of course!
B. Only redox enzymes
C. No, only biological enzymes
D. No, only oxidases
E. No, only oxidases and cytochromes

ATP detection



Redox with P450

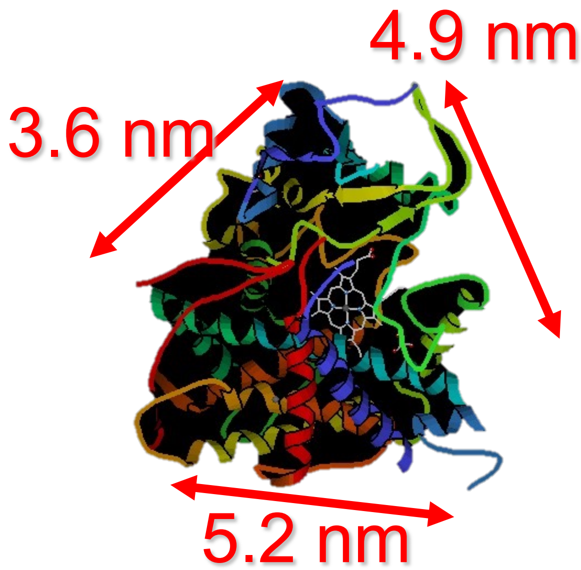
The typical redox involving a cytochrome P450 is as follows:

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

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 working Principle

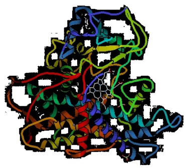


RH (e.g. benzphetamine)

O_2

2e$^-$

NADPH+H$^+$



Cytochrome P450 2B4

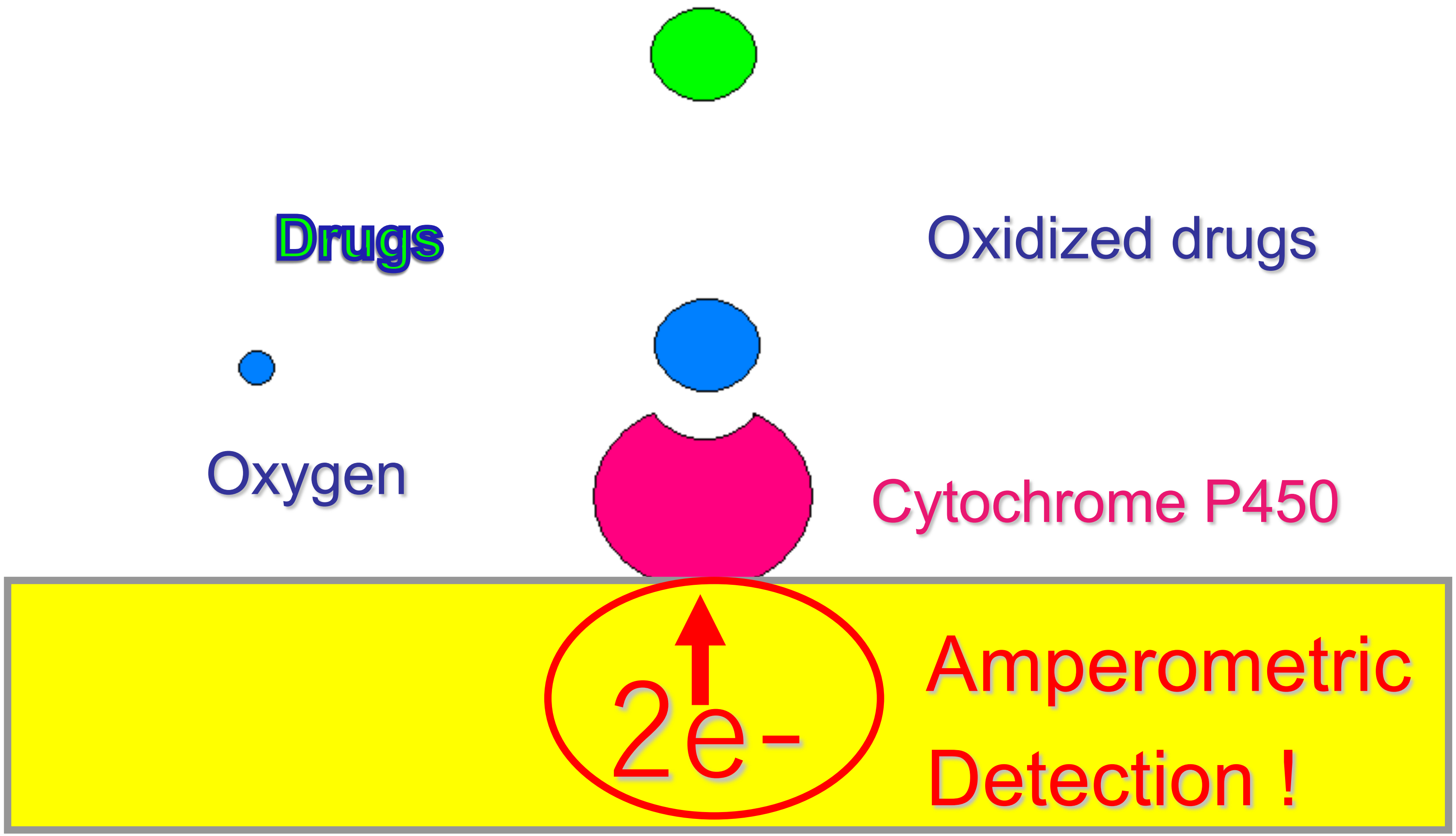
NADP$^+$

H-O-H

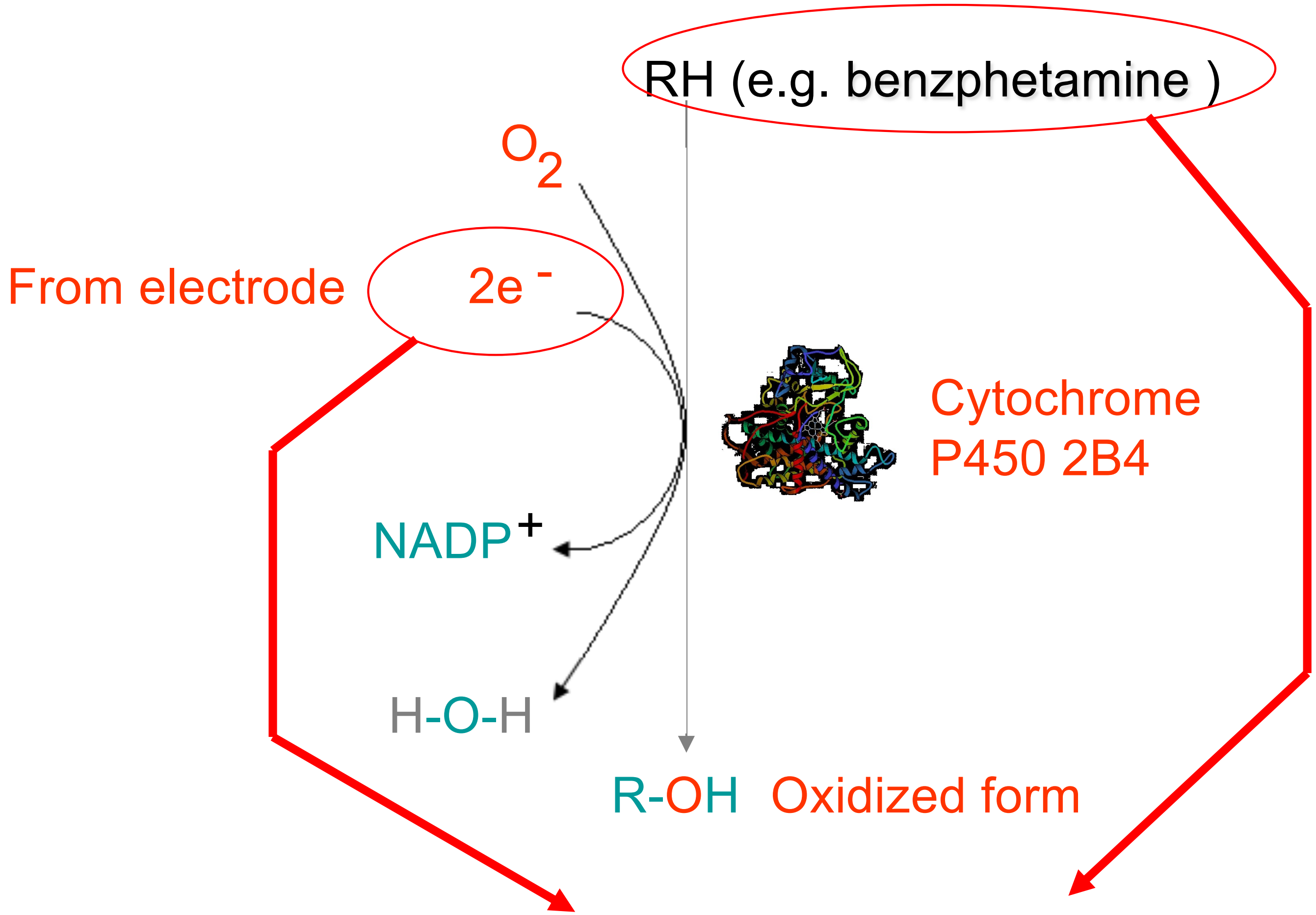
R-OH Oxidized form

more soluble then faster secreted

P450-based Detection

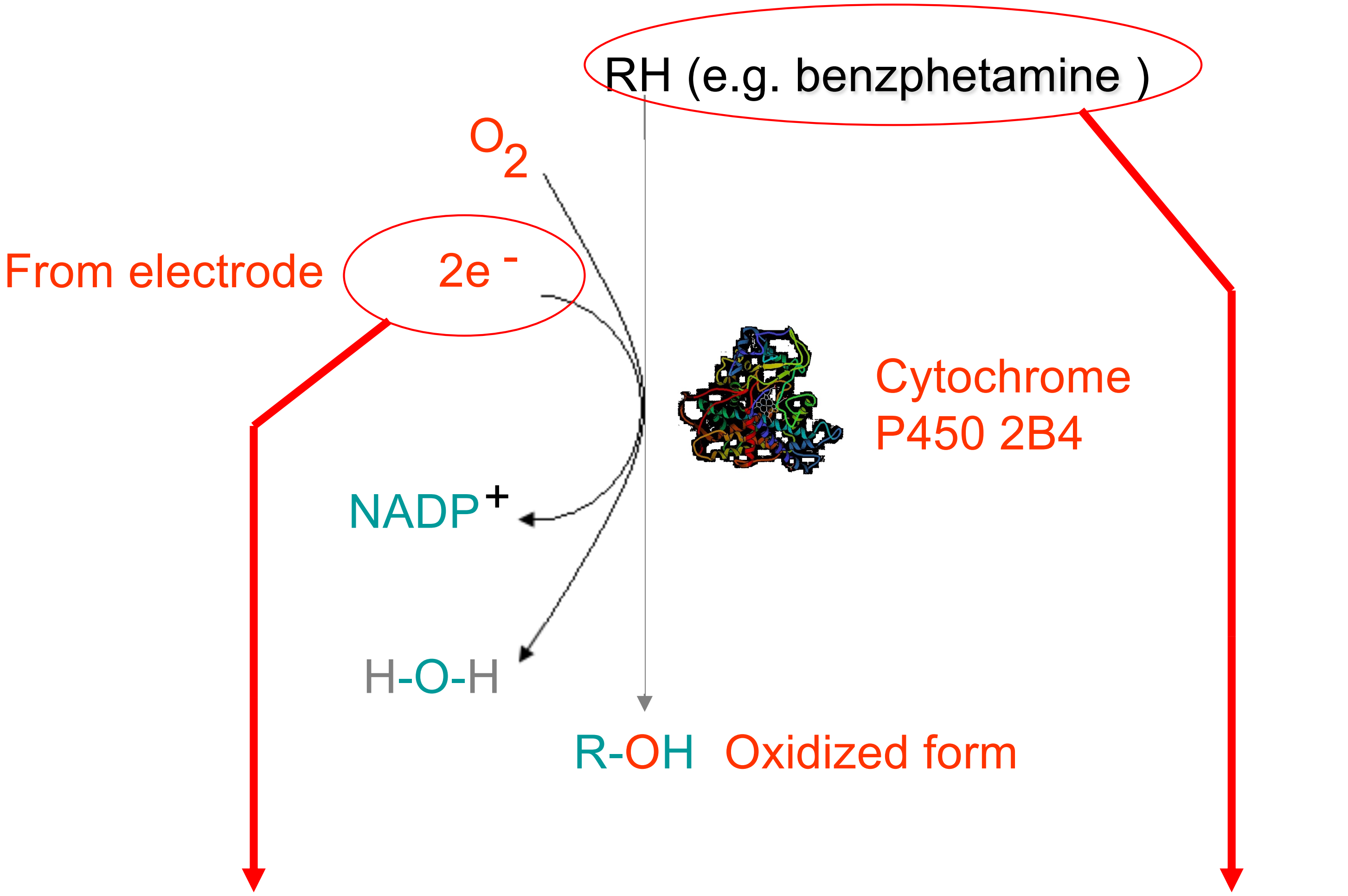


Enzyme based Detection



What's about the electro-CHEMICAL properties?

Enzyme based Detection



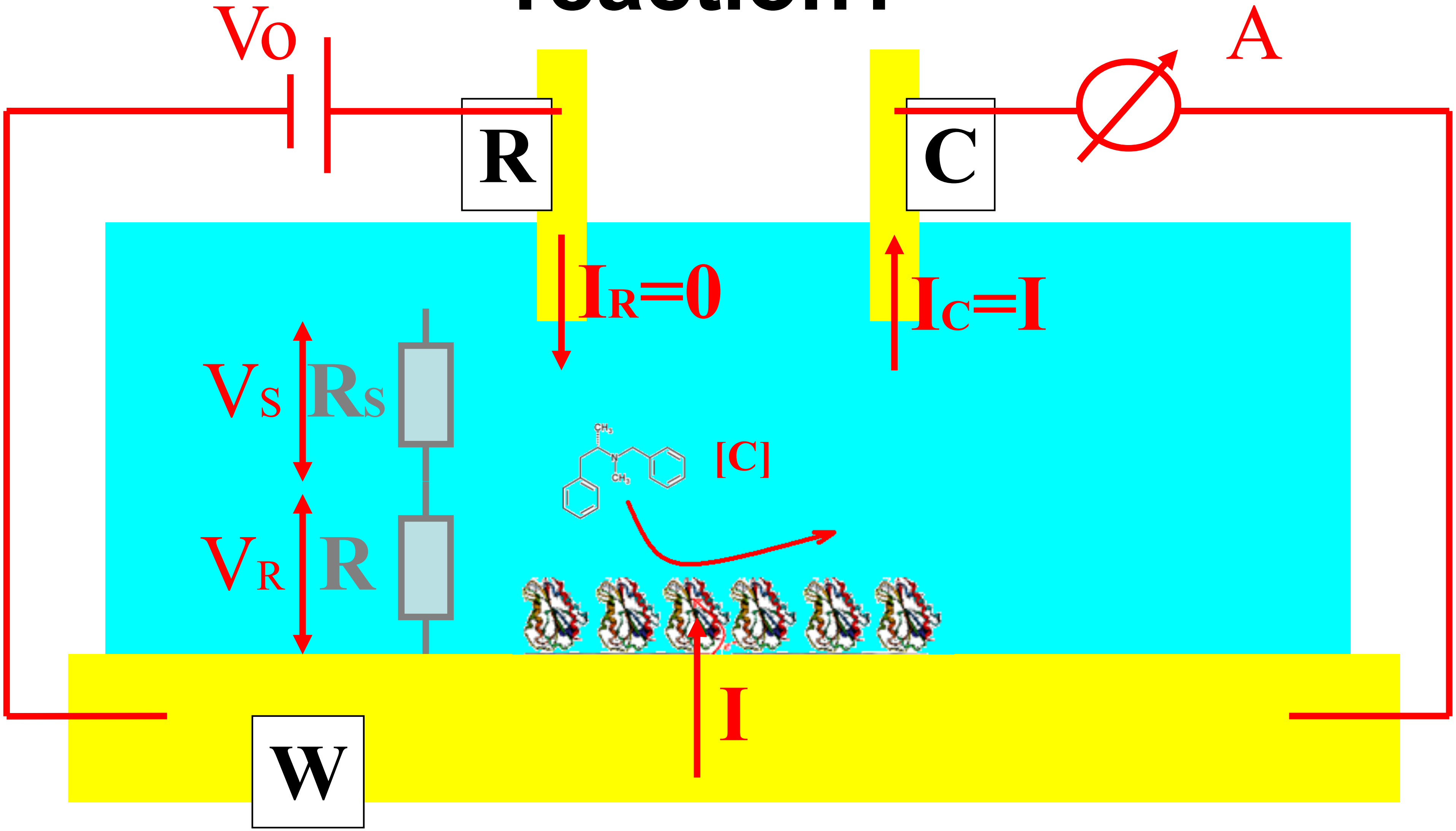
Current versus the redox species amount [concentration] upon the time

Q5

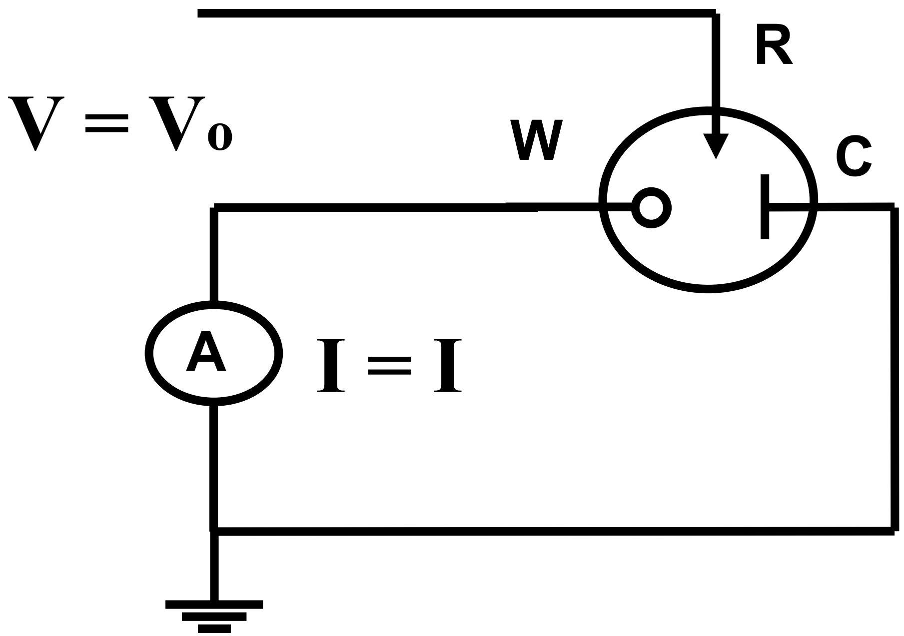
How many electrodes are required to measure at best a current vs a redox?

A. 1
B. 2
C. 3
D. 4
E. 5
F. 5+

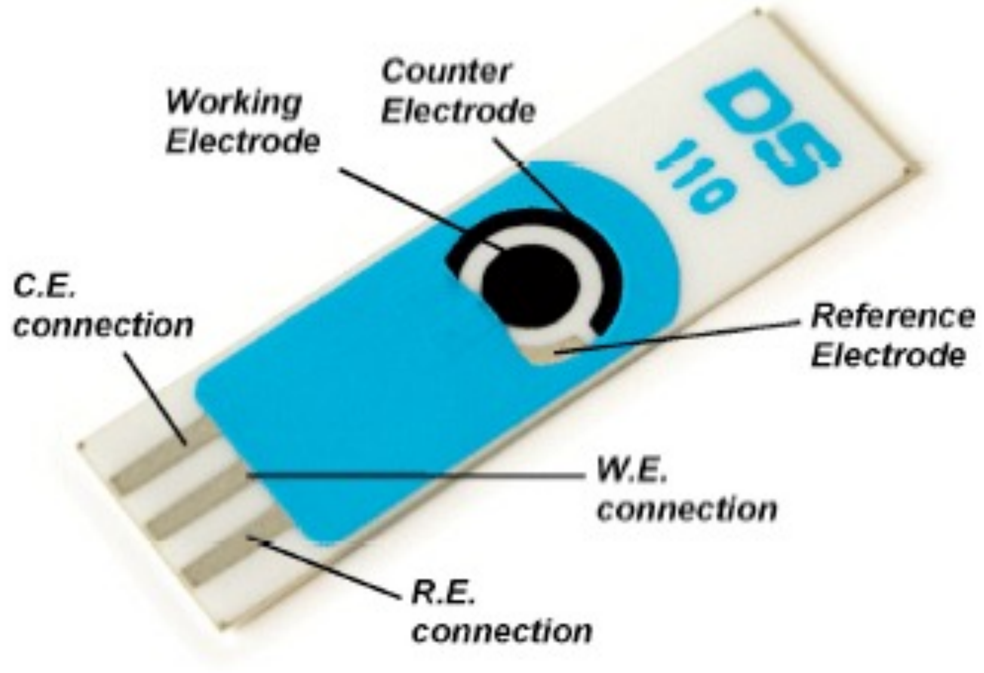
How to measure a redox reaction?



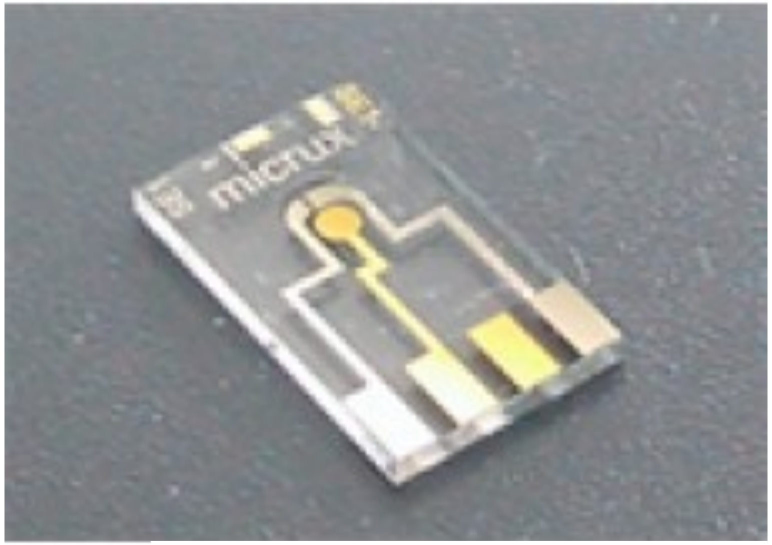
The three-electrode Electrochemical cell

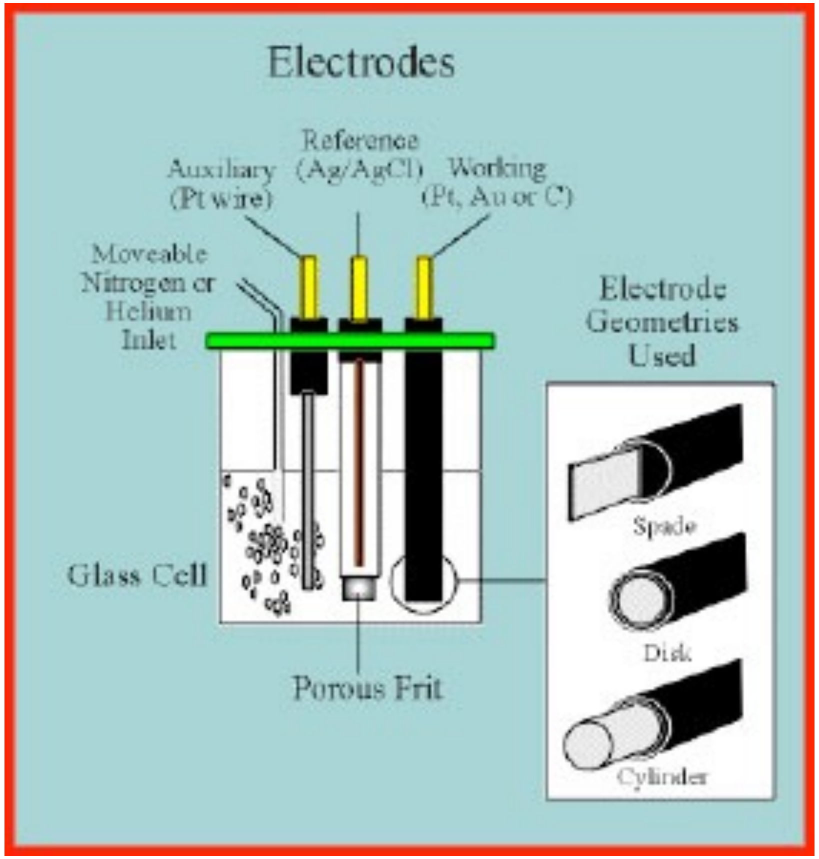


The three-electrode Electrochemical cell



Different kinds of three-electrode Electrochemical cell



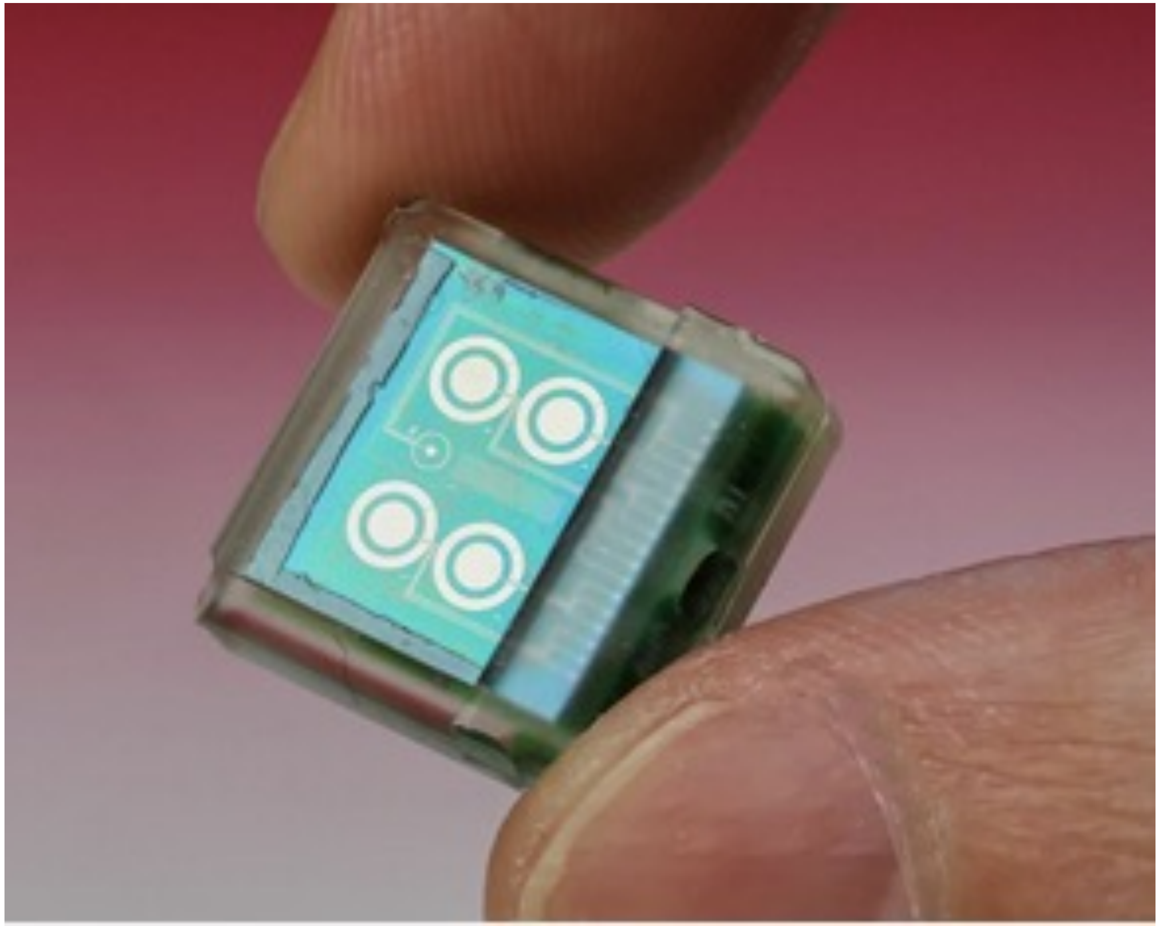


Q6

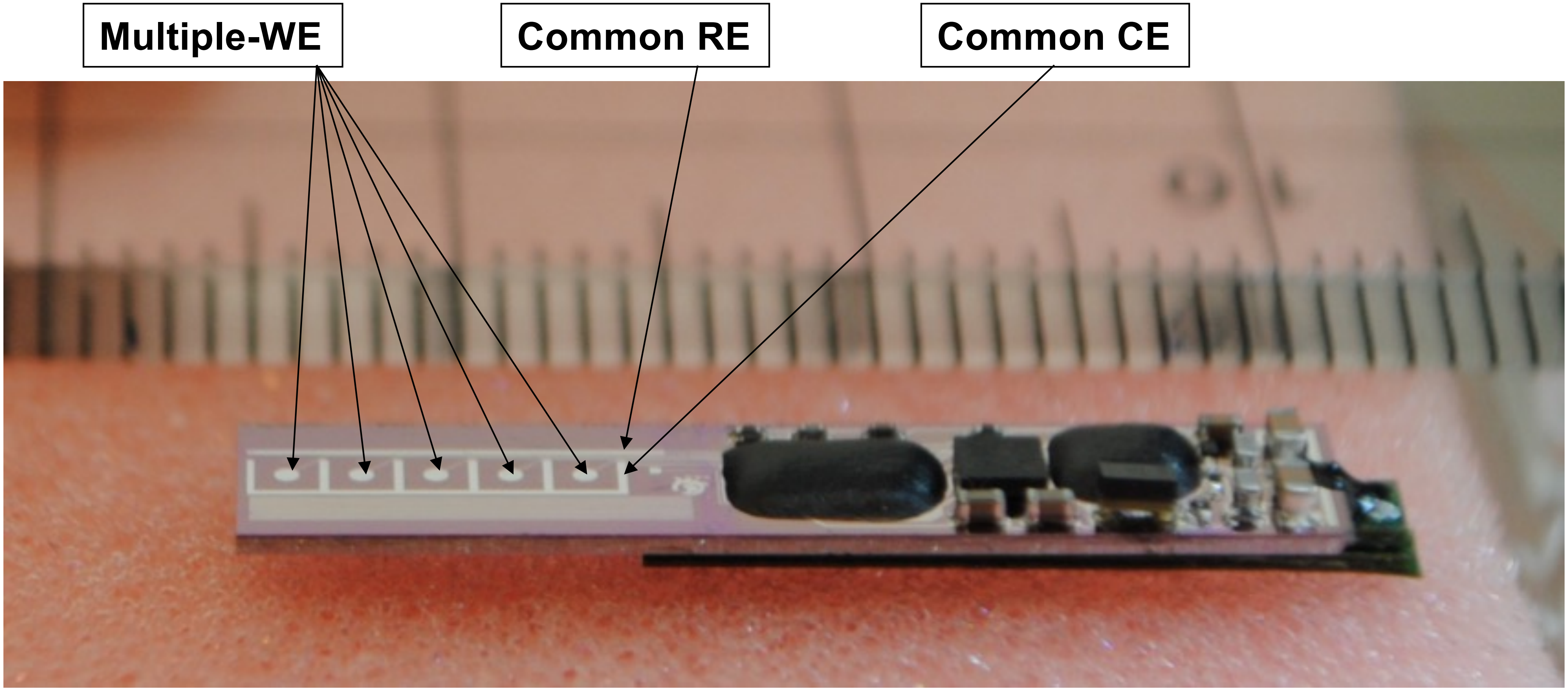
How many electrodes might have an electrochemical cell?

A. 1
B. 2
C. 3
D. 4
E. 5
F. 5+

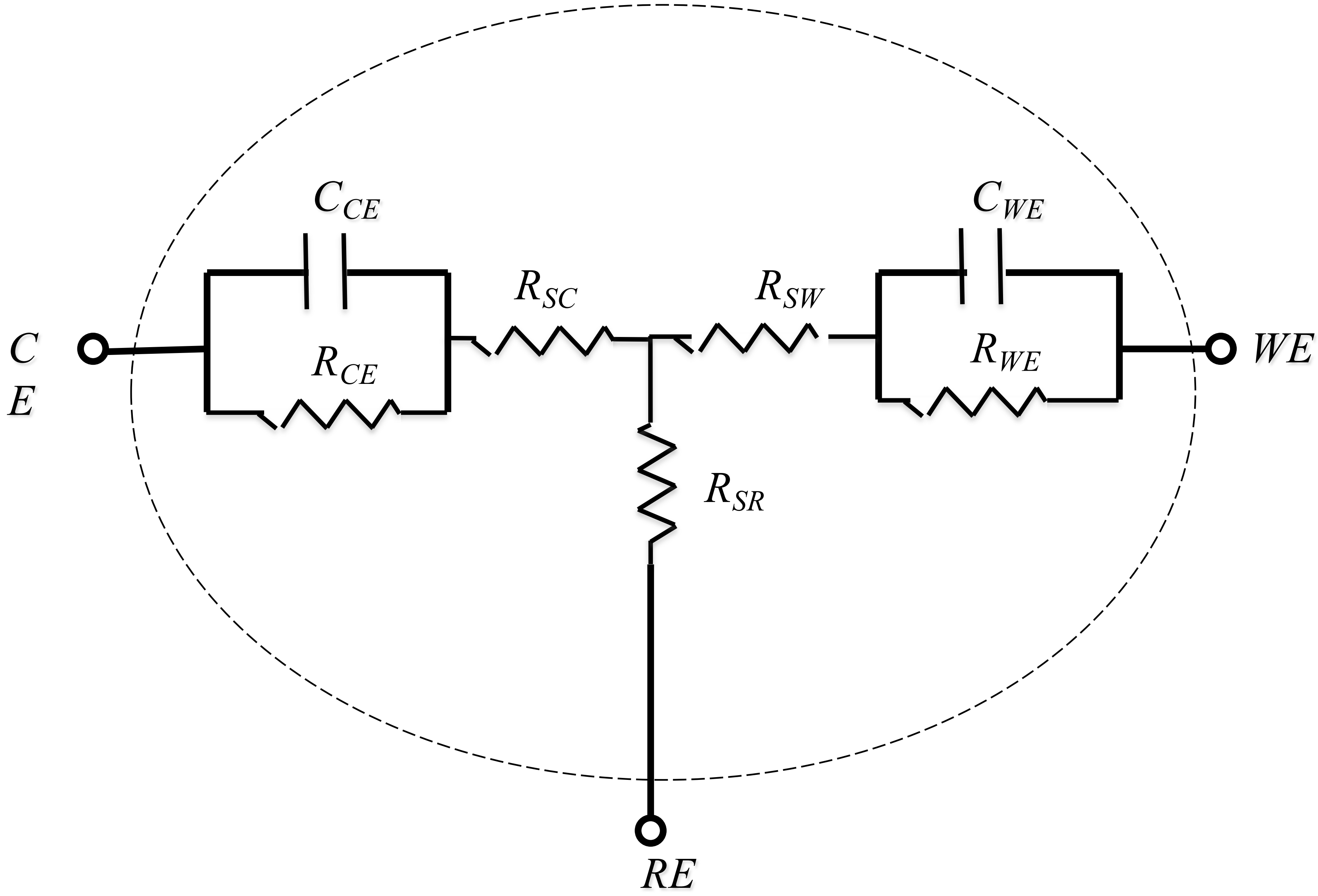
The three-electrode Electrochemical cells



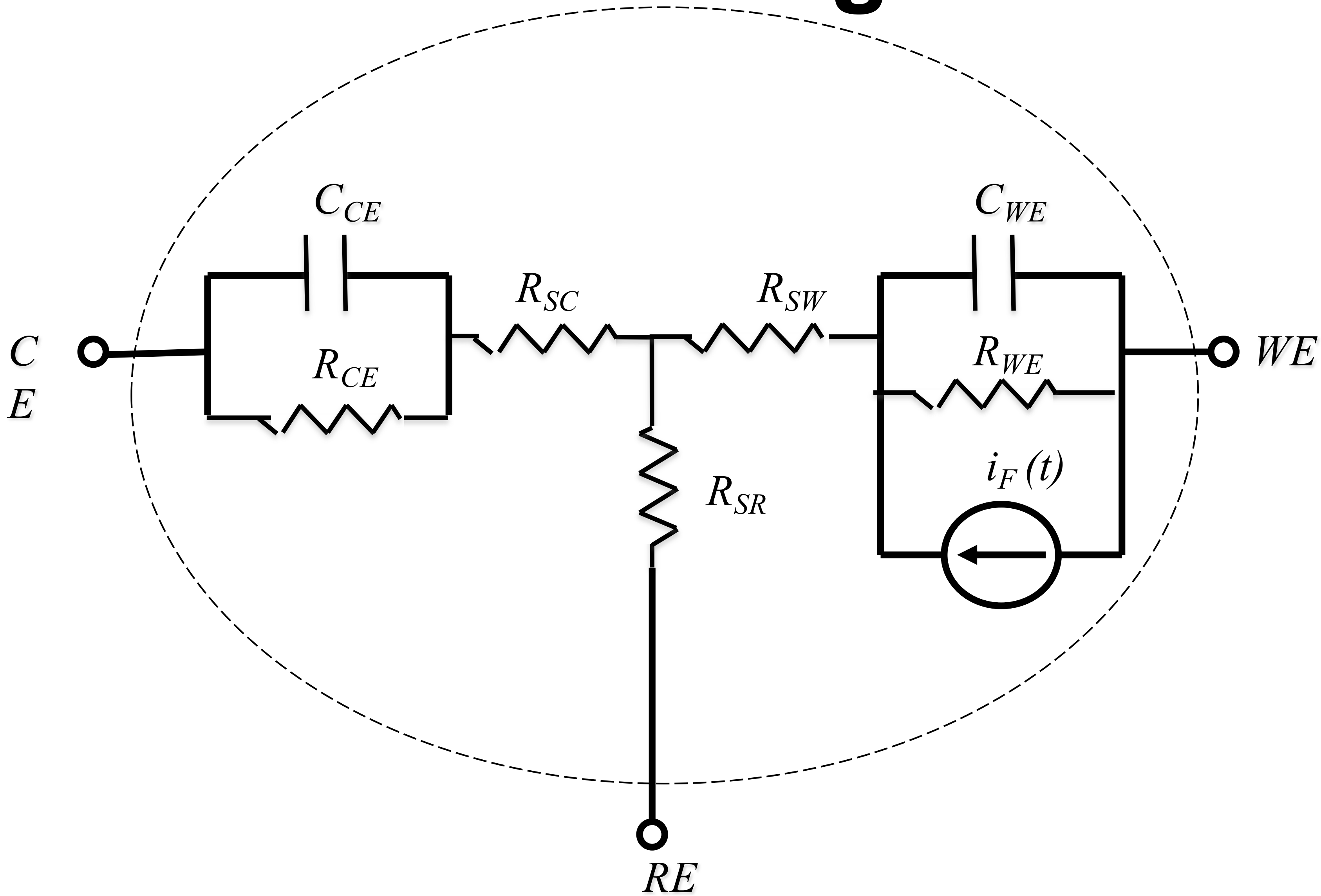
Electrochemical cells with multiple-electrodes



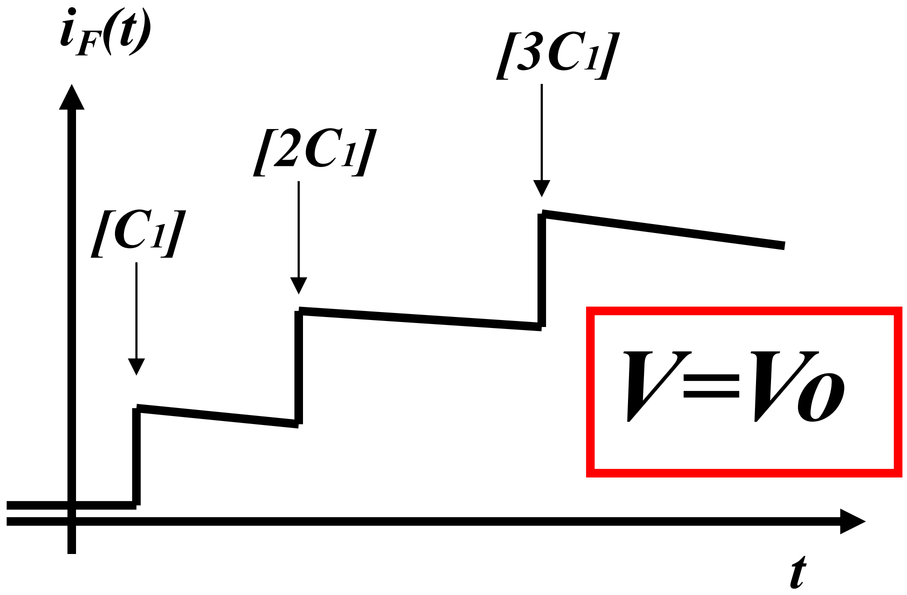
Equivalent circuit



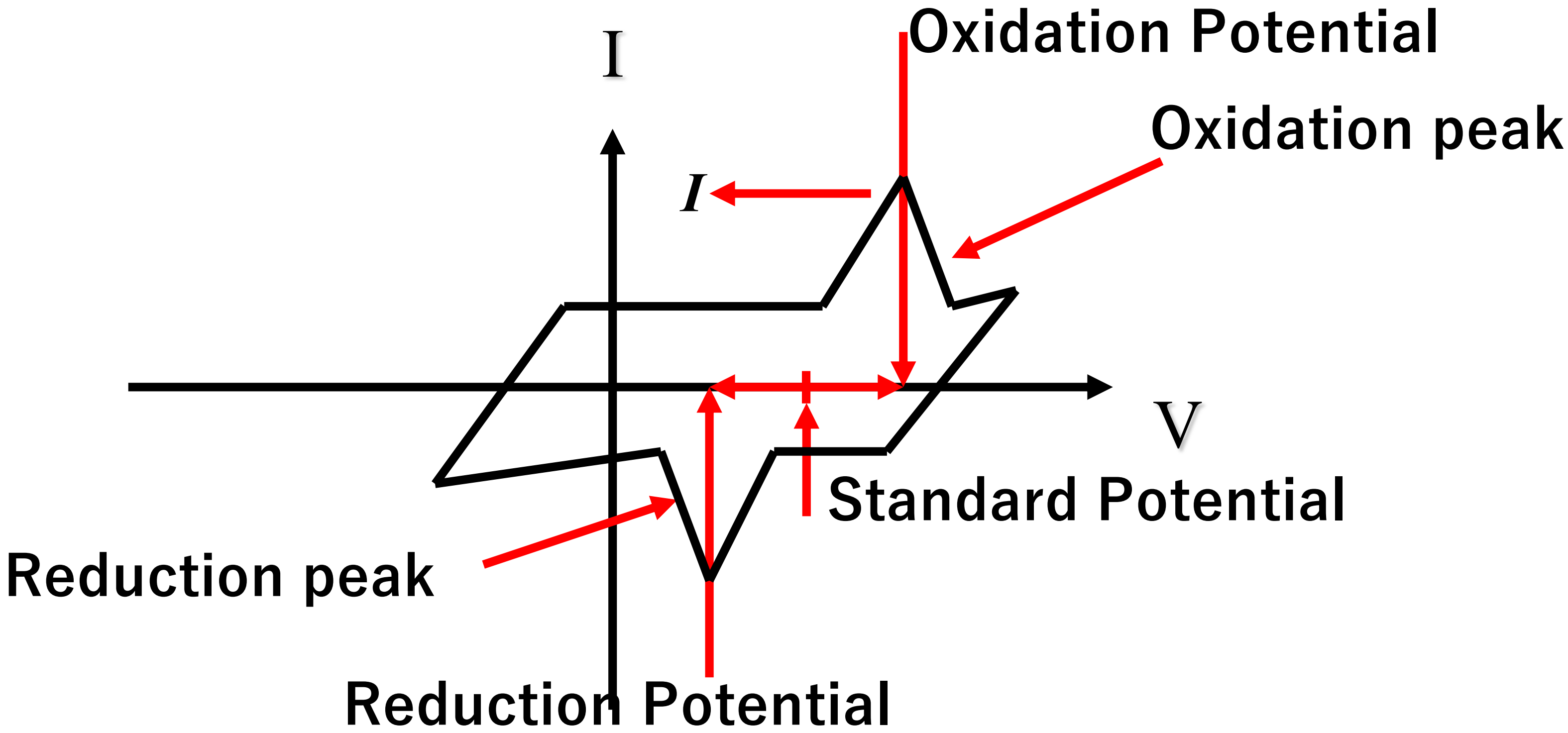
Equivalent circuit with Faradaic current-generator



Faradaic currents from Crono-Amperometry



Faradaic currents from Cyclic Voltammetry



Redox with oxidases

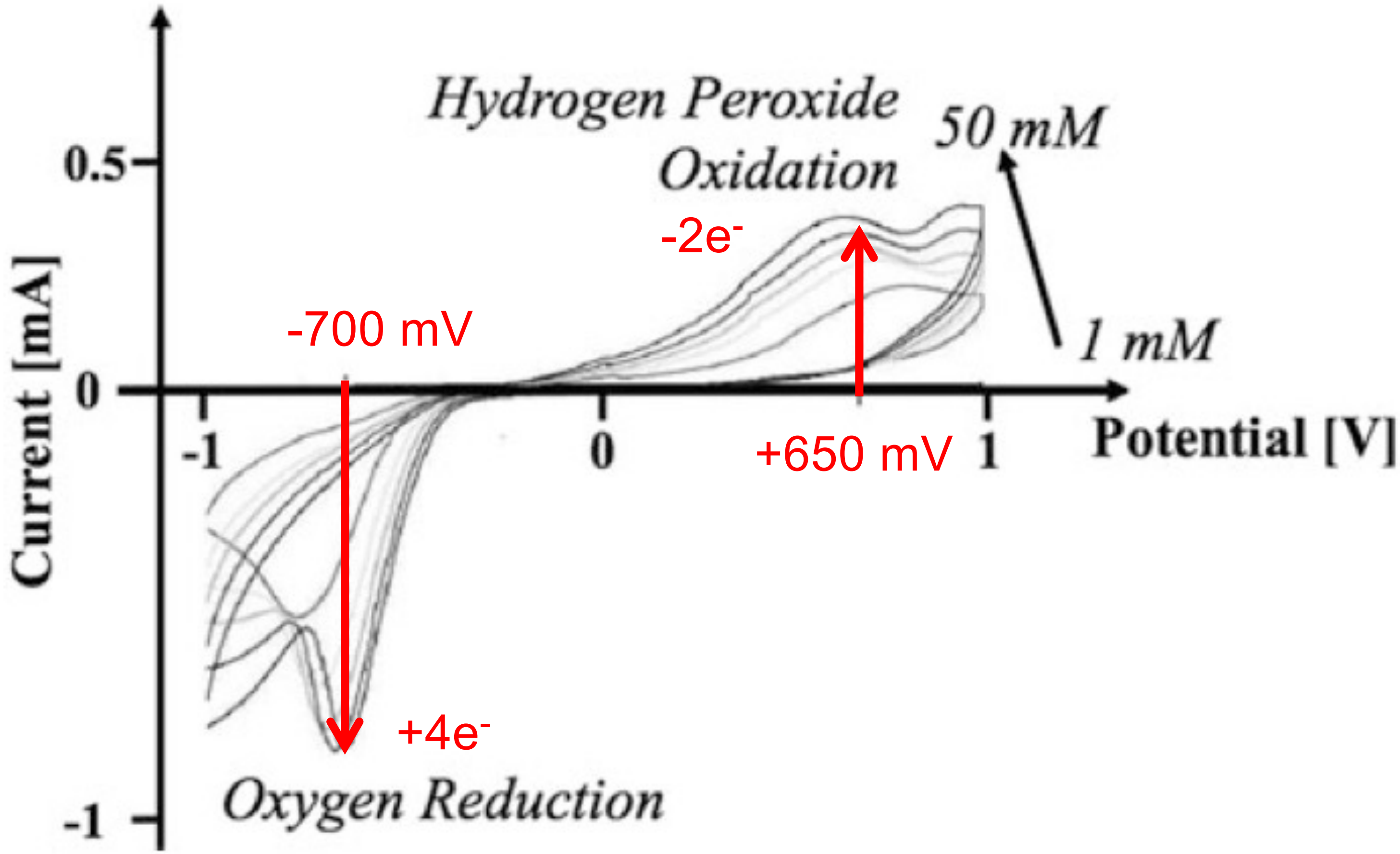
The hydrogen peroxide provides two possible redox reactions.
An oxidation:

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

And a reduction (of the oxygen):

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

Redox with hydrogen peroxide



O_2 reduction and H_2O_2 oxidation observed by potential sweeping

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

Q7

What is the Laplace Transform?

A. An integral transform only for periodical functions
B. An integral transform only for converging functions
C. An integral transform only for non-converging functions
D. An integral transform to simplify differential equations

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

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

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

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

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

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

Flick'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):

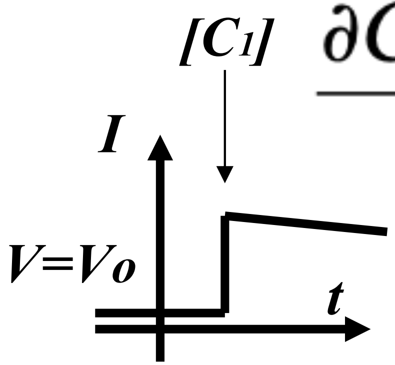
$$j_m \cong -D\,\frac{\partial C(x,t)}{\partial x}$$

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

Linear diffusion equation



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

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

$$s\hat{C}_0(x,s) - C(x,0) = D\frac{\partial \hat{C}_0(x,s)}{\partial x^2}$$

Boundary conditions

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

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

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

The Cottrell Equation

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

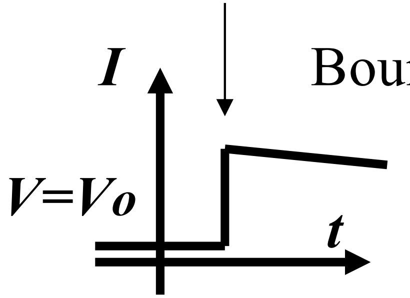
$$\widehat{C}(x,s) = \frac{C_0}{s} + A(s)e^{-\sqrt{s/D}x} + B(s)e^{+\sqrt{s/D}x}$$

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

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

The Cottrell Equation

[C₁] Linear diffusion equation



Boundary conditions

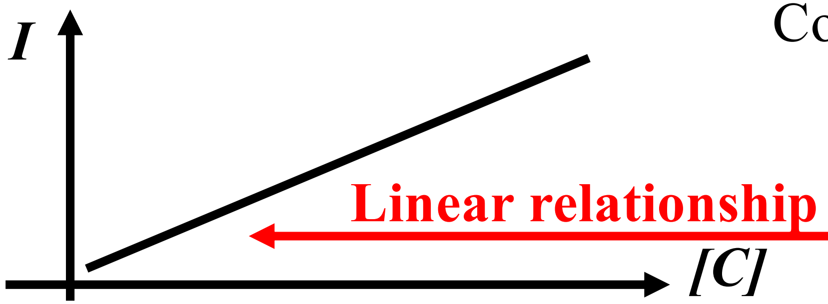
$$\widehat{C}(x,s)=\frac{C_0}{s}+A(s)e^{-\sqrt{s/D}x}$$

By definition

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

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

$$\widehat{i}(s)=nFA\frac{\sqrt{D}C(x,s)}{\sqrt{s}}$$



Cottrell equation

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

Q8

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

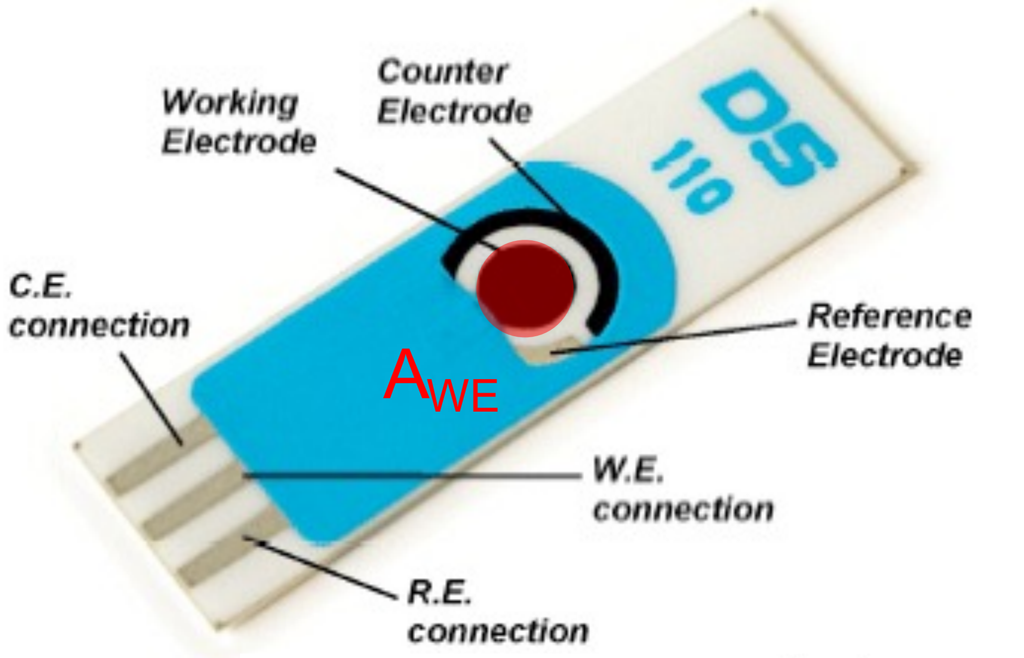
What is the “A” in the Cottrell Equation?

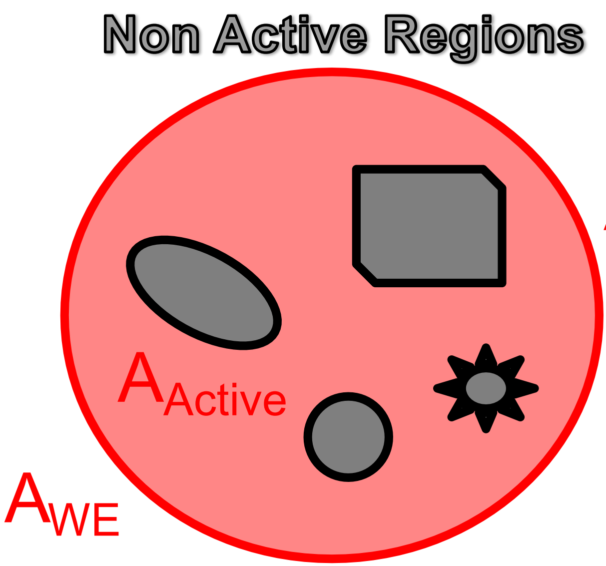
A. The geometrical area of the WE
B. The active area of the WE
C. The geometrical area of the CE
D. The active area of the CE
E. The geometrical area of the RE
F. The active area of the RE

Geometrical Area vs Active Area

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

?



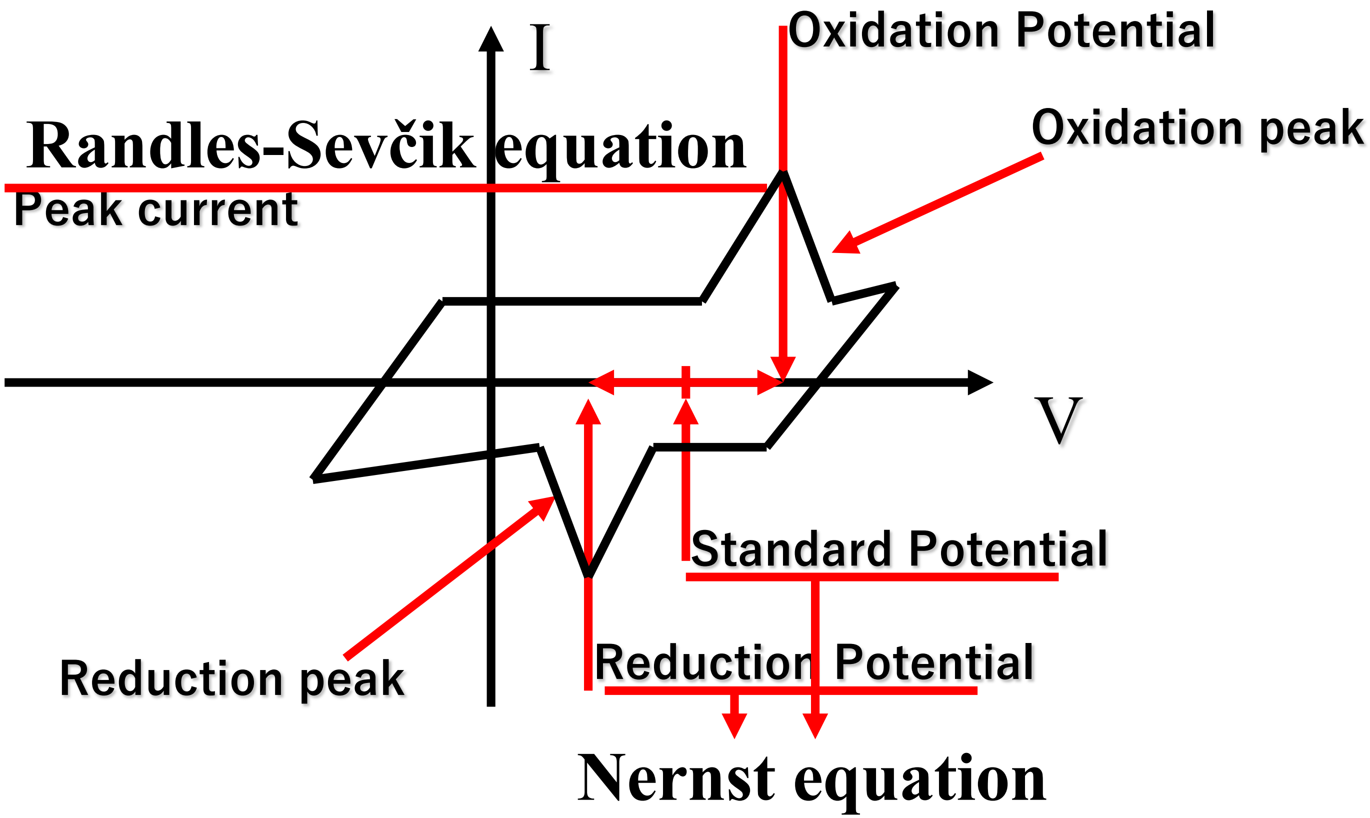


$A_{Active} < A_{WE}$

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

Electrochemically Active Area

Redox reactions from Voltammetry



Q9

What is the meaning of the "Oxidation Potential"?

A. The Energy of the current generated at the WE
B. The Energy of the current collected at the CE
C. The Energy provided by the RE
D. The Energy required by the Oxidation-reaction
E. A reference potential due to materials used for the RE

Q10

What is the meaning of the “Standard Potential”?

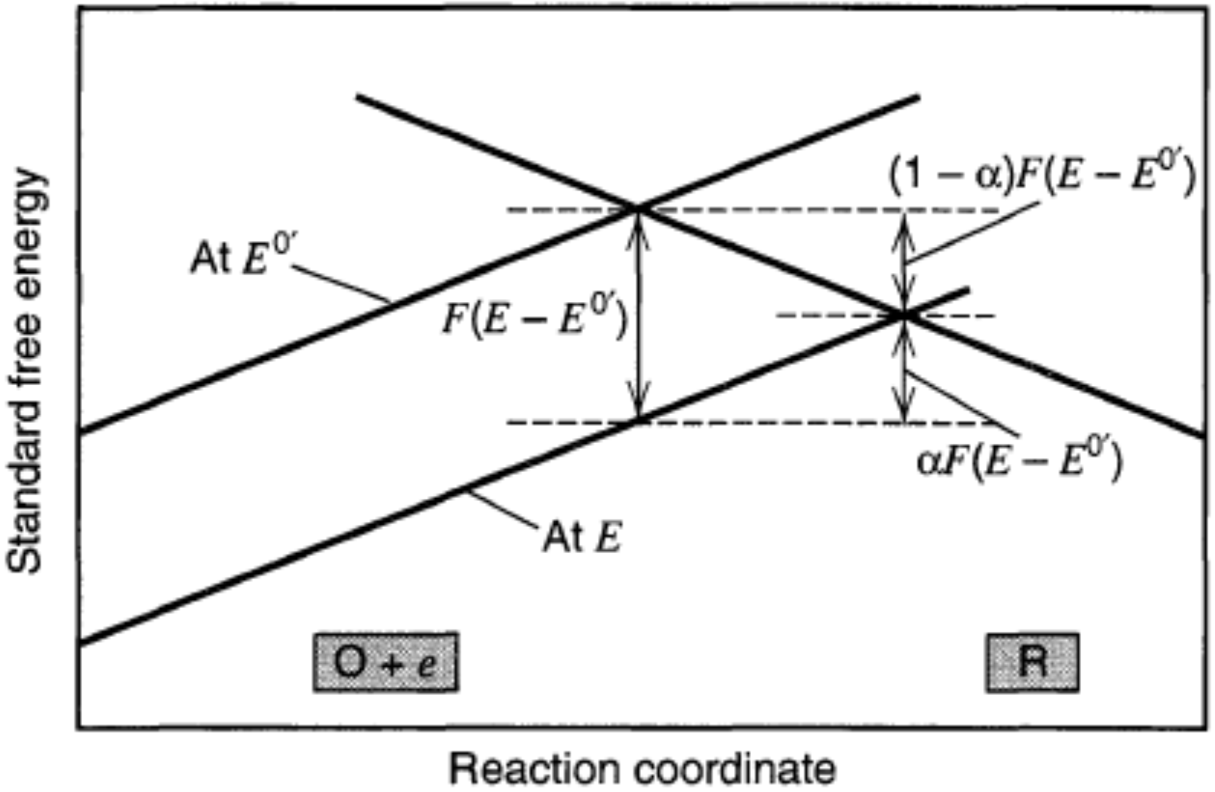
A. The Energy of the current generated at the WE
B. The Energy of the current collected at the CE
C. The Energy provided by the RE
D. The Energy required by the Oxidation-reaction
E. A reference potential due to materials used for the RE

Q11

What is the meaning of the difference between "Standard" and "Oxidation" Potential?

A. The Energy of the current generated at the WE
B. The Energy of the current collected at the CE
C. The Energy provided by the RE
D. The Energy required by the Oxidation-reaction
E. A reference potential due to materials used for the RE

Redox Reactions



Nernst Equation

Redox Reaction

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

Equilibrium Constants

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

@ Equilibrium:

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

Nernst Equation

Redox Reaction

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

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

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:

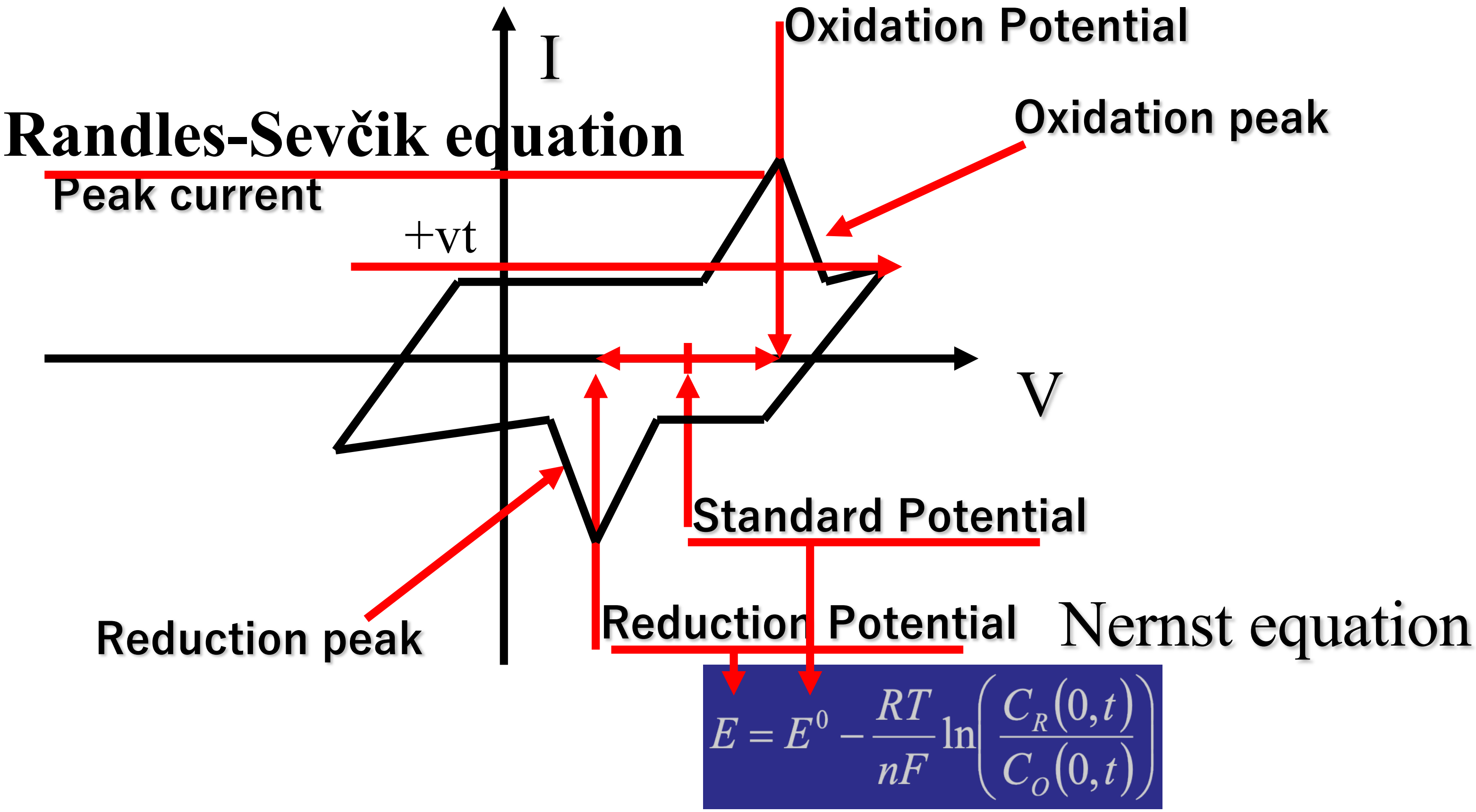
$$i = 0 \Rightarrow C_O(0,t)e^{-\frac{\alpha F(E-E^0)}{RT}} = C_R(0,t)e^{\frac{(1-\alpha)F(E-E^0)}{RT}}$$

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

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

If *n* electrons are involved!

Redox reactions from Voltammetry



Randles-Sevčik Equation

Voltage Sweep

$$E = E_i + \upsilon t$$

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

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

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

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

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

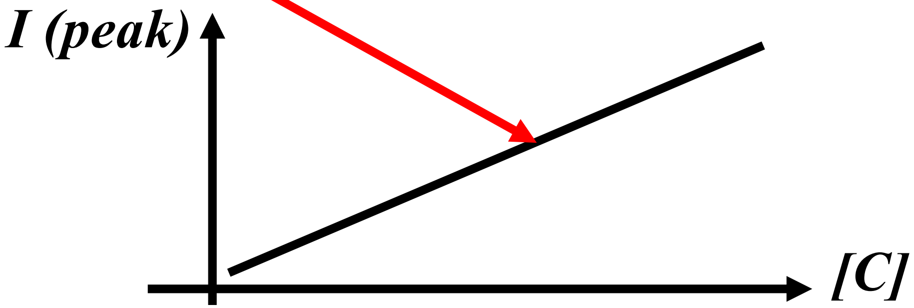
Randles-Sevčik Equation

Voltage Sweep

$$E = E_i + \upsilon t$$

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

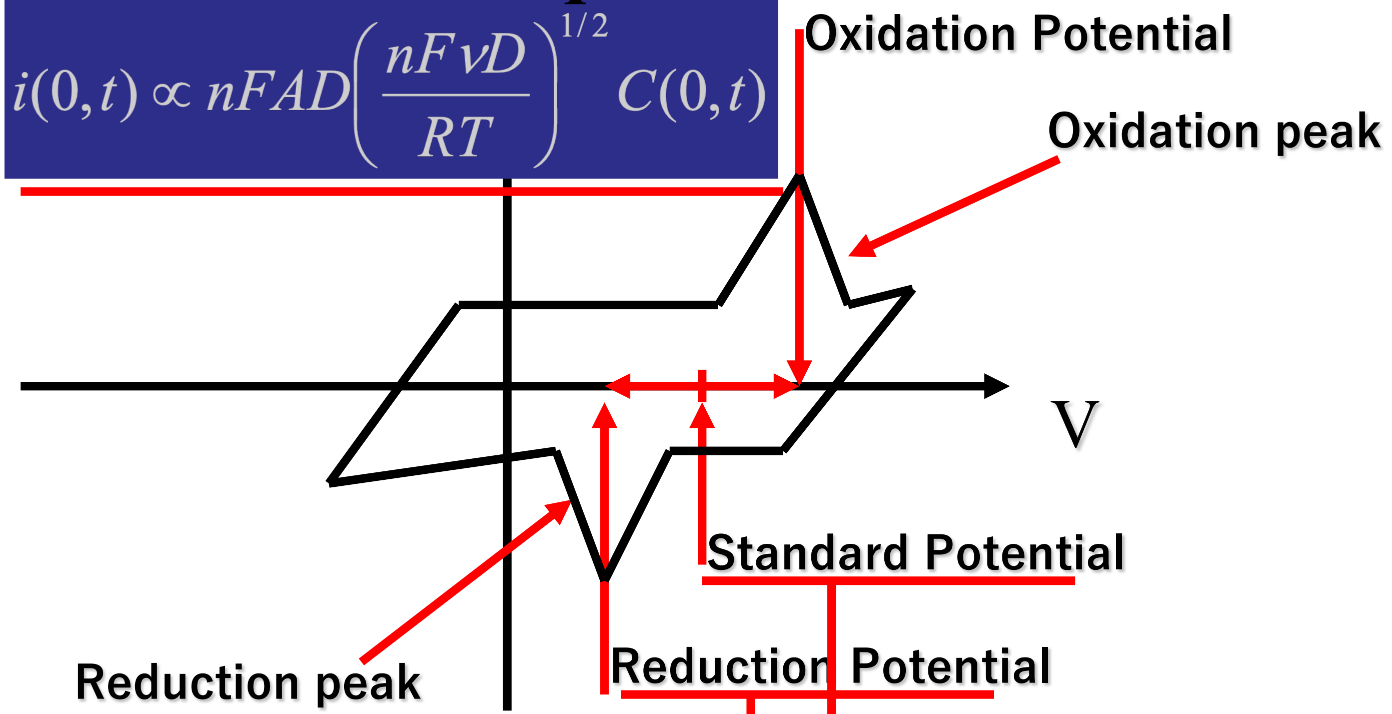
$$i(t) = nFAD\sqrt{\frac{nFDv}{RT}}C(0,t)$$



Redox reactions from Voltammetry

Randles-Sevčik equation for the Peak current

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



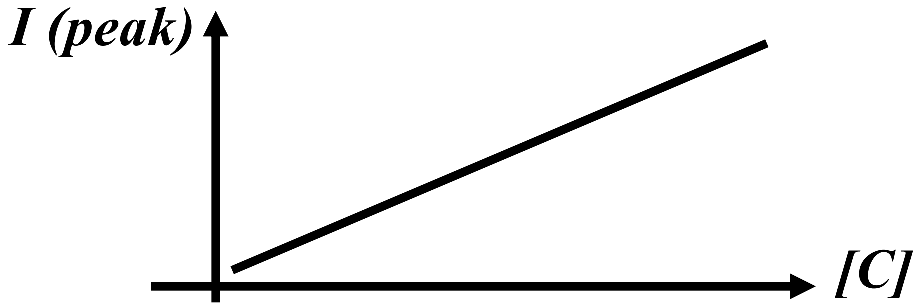
$$E = E^0 - \frac{RT}{nF}\ln\left(\frac{C_R(0,t)}{C_O(0,t)}\right)$$

Nernst equation

Detection Principle

- Sensitivity: definition

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

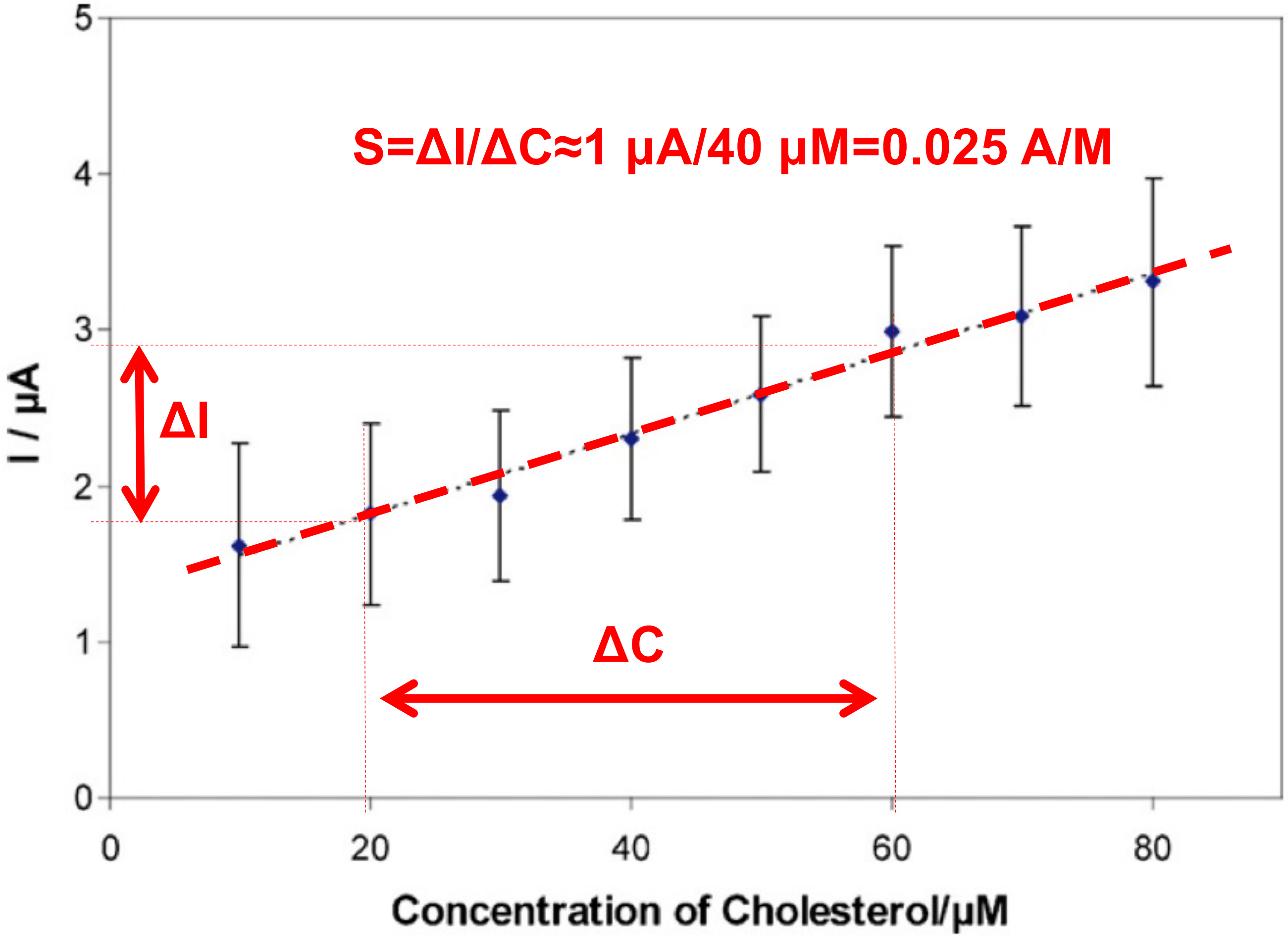


$$\Delta i = nFAD\sqrt{\frac{nFDv}{RT}}\Delta C \rightarrow \frac{\Delta i}{\Delta C} \equiv S$$

Detection Sensitivity!

Detection Principle

- Sensitivity: example – A linear sensor



Q12

In biosensing, is 25 mA/M fully equivalent to 25 nA/µM?

A. Yes, since it is mathematically correct
B. Yes, since units are equivalent
C. In some cases, depending on the kind of sensing
D. No, since units matter
E. No, since it is not mathematically correct

Detection Principle

- Sensitivity: metric considerations

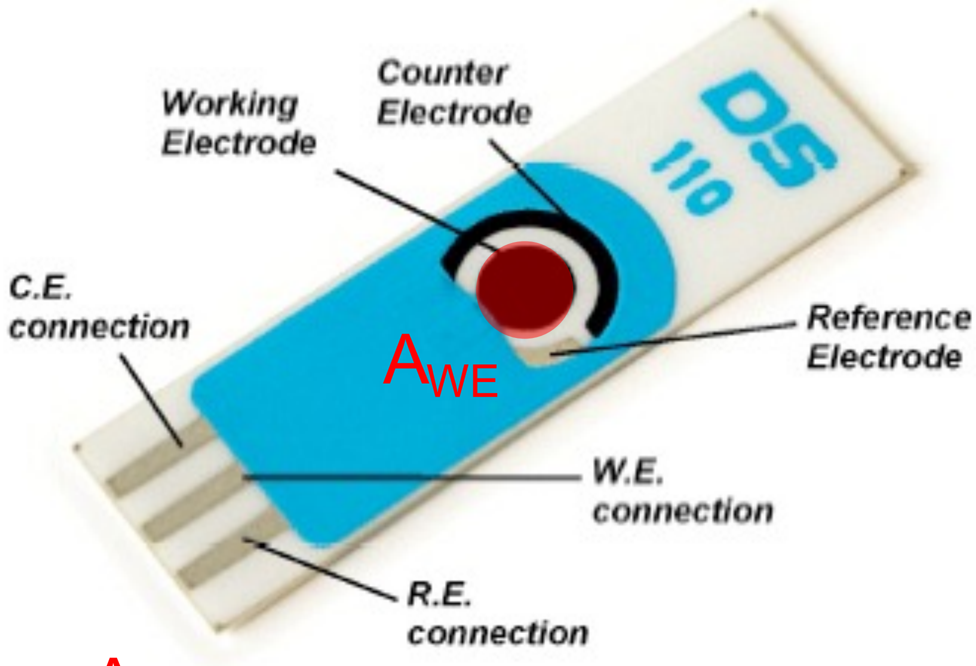
$$S = \frac{\Delta i}{\Delta C} = \frac{1\,\mu A}{40\,\mu M} = 25\frac{mA}{M}$$

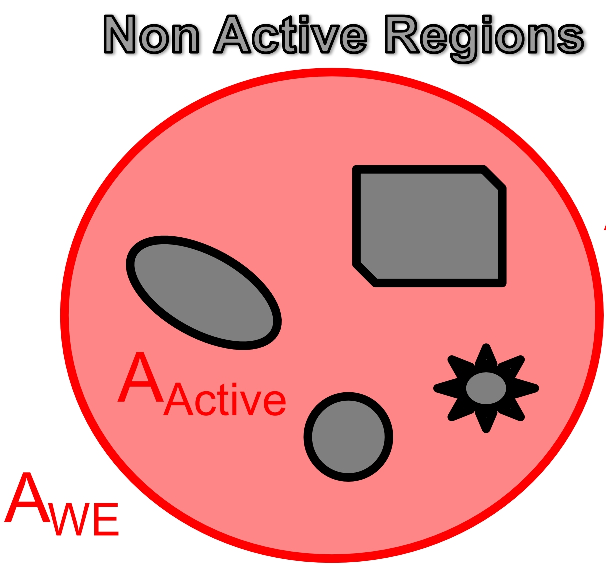
Mathematically correct, but are we going to measure mAmpere each Molar of concentration?

$$S = \frac{\Delta i}{\Delta C} = \frac{1\,\mu A}{40\,\mu M} = 25\frac{nA}{\mu M}$$

More correct, because we are going to require a precision of sub-µAmpere meanwhile facing variations on µM range

Geometrical Area vs Active Area





$A_{Active} < A_{WE}$

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

Electrochemically Active Area

Detection Principle

- Sensitivity: metric considerations

$$S = \frac{\Delta i}{\Delta C} = \frac{1\,\mu A}{40\,\mu M} = 25\frac{mA}{M}$$

Correct, but we cannot compare biosensors with different geometry on working electrodes

$$S_A = \frac{\Delta i}{\Delta C \cdot A} = \frac{1\,\mu A}{40\,\mu M \cdot 0.2cm^2} = 125\frac{nA}{\mu M \cdot cm^2}$$

More useful to compare biosensors with different geometry on working electrodes

Q13

$$S_A = \frac{\Delta i}{\Delta C \cdot A}$$

What is the "A" in the definition of S_A?

A. The geometrical area of the WE
B. The active area of the WE
C. The geometrical area of the CE
D. The active area of the CE
E. The geometrical area of the RE
F. The active area of the RE

Sensitivity per Unit Area

$$S = \frac{\Delta i}{\Delta C}$$ Sensitivity

$$S_A = \frac{S}{A_{WE}}$$ Sensitivity per unit of Area

$$S_A = \frac{\Delta i}{A_{WE}\Delta C} = \frac{nFA_{Active}\sqrt{D}}{A_{WE}\sqrt{\pi t}} \xrightarrow[A_{Active} \to A_{WE}]{} \frac{nF\sqrt{D}}{\sqrt{\pi t}}$$

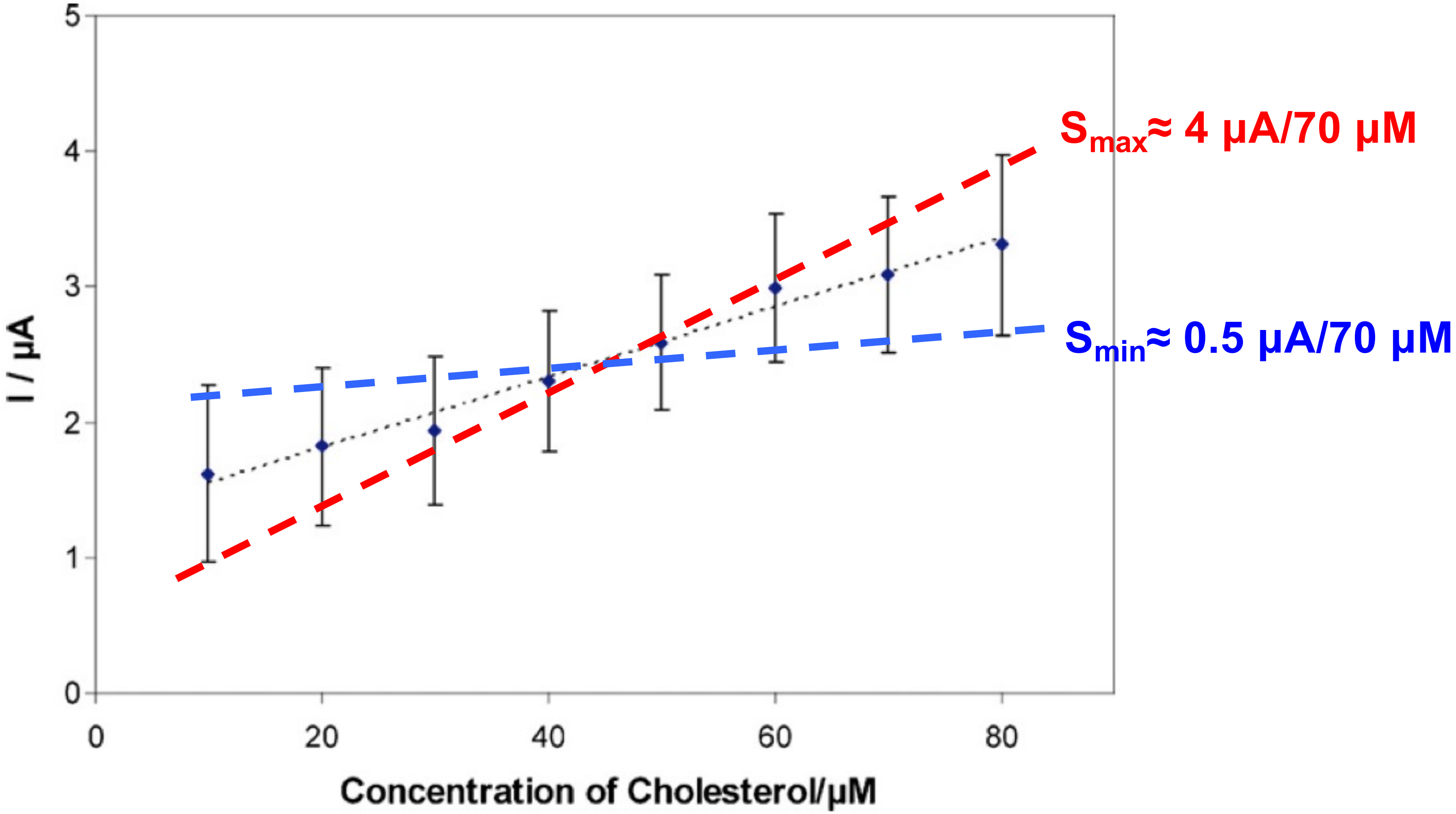
Q14

Does the S need to be estimated with standard deviation?

A. Not at all !!!
B. No, since S is calculated by a linear regression
C. In some cases, when measures are very noisy
D. Yes, but not when measures are not so noisy
E. Yes, of course!!!

Detection Principle

- Sensitivity: Measurement errors



Detection Principle

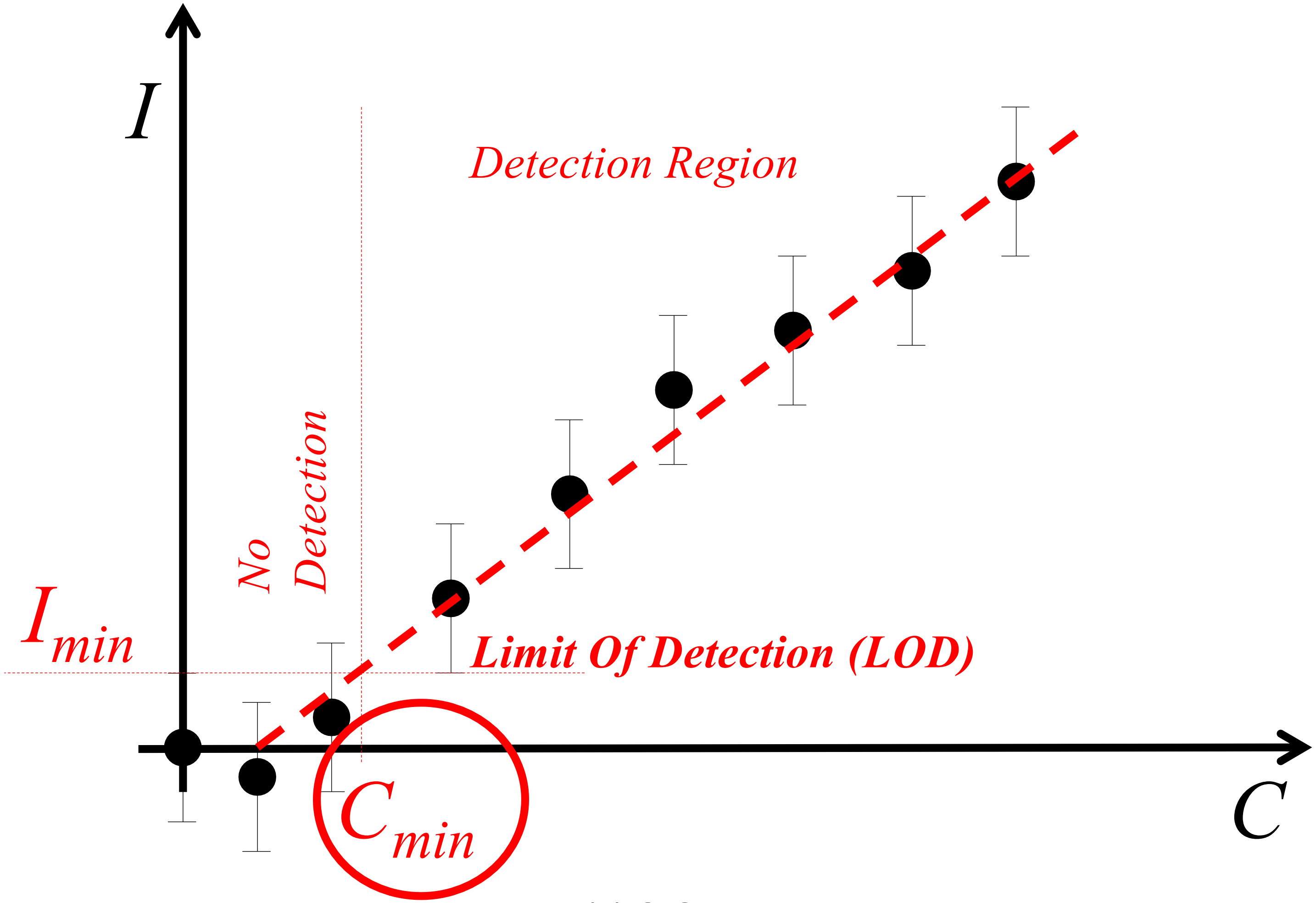
- Sensitivity: Average value and standard its deviation

$$\left.\begin{aligned} S_{\max} &\approx \frac{4\,\mu A}{70\,\mu M} = 57\,\frac{nA}{\mu M} \\ S_{\min} &\approx \frac{0.5\,\mu A}{70\,\mu M} = 7\,\frac{nA}{\mu M} \end{aligned}\right\} \rightarrow \bar{S} = 32 \pm 25\,\frac{nA}{\mu M}$$

More correct, because we have measurement errors that do not allow a precise estimation of the sensitivity

Detection Principle

- Detection Limit: a graphic interpretation

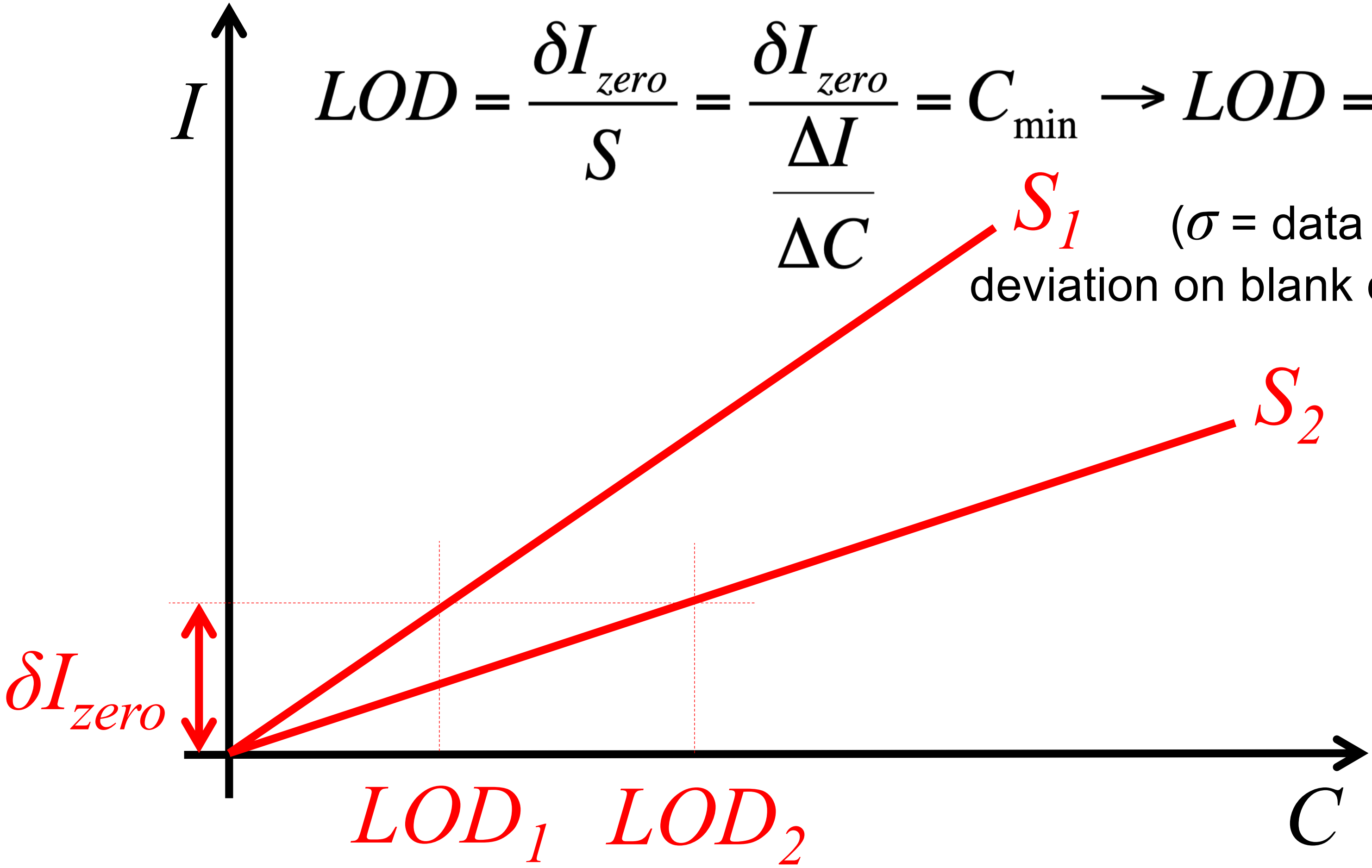


Detection Principle

- Detection Limit: a precise definition

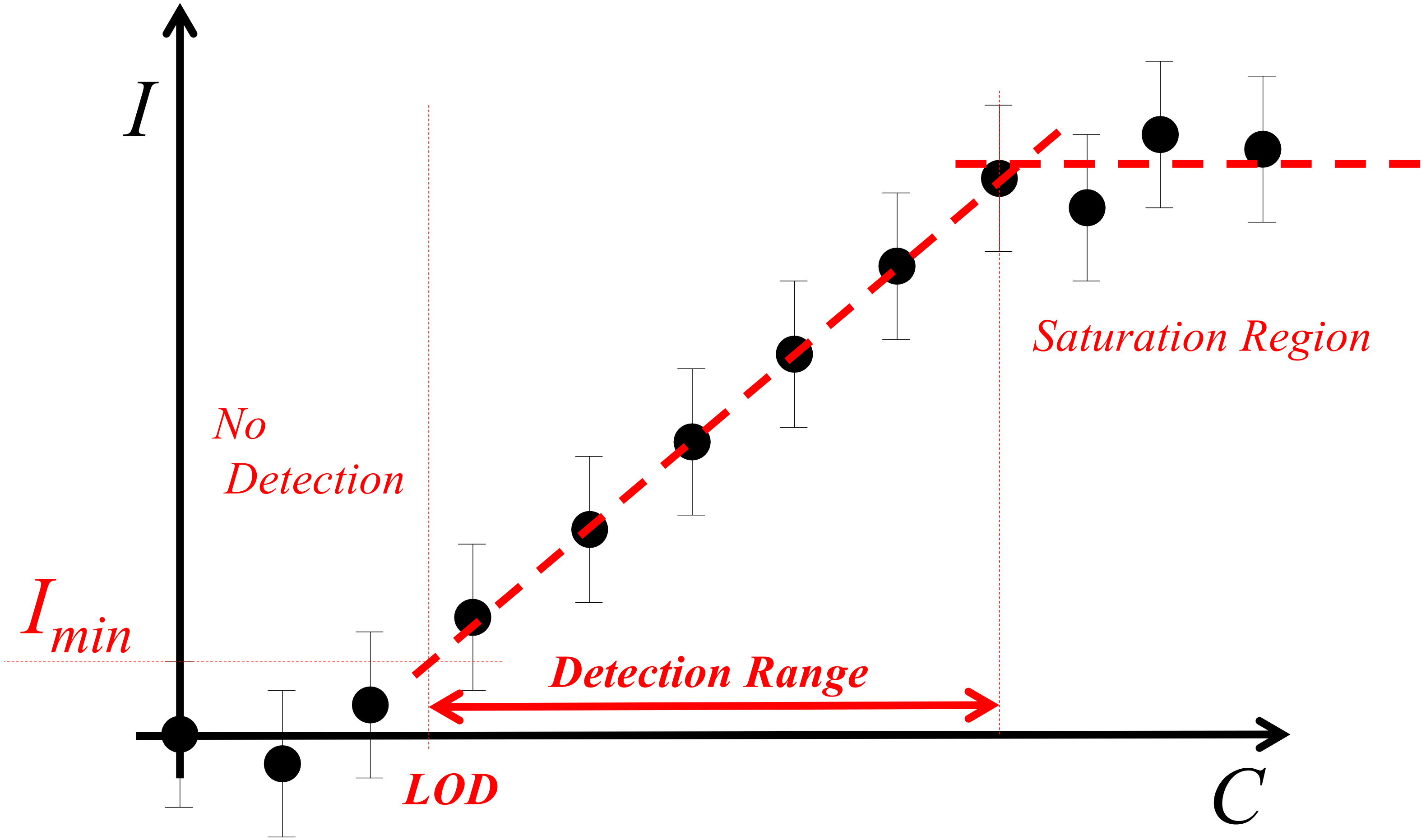
$$LOD = \frac{\delta I_{zero}}{S} = \frac{\delta I_{zero}}{\frac{\Delta I}{\Delta C}} = C_{\min} \rightarrow LOD = \frac{3\sigma}{S}$$

(σ = data standard deviation on blank detection)



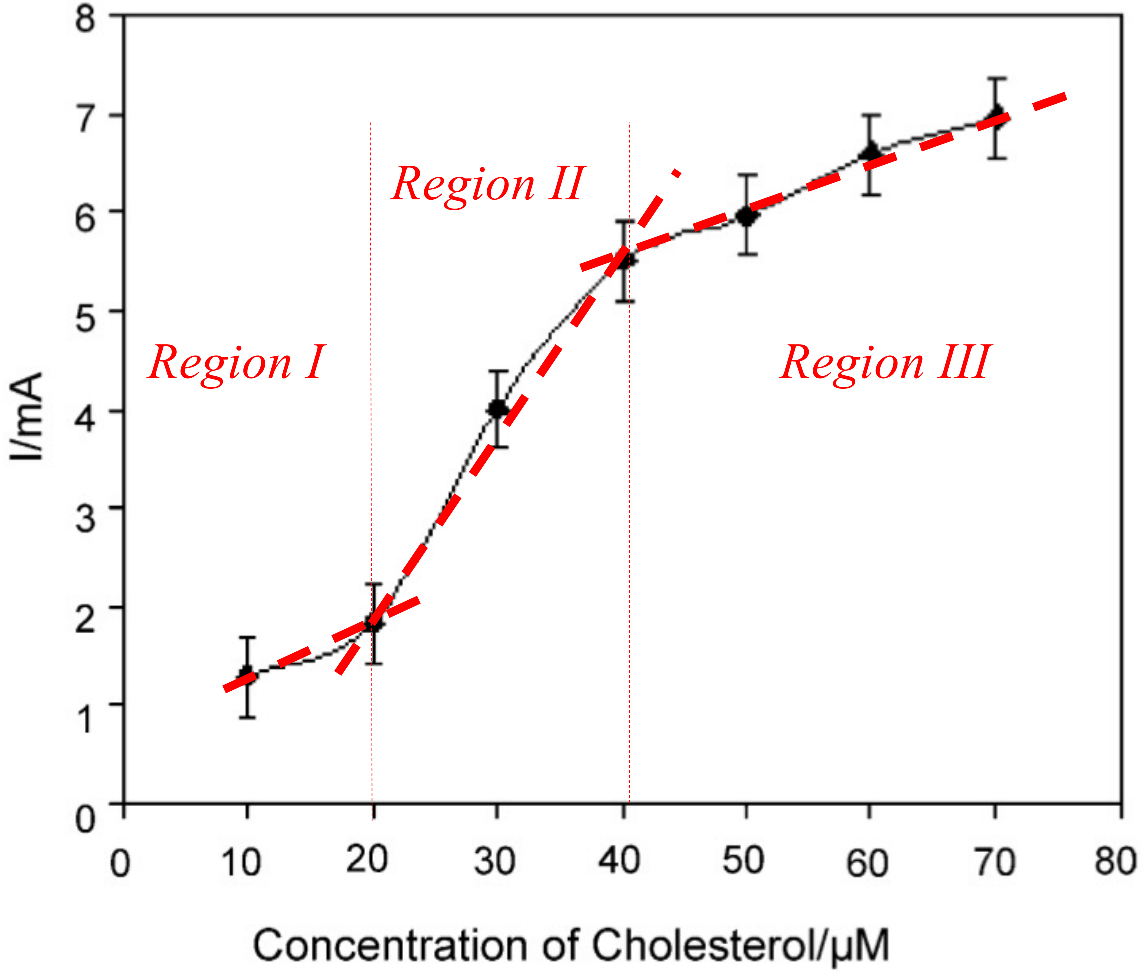
Detection Principle

- Saturation region: a graphic interpretation



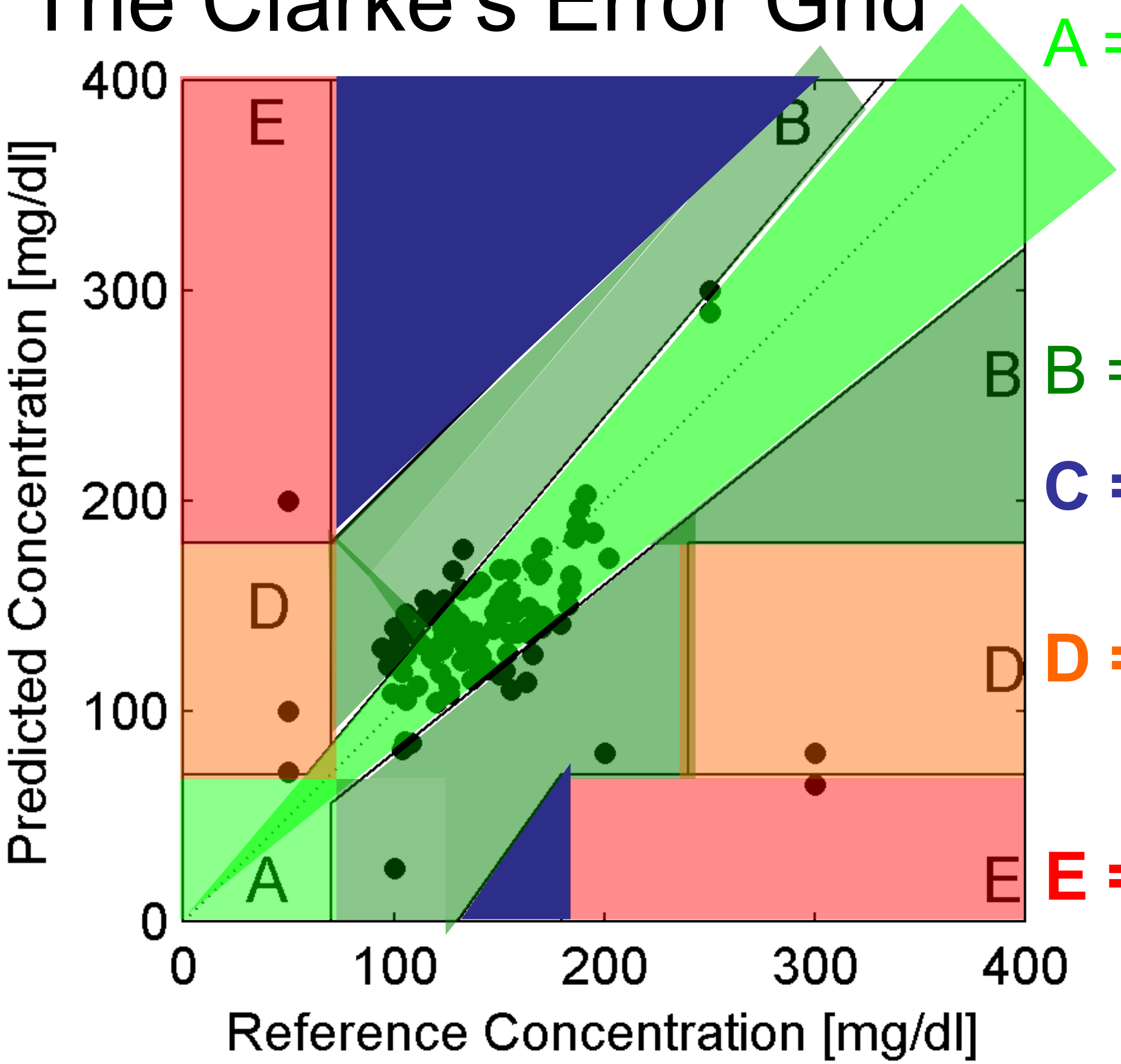
Detection Principle

- Sensitivity: example – A non linear sensor



Sensitivity

- The Clarke's Error Grid



A = Clinically Accurate

B = Clinically Acceptable

C = Overcorrection

D = Dangerous Failure

E = Serious Errors