Elektrokemijske metode za rad s biosenzorima

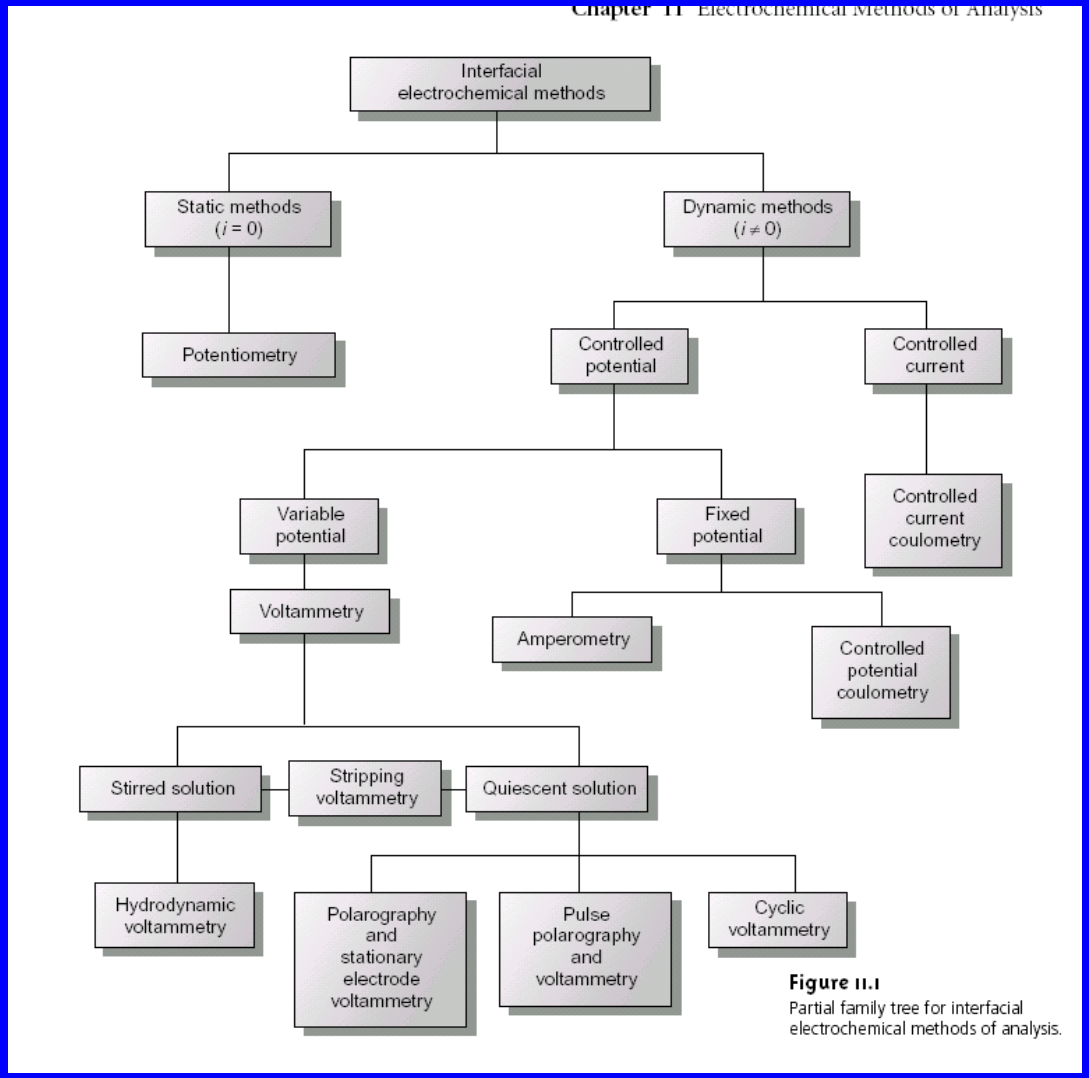


Figure 11.1
Partial family tree for interfacial electrochemical methods of analysis.

POTENCIOSTAT

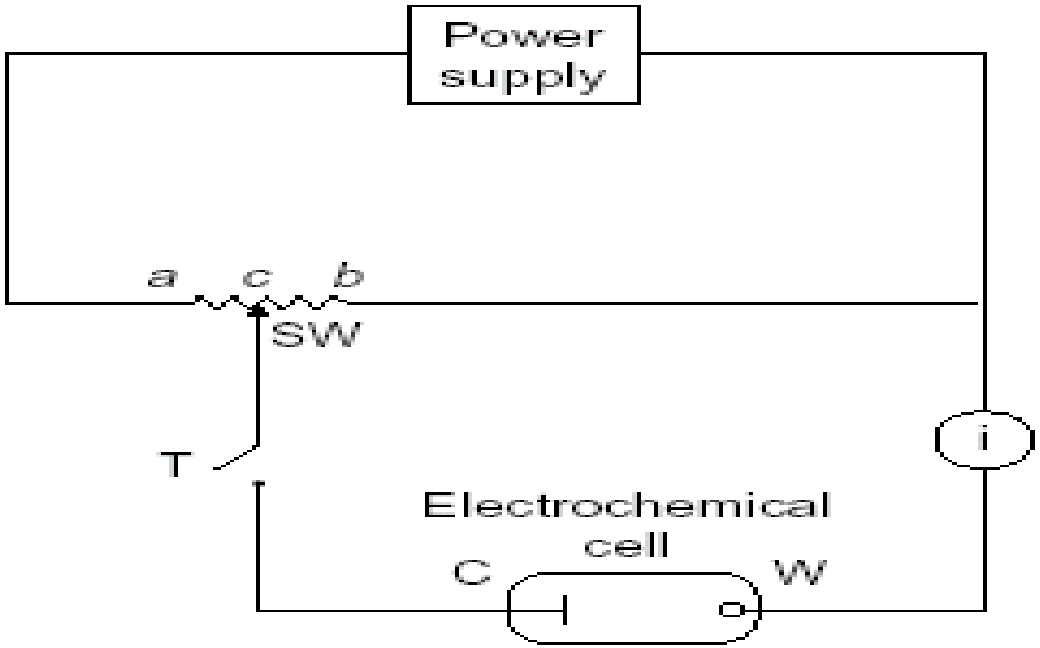


Figure 11.2
Schematic diagram of a manual potentiostat: C = counter electrode; W = working electrode; SW = slide-wire resistor; T = tap key; i = galvanometer.

GALVANOSTAT

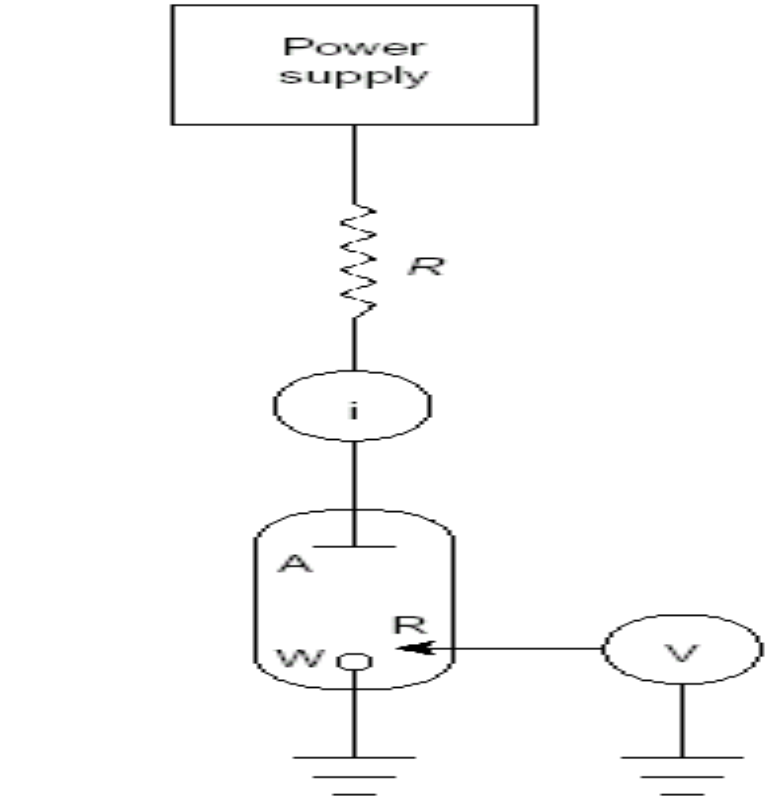
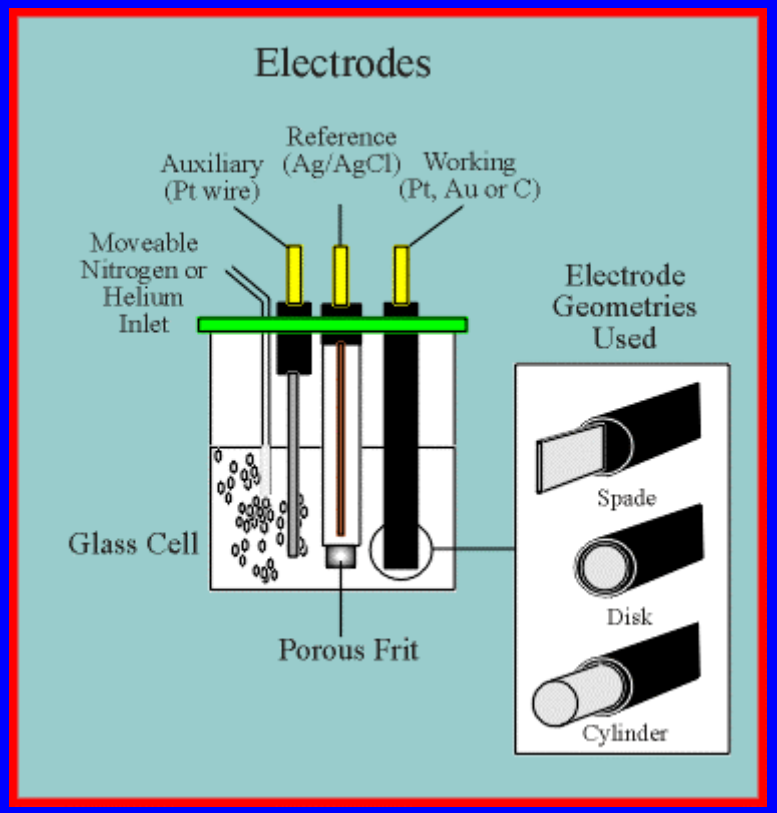
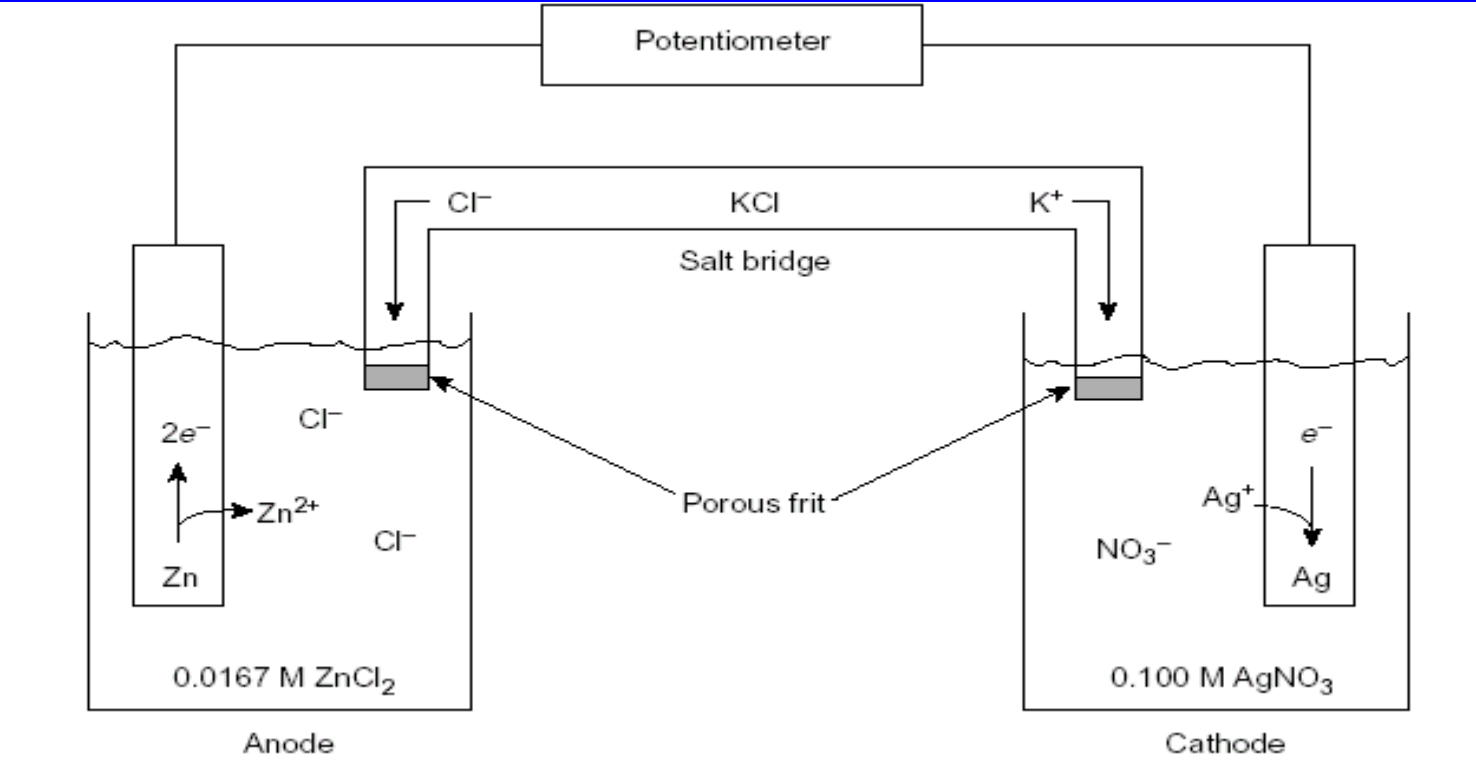


Figure 11.3
Schematic diagram of a galvanostat: *R* = resistor; i = galvanometer; A = auxiliary electrode; W = working electrode; R = reference electrode; V = voltmeter or potentiometer (optional).

OPREMA ZA ELEKTROKEMIJSKA MJERENJA



POTENCIOMETRIJSKA MJERENJA



- **Anodna reakcija:**
  - $Zn(s) \Leftrightarrow Zn^{2+}(aq) + 2e-$
- **Katodna reakcija:**
  - $Ag+(aq) + e- \Leftrightarrow Ag(s)$
  -
- **Sumarna reakcija:**
  - $Zn(s) + 2Ag+(aq) \Leftrightarrow 2Ag(s) + Zn^{2}+(aq)$

Prikaz elektrokemijskog članka:

- Zn(s) | ZnCl2 (*aq*, 0.0167 M) || AgNO3 (*aq*, 0.100 M) | Ag(s)
- Elektromotorna sila elektrokemijskog članka:
  - *E*cell = *E*k– *E*a
- Elektrokemijski potencijal polučlanka :

$$E = E^\circ - \frac{RT}{nF} \ln Q$$

- Q = [O] /[R]
- [O]- ravnotežna koncentracija oksidiranmog oblika
- [R]- ravnotežna koncentracija reduciranog oblika

- **Pri standardnim uvjetima Nernstova jednadžba ima oblik:**

$$E = E^\circ - \frac{0.05916}{n} \log Q$$

Primjena Nernstove jednadžbe za prethodni elektrokemijski članak:

$$E_a = E^\circ_{Zn^{2+}/Zn} - \frac{0.05916}{2} \log \frac{1}{[Zn^{2+}]}$$

$$E_c = E^\circ_{Ag^+/Ag} - 0.05916 \log \frac{1}{[Ag^+]}$$

- **Sumarno:**

$$E_{cell} = \left(E^{\circ}_{Ag^+/Ag} - 0.05916 \log \frac{1}{[Ag^+]} \right) - \left(E^{\circ}_{Zn^{2+}/Zn} - \frac{0.05916}{2} \log \frac{1}{[Zn^{2+}]} \right)$$

$$E_{cell} = \left(+0.7996 - 0.05916 \log \frac{1}{0.100} \right) - \left(-0.7618 - \frac{0.05916}{2} \log \frac{1}{0.0167} \right)$$

$$= +1.555 \text{ V}$$

REFERENTNE ELEKTRODE

- **Referentna elektroda || Indikatorska elektroda**
- *E*cell = *E*ind – *E*ref + *E*lj
- *E*lj – (liquid junction) difuzijski potencijal
- **Kalomel elektroda:**
- **$Hg_2Cl_2(s) + 2e^- \Leftrightarrow 2Hg(l) + 2Cl^-(aq)$**

$$E = E^{\circ}_{Hg_2Cl_2/Hg} - \frac{0.05916}{2}\log[Cl^-]^2 = +0.2682 - \frac{0.05916}{2}\log[Cl^-]^2$$

- **pri 25 °C, *E* = + 0.2444V**
-
  - **Hg(l) | Hg_2Cl_2 (sat'd) | KCl (*aq*, sat'd) ||**
-

Izvedba referentne kalomel elektrode

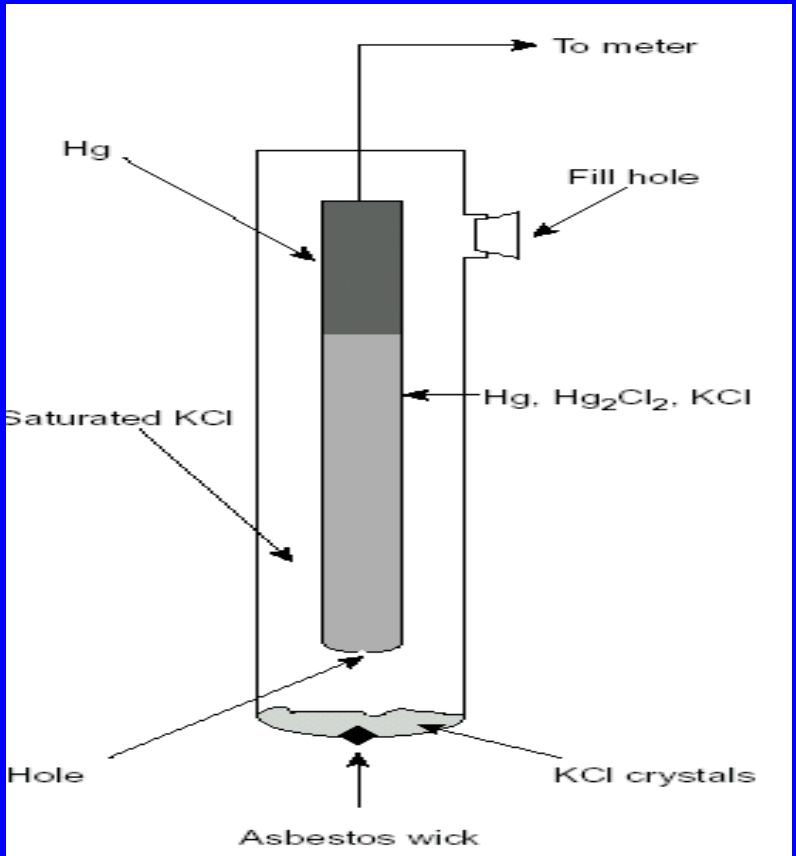


Figure 11.8
Schematic diagram of the saturated calomel electrode (SCE).

Ag/AgCl referentna elektroda

AgCl(*s*) + *e*– ⇔ Ag(*s*) + Cl–(*aq*)

Ag(*s*) | AgCl (sat'd) | KCl (*x* M) ||

$$E + E^{\circ}_{\mathrm{AgCl/Ag}} - 0.05916 \log [\mathrm{Cl}^-] = +0.2223 - 0.05916 \log [\mathrm{Cl}^-]$$

Ag/AgCl referentna elektroda

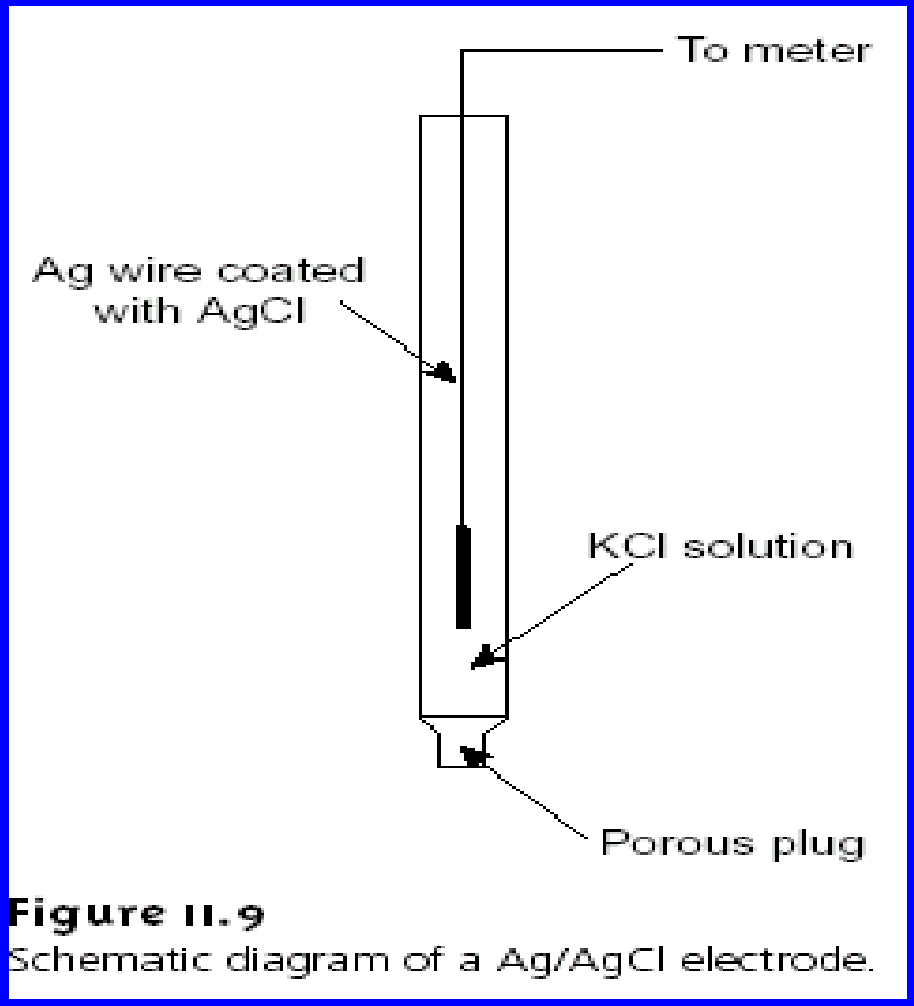


Figure 11.9
Schematic diagram of a Ag/AgCl electrode.

Elektrode prve vrste

- **METALNE ELEKTRODE:**
-
  - **$Cu^{2+}(aq) + 2e^- \Leftrightarrow Cu(s)$**

$$E = E^{\circ}_{Cu^{2+}/Cu} - \frac{0.05916}{2}\log\frac{1}{[Cu^{2+}]} = +0.3419 - \frac{0.05916}{2}\log\frac{1}{[Cu^{2+}]}$$

Elektrode druge vrste

- **REF || AgI (sat'd) | I– (unk) | Ag(s)**
- **Srebro/ srebrov jodid elektroda:**
- **Potencijal Ag/Ag+ elektrode:**

$$E = E^{\circ}_{Ag^+/Ag} - 0.05916 \log \frac{1}{[Ag^+]} = +0.7996 - 0.05916 \log \frac{1}{[Ag^+]}$$

AgI(*s*) ⇔ Ag+(*aq*) + I–(*aq*)

$$[\mathrm{Ag^+}] = \frac{K_{\mathrm{sp,AgI}}}{[\mathrm{I^-}]}$$

$$E = +0.7996 - 0.05916 \log \frac{[\mathrm{I^-}]}{K_{\mathrm{sp,AgI}}}$$

Redoks elektrode

- Potencijal elektrode ovisi o prisutnosti oksidiranog i reduciranog oblika redoks para

Membranske elektrode

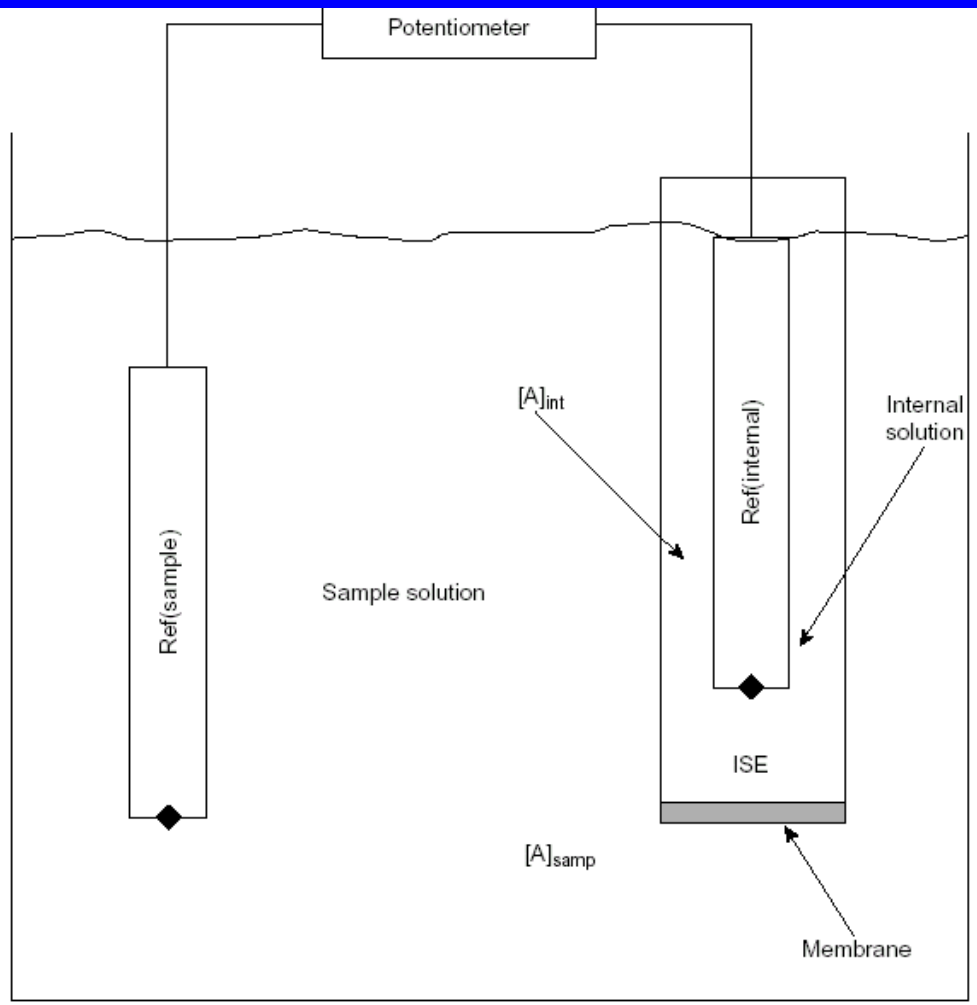


Figure 11.10
Electrochemical cell for potentiometry with an ion-selective membrane electrode.

- Ref(samp) || [A]samp | [A]int || Ref(int)
- *E*cell = *E*Ref(int) – *E*Ref(samp) + *E*mem + *E*lj

$$E_{\text{cell}} = K + \frac{0.05916}{z} \log [\text{A}]_{\text{samp}}$$

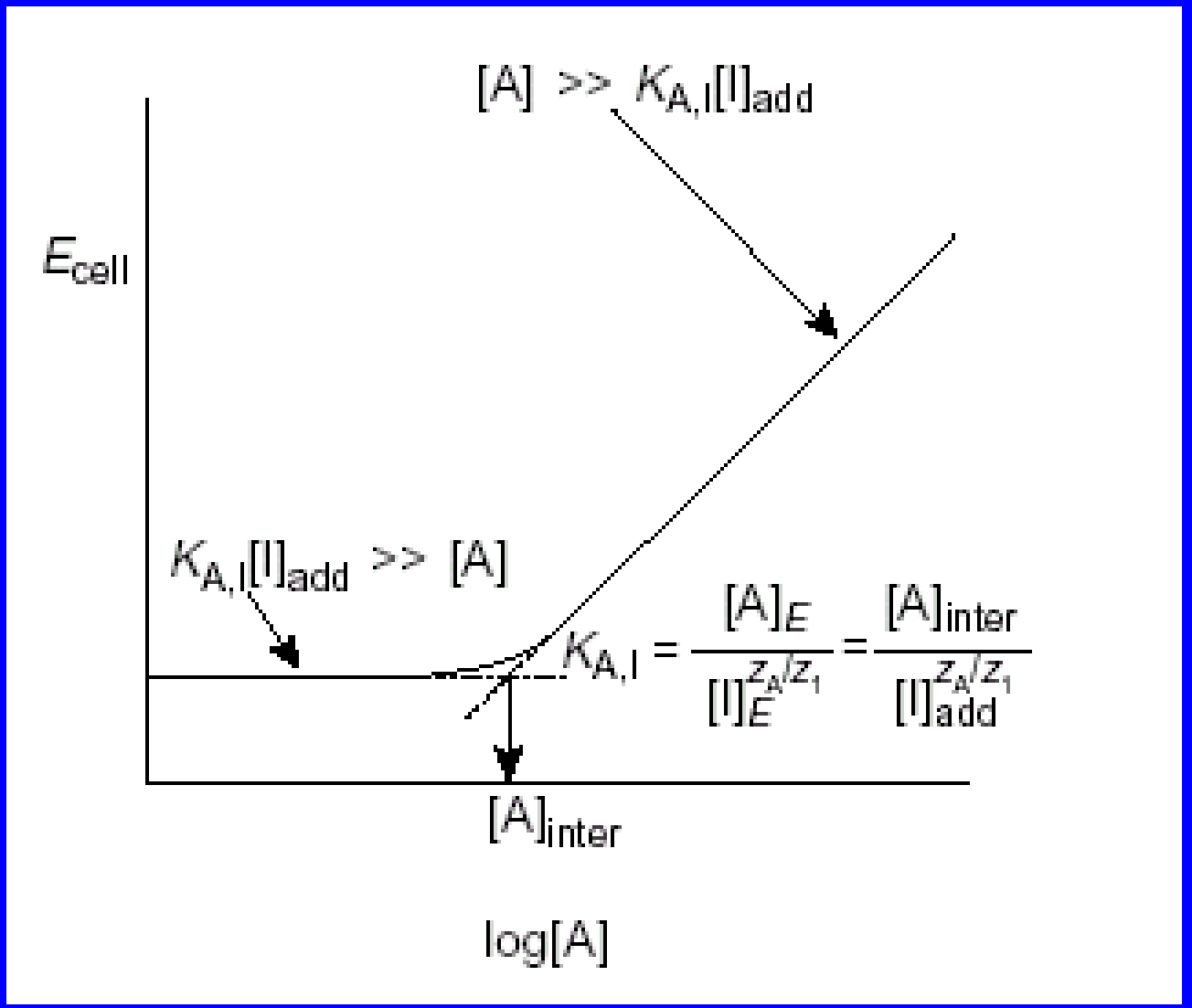
$$E_{\text{mem}} = E_{\text{asym}} - \frac{RT}{zF} \ln \frac{[\text{A}]_{\text{int}}}{[\text{A}]_{\text{samp}}}$$

Selektivnost

- **Nickolsky-Eissemanova jednadžba :**

$$E_{\text{cell}} = K + \frac{0.05916}{z_{\text{A}}} \log([\text{A}] + K_{\text{A,I}}[\text{I}]^{z_{\text{A}}/z_{\text{I}}})$$

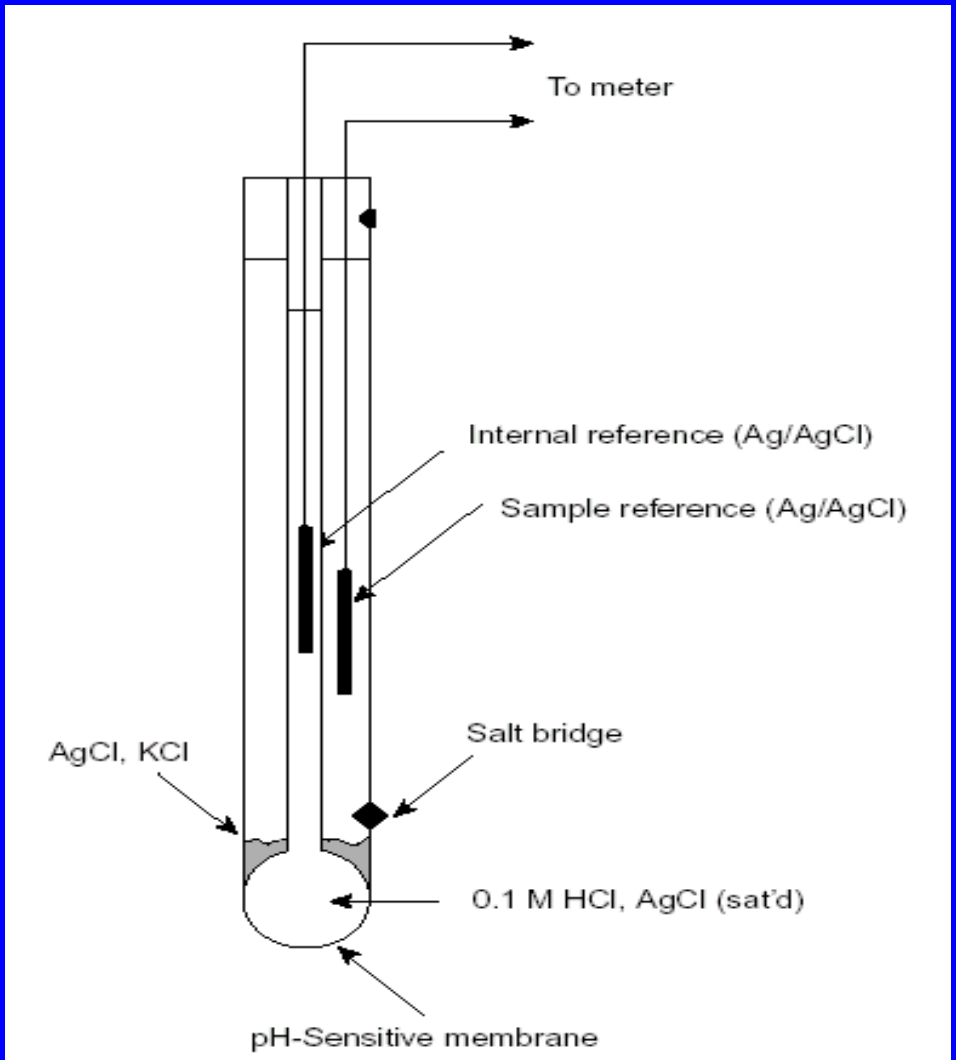
$$K_{\text{A,I}} = \frac{[\text{A}]_E}{[\text{I}]_E^{z_{\text{A}}/z_{\text{I}}}}$$


[A] >> $K_{A,I}[I]_{add}$
E_{cell}
$K_{A,I}[I]_{add}$ >> [A]
$K_{A,I} = \frac{[A]_E}{[I]_E^{z_A/z_I}} = \frac{[A]_{inter}}{[I]_{add}^{z_A/z_I}}$
$[A]_{inter}$
log[A]

Analyte	Membrane Composition	Selectivity Coefficients[a]
Ag^+	Ag_2S	$K_{Ag^+/Cu^{2+}} = 10^{-6}$ $K_{Ag^+/Pb^{2+}} = 10^{-10}$ Hg^{2+} interferes
Cd^{2+}	CdS/Ag_2S	$K_{Cd^{2+}/Fe^{2+}} = 200$ $K_{Cd^{2+}/Pb^{2+}} = 6$ Ag^+, Hg^{2+}, Cu^{2+} must be absent
Cu^{2+}	CuS/Ag_2S	$K_{Cu^{2+}/Fe^{3+}} = 10$ $K_{Cu^{2+}/Cu^+} = 1$ Ag^+, Hg^{2+} must be absent
Pb^{2+}	PbS/Ag_2S	$K_{Pb^{2+}/Fe^{3+}} = 1$ $K_{Pb^{2+}/Cd^{2+}} = 1$ Ag^+, Hg^{2+}, Cu^{2+} must be absent
Br^-	$AgBr/Ag_2S$	$K_{Br^-/I^-} = 5000$ $K_{Br^-/CN^-} = 100$ $K_{Br^-/Cl^-} = 5 \times 10^{-3}$ $K_{Br^-/OH^-} = 1 \times 10^{-5}$ S^{2-} must be absent
Cl^-	$AgCl/Ag_2S$	$K_{Cl^-/I^-} = 1 \times 10^6$ $K_{Cl^-/CN^-} = 1 \times 10^4$ $K_{Cl^-/Br^-} = 100$ $K_{Cl^-/OH^-} = 0.01$ S^{2-} must be absent
CN^-	AgI/Ag_2S	$K_{CN^-/I^-} = 100$ $K_{CN^-/Br^-} = 1 \times 10^{-4}$ $K_{CN^-/Cl^-} = 1 \times 10^{-6}$ $K_{CN^-/OH^-} = 1 \times 10^{-8}$ S^{2-} must be absent
I^-	AgI/Ag_2S	$K_{I^-/S^{2-}} = 30$ $K_{I^-/CN^-} = 0.01$ $K_{I^-/Br^-} = 1 \times 10^{-4}$ $K_{I^-/Cl^-} = 1 \times 10^{-6}$ $K_{I^-/OH^-} = 1 \times 10^{-7}$
SCN^-	$AgSCN/Ag_2S$	$K_{SCN^-/I^-} = 1000$ $K_{SCN^-/Br^-} = 100$ $K_{SCN^-/CN^-} = 100$ $K_{SCN^-/Cl^-} = 0.1$ $K_{SCN^-/OH^-} = 0.01$ S^{2-} must be absent
S^{2-}	Ag_2S	Hg^{2+} interferes

Analyte	Membrane Composition	Selectivity Coefficients[a]
Ca^{2+}	di-(*n*-decyl) phosphate in PVC	$K_{Ca^{2+}/Zn^{2+}} = 1–5$ $K_{Ca^{2+}/Al^{3+}} = 0.90$ $K_{Ca^{2+}/Mn^{2+}} = 0.38$ $K_{Ca^{2+}/Cu^{2+}} = 0.070$ $K_{Ca^{2+}/Mg^{2+}} = 0.032$
K^+	Valinomycin in PVC	$K_{K^+/Rb^+} = 1.9$ $K_{K^+/Cs^+} = 0.38$ $K_{K^+/Li^+} = 1 \times 10^{-4}$ $K_{K^+/Na^+} = 1 \times 10^{-5}$
Li^+	ETH 149 in PVC	$K_{Li^+/H^+} = 1$ $K_{Li^+/Na^+} = 0.05$ $K_{Li^+/K^+} = 7 \times 10^{-3}$
NH_4^+	Nonactin and monactin in PVC	$K_{NH_4^+/K^+} = 0.12$ $K_{NH_4^+/H^+} = 0.016$ $K_{NH_4^+/Li^+} = 4.2 \times 10^{-3}$ $K_{NH_4^+/Na^+} = 2 \times 10^{-3}$
ClO_4^-	$Fe(o\text{-phen})_3^{3+}$ in *p*-nitrocymene with porous membrane	$K_{ClO_4^-/OH^-} = 1$ $K_{ClO_4^-/I^-} = 0.012$ $K_{ClO_4^-/NO_3^-} = 1.5 \times 10^{-3}$ $K_{ClO_4^-/Br^-} = 5.6 \times 10^{-4}$ $K_{ClO_4^-/Cl^-} = 2.2 \times 10^{-4}$
NO_3^-	tetradodecyl ammonium nitrate in PVC	$K_{NO_3^-/Cl^-} = 6 \times 10^{-3}$ $K_{NO_3^-/F^-} = 9 \times 10^{-4}$

pH- elektroda



Mjerenje pH

- *pH* = –log *[H^+]*
- *pH* = –log *(aH^+)*

$$E_X = K - \frac{RT}{F} \ln \frac{1}{a_{H^+}} = K - \frac{2.303RT}{F} pH_X$$

$$E_S = K - \frac{2.303RT}{F} pH_S$$

$$pH_X = pH_S - \frac{(E_X - E_S)F}{2.303RT}$$

Plinske elektrode (senzori)

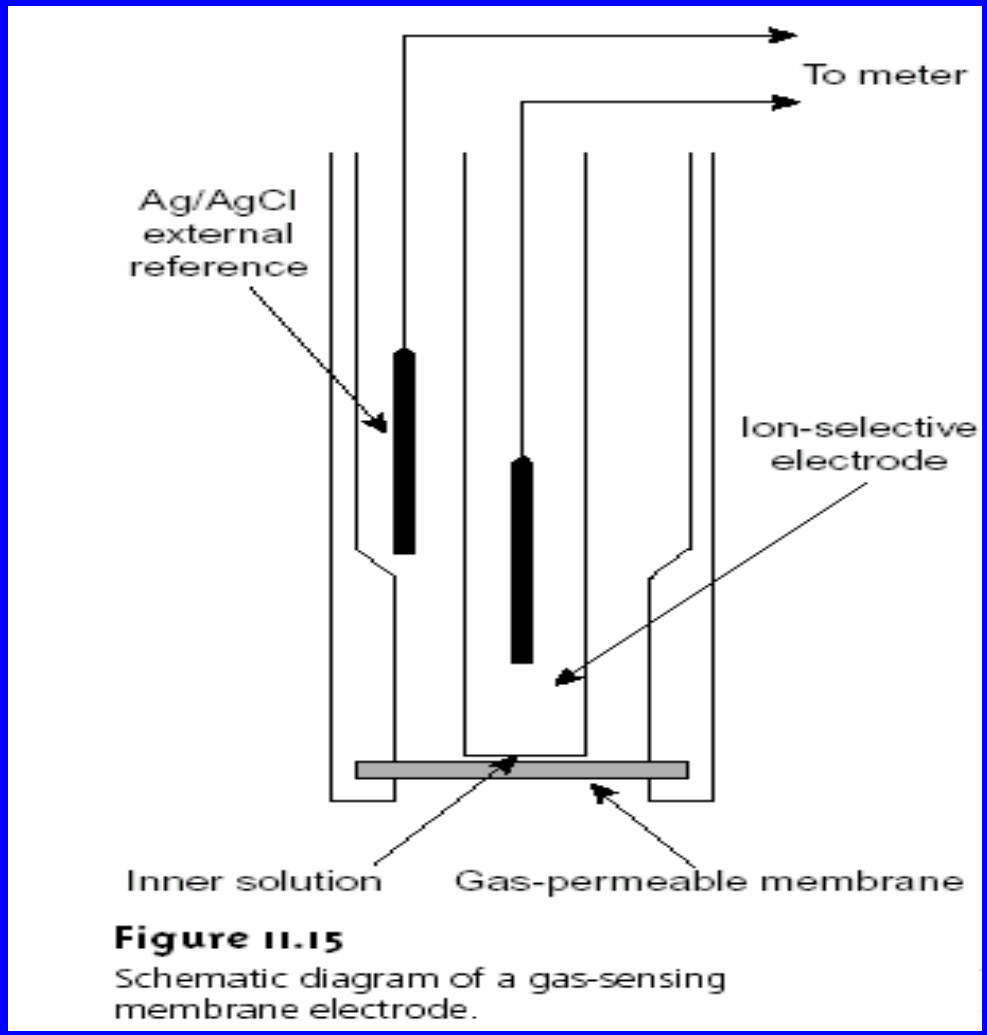


Figure 11.15
Schematic diagram of a gas-sensing membrane electrode.

- $CO_2(aq) + 2H_2O(l) \Leftrightarrow HCO3^-(aq) + H_3O^+(aq)$

$$[H_3O^+] = K\frac{[CO_2]}{[HCO_3^-]}$$

$$E\text{cell} = K' + 0.05916 \log [CO_2]$$

Biosenzor za ureu

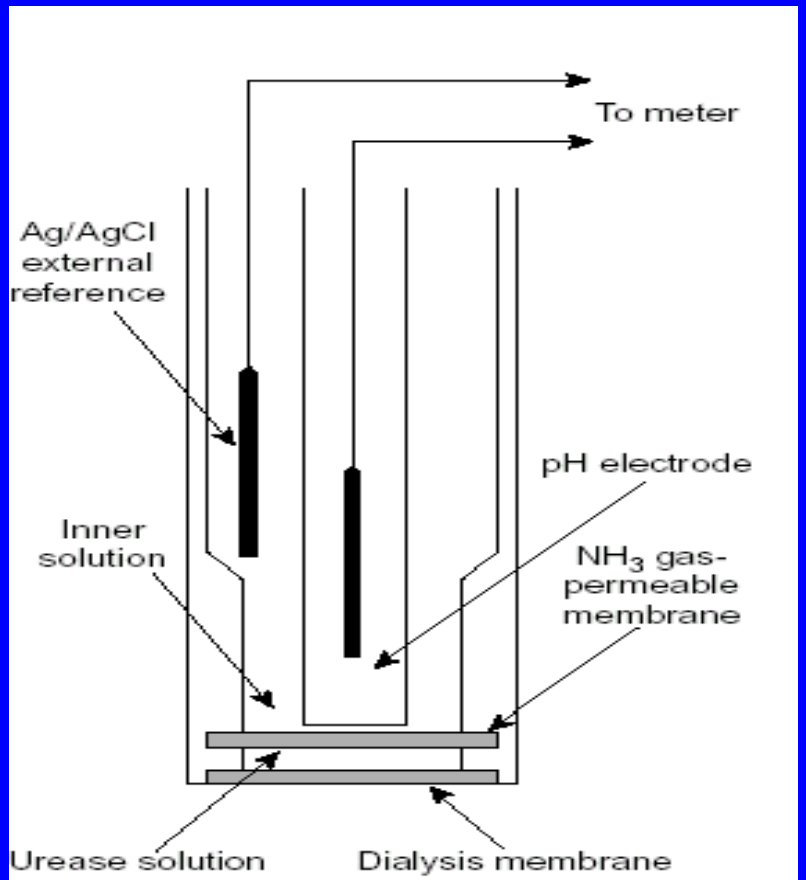


Figure 11.16
Schematic diagram of an enzyme-based potentiometric biosensor for urea in which urease is trapped between two membranes.

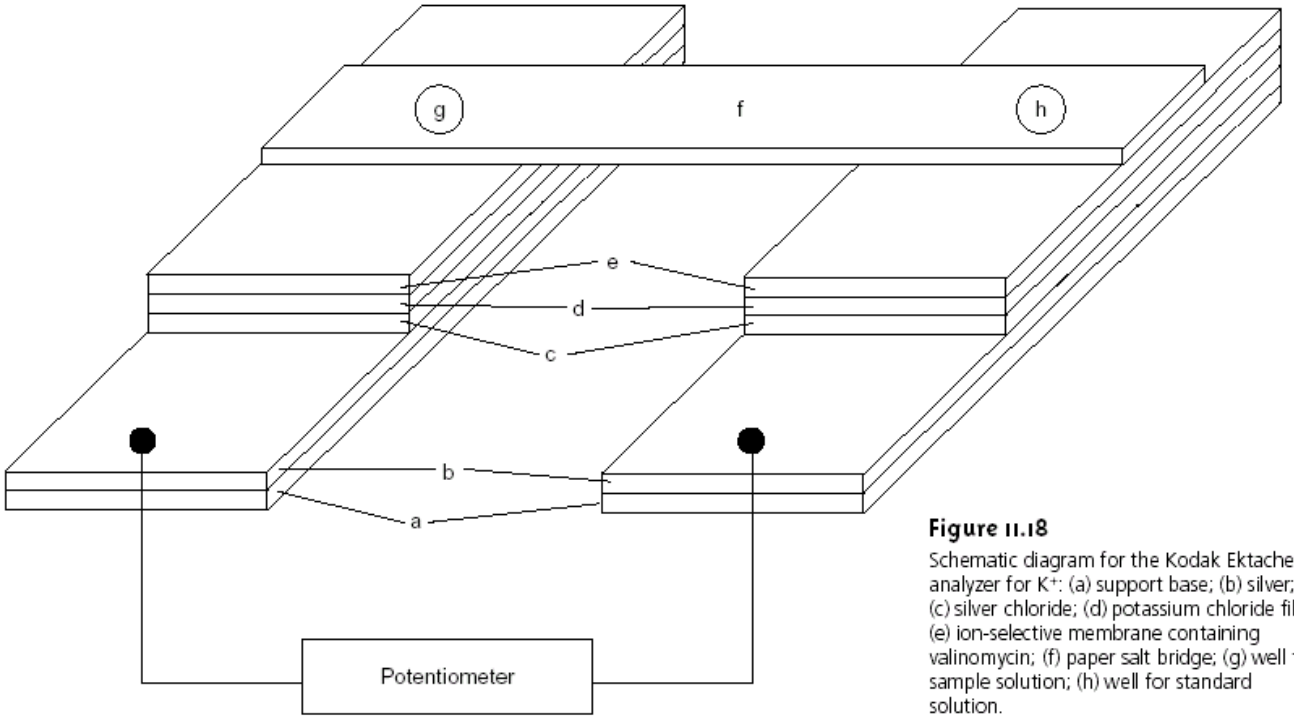


Figure 11.18

Schematic diagram for the Kodak Ektachem analyzer for K^+: (a) support base; (b) silver; (c) silver chloride; (d) potassium chloride film; (e) ion-selective membrane containing valinomycin; (f) paper salt bridge; (g) well for sample solution; (h) well for standard solution.

Primjeri potenciometrijskih biosenzora

a) H^+ cation

$$D-\text{glucose}+O_2 \xrightarrow{\text{glucose oxidase}} D-\text{glucono-1,5-lactone} + H_2O_2 \xrightarrow{H_2O} D-\text{gluconate}+H^+$$

$$\text{penicillin} \xrightarrow{\text{penicillinase}} \text{penicilloic acid} + H^+$$

$$H_2NCONH_2 + H_2O + 2H^+ \xrightarrow{\text{urease (pH=6,0)}} 2NH_4^+ + CO_2$$

$$H_2NCONH_2 + 2H_2O \xrightarrow{\text{urease (pH=9,5)}} 2NH_3 + HCO_3^- + H^+$$

$$\text{neutral lipids} + H_2O \xrightarrow{\text{lipase}} \text{glycerol} + \text{fatty acids} + H^+$$

b) NH_4^+ cation

$$\text{L-amino acid} + O_2 + H_2O \xrightarrow{\text{L-amino acid oxidase}} \text{keto acid} + NH_4^+ + H_2O_2$$

$$\text{L-aspargin} + H_2O \xrightarrow{\text{asparginase}} L - \text{aspartate} + NH_4^+$$

$$H_2NCONH_2 + 2H_2O + H^+ \xrightarrow{\text{urease (pH=7,5)}} 2NH_3 + HCO_3^-$$

c) I^- anion

$$H_2O + 2H^+ + 2I^- \xrightarrow{\text{peroxidase}} I_2 + H_2O$$

d) CN^- anion

$$\text{amygdalin} + 2H_2O \xrightarrow{\beta-\text{glucosidase}} 2\ \text{glucosidase} + \text{benzaldehyde} + H^+ + CN^-$$

[a] can also be used in NH_4^+ and CO_2 (gas) potentiometric biosensors

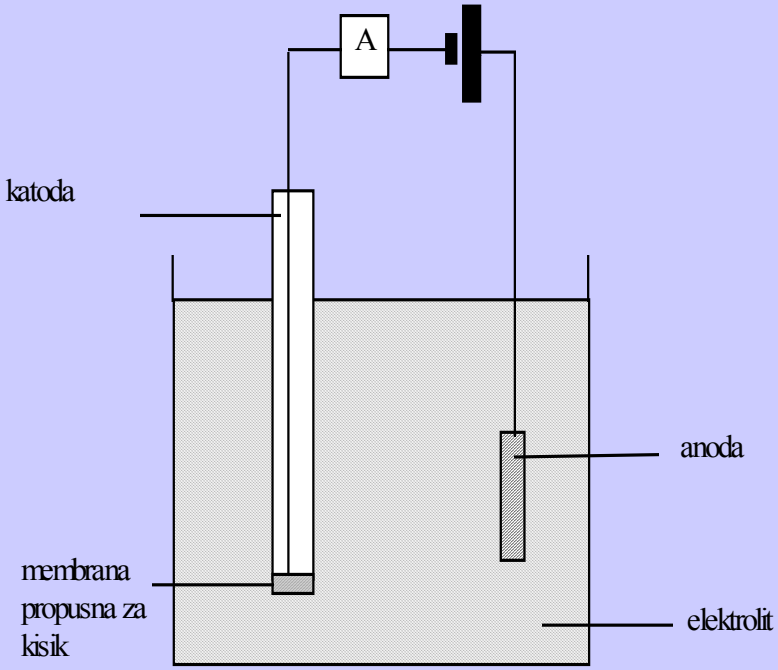
[b] can also be used in NH_3 (gas) potentiometric biosensors

Analyte	Biologically Active Phase[a]	Substance Determined
5′-adenosinemonophosphate (5′-AMP)	AMP-deaminase (E)	NH_3
L-arginine	arginase + urease (E)	NH_3
asparagine	asparaginase (E)	NH_4^+
L-cysteine	*Proteus morganii* (B)	H_2S
L-glutamate	yellow squash (T)	CO_2
L-glutamine	*Sarcina flava* (B)	NH_3
oxalate	oxalate decarboxylase (E)	CO_2
penicillin	penicillinase (E)	H_3O^+
L-phenylalanine	L-amino acid oxidase and horseradish peroxidase (E)	I^-
sugars	bacteria from human dental plaque (B)	H_3O^+
urea	urease (E)	NH_3 or H_3O^+

Source: Compiled from Cammann, K. *Working with Ion-Selective Electrodes.* Springer-Verlag: Berlin, 1977; and Lunte, C. E.; Heineman, W. R. "Electrochemical Techniques in Bioanalysis." In Steckham, E., ed. *Topics in Current Chemistry,* Vol. 143, Springer-Verlag: Berlin, 1988, p. 8.[3,6]

[a]*Abbreviations:* E = enzyme; B = bacterial particle; T = tissue.

Amperometrijski biosenzori



- Clark (1956)
- Katodna reakcija:
- $O_2 + H_2O + 4\ e^- \rightarrow 4OH^-$
- Anodna reakcija:
- $Ag \rightarrow Ag^+ + e^-$

- $Ag^+ + Cl^- \rightarrow AgCl$

Struju redukcije može se prikazati Faradayevim zakonom:

$$I = \frac{dQ}{dt} = z * F * \frac{dn}{dt}$$

Povezivanjem Fickovog i Faradayevog zakona dolazi se do izraza:

$$\frac{dQ}{dt} = -z * F * D * A * \frac{dc}{dx}$$

$$I = \frac{n * F}{V_m} * A * \frac{pO_2}{de/\ pe + dm/\ pm}$$

- **gdje su dm i de debljina sloja elektrolita i membrane, pe i pm su permeabilnost elektrolita i membrane, a V_m je molarni volumen plina.**
- **Budući je *dm/pm > de/pe* gornji se izraz može napisati :**

$$I = \frac{nF}{V_m} * A * \frac{p_m pO_2}{d_m}$$

odnosno jednostavnije *i = konstanta x* pO_2

Amperometrijski senzor za glukozu

- Kemijsku reakciju u enzimskom sloju objasnio je Romette
-
- glukoza+ O_2 $\rightarrow$ glukolakton + H_2O_2