including the use of the Nicholskii–Eisenman relationship, are presented. The range, linear range, and detection limit are described, and various time-related criteria, including the response time, recovery time and lifetime are discussed. The importance of accuracy, precision and reproducibility are outlined. These factors are exemplified by application to potentiometric and amperometric sensors and biosensors, including those used for urea, glucose, uric acid and amino acids. The effects of enzyme amount, immobilization method, transducer and pH on these criteria are also presented.

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Chapter 5

Electrochemical Sensors and Biosensors

Learning Objectives

- To appreciate the operation and application of potentiometry to ion-selective electrodes.
- To apply the concept of activities to the determination of concentrations and interference effects.
- To understand how potentiometry can be used in gas sensors.
- To know how these concepts are applied to biosensors.
- To appreciate the development of the three generations of amperometric biosensors.
- To know how mediators are used in biosensors.
- To acquire a knowledge of a wide range of different amperometric biosensors.
- To understand the limitations and uses of conductivity measurements in gas sensors and biosensors.
- To know how field-effect transistors are used in a variety of sensors.

5.1 Potentiometric Sensors – Ion-Selective Electrodes

5.1.1 Concentrations and Activities

Ion-selective electrodes (introduced earlier in Chapter 2) are based on the principle of concentration cells, i.e. electrochemical cells linked by a membrane and containing the same half-cell electrode in each half of the cell, and differing only in the concentration of the analyte (as shown above in Figure 2.2). The

membrane is *selective*, i.e. it responds to the analyte ion more than to other ions. The relationship between the emf of the cell and the analyte concentration is derived from the Nernst equation (see Section 2.2.3 above) and can be expressed in the following general form:

$$E = K + S \log [\text{ion}]$$

where E is the emf of the cell, S is the slope of the calibration graph (ideally 59.1 mV per decade of concentration), and [ion] is the concentration of the ion. Strictly speaking, the latter parameter should be the 'activity' of the ion (a_i), which gives the true thermodynamic Nernstian response. The activity is related to the concentration by the activity coefficient, γ, so that:

$$a_i = \gamma [\text{ion}]$$

where γ can be calculated from the Debye–Huckel theory, which estimates the effects of interaction between ions in a solution. The Debye–Huckel equation is given as follows:

$$-\log \gamma_i = (Az_i^2 \sqrt{I})/(1 + Ba\sqrt{I}) \qquad (5.1)$$

where A and B are constants arising from the theory, with values of 0.51 and 3.3×10^7, respectively, at 298 K, a is the ion size parameter (see Table 5.1), and z is the charge on the ion. The ionic strength, I, is a measure of the total ions in solution, weighted according to their charges, as in the following equation:

$$I = 1/2 \Sigma [\text{ion}]_{i,j} z_{ij}^2$$

Table 5.1 Ion sizes for use in the Debye–Huckel equation

Ion	Size parameter (pm)
Sn^{4+}, Ce^{4+}	1100
H^+, Al^{3+}, Fe^{3+}, Cr^{3+}	900
Mg^{2+}	800
Li^+, Ca^{2+}, Cu^{2+}, Zn^{2+}, Sn^{2+}, Fe^{2+}	600
Sr^{2+}, Cd^{2+}, Hg^{2+}, S^{2-}, OAc^-	500
Na^+, Pb^{2+}, CO_3^{2-}, SO_4^{2-}, HPO_4^-	400
K^+, Ag^+, NH_4^+	300
Cl^-, F^-, Br^-, I^-, OH^-, NO_3^-, SH^-, ClO_4^-	300

DQ 5.1

Calculate the activity of the sodium and sulfate ions in a 0.01 M solution of sodium sulfate.

Answer

The ionic strength of a 0.01 M solution of sodium sulfate is given by:

$$I = 1/2\{(0.02 \times 1^2) + [0.01 \times (-2)^2]\} = 0.03 \ M$$

while the activity coefficients can be calculated as follows:

$$-\log \gamma(Na^+) = [0.51 \times (+1)^2 \times \sqrt{0.03}]/[1 + (3.3 \times 10^7 \times 4 \times 10^{-8} \times \sqrt{0.03})] = 0.0719$$

and therefore $\gamma(Na^+) = 0.847$

$$-\log \gamma(SO_4^{2-}) = [0.51 \times (-2)^2 \times \sqrt{0.03}]/[1 + (3.3 \times 10^7 \times 4 \times 10^{-8} \times \sqrt{0.03})] = 0.2876$$

and therefore $\gamma(SO_4^{2-}) = 0.516$

Hence, the activities are given as follows:

$$Na^+ = 0.847 \times 0.02 \ M = 1.694 \times 10^{-2} \ M$$

$$SO_4^{2-} = 0.516 \times 0.01 \ M = 5.16 \times 10^{-3} \ M$$

In practice, one can eliminate the effects of activity coefficients by making up all of the test solutions with a high concentration of the same ion which does not interfere (an ionic-strength adjuster*). Thus, the ionic strength is constant for each sample and so the activity coefficient is also constant. Therefore:*

$$E = K + S \log a_i \text{ becomes:}$$

$$E = K + S \log (\gamma [ion])$$

giving:

$$E = (K - S \log \gamma) + S \log [ion]$$

This can be written as follows:

$$E = K' + S \log [ion]$$

5.1.2 Calibration Graphs

When employing calibration graphs in the study of ISEs, the following important points should be noted:

1. The slope of a (calibration) graph is *Nernstian* if the slope, S, is $59.1/z$ mV ($\pm(1–2)$ mV). Below this level of S, the slope is termed *sub-Nernstian* (the usual case), or is called *hyper-Nernstian* if greater (than $59.1/z$ mV). Improved performance may be achieved if the electrode is conditioned for 1–2 h in a solution of the ion of interest (ca. 0.01 M).

2. The linear range is usually between 10^{-5} and 10^{-1} M (depending on the ion), thus making ISEs suitable for many environmental and biological measurements.

3. Below 10^{-5} M, there may be curvature (see Figure 4.1 above), due to either approaching the detection limit or to the effect of an interferant.

4. Conditioning of the ISE should be carried out before preparation of calibration curves. To achieve this, the electrode is steeped in a 0.01 M solution of the ion to be analysed for 1–2 h, followed by 30 min in deionized water.

5. A criterion of stability for an ISE is generally that the cell potential does not vary by more than ±0.1 mV over a period of 60 s. At low concentrations, a more exact standard may be necessary, such as requiring that the potential should be stable within ±0.1 mV for 120 s. For example, a fluoride electrode may take 15–30 min to reach a steady-state condition at a concentration of 0.1 mg dm^{-3}.

6. The effect of interfering ions can be described by the Nicholskii–Eisenman equation, as discussed above in Sections 3.2.2 and 4.2.1.

SAQ 5.1

50 cm^3 of a solution of Cu(II) was analysed by using a multiple standard addition (potentiometric) method. When 1.00 cm^3 increments of 0.1 M Cu(II) were added to the test sample, the following readings were obtained:

Volume added (cm^3)	E (mV)
1.0	99.8
2.0	102.5
3.0	104.6
4.0	106.3
5.0	107.9

A blank solution gave a reading of 70.0 mV.
Estimate the concentration of copper in the original solution.

DQ 5.2

For a calcium ISE, the calibration slope, S, was +29.6 mV/decade in a 0.001 M solution. In a 0.001 M calcium chloride solution, the cell potential was −20.1 mV, while the potential in a solution containing a mixture of 0.001 M calcium chloride and 0.1 M sodium chloride was −19.8 mV.

Calculate the selectivity coefficient for calcium ions in the presence of sodium.

Answer

The ionic strengths of the analyte and mixed solutions are determined as in DQ 5.1 above.

$$I(CaCl_2) = 0.5(0.001 \times 2^2 + 0.002 \times 1^2) = 0.003$$

$$I(CaCl_2 + NaCl) = 0.5(0.001 \times 2^2 + 0.002 \times 1^2 + 0.1 \times 1^2 + 0.1 \times 1^2) = 0.103$$

The activity coefficient of Ca^{2+} in the analyte is given by:

$$-\log \gamma = 0.51 \times 2^2 \times \sqrt{(0.003)}/[1 + 3.3 \times 10^7 \times 6 \times 10^{-8}\sqrt{(0.003)}] = 0.100\,80$$

and so $\gamma(Ca^{2+}) = 0.793$.

Hence, the activity of Ca^{2+} in the analyte $= 0.001 \times 0.793 = 7.93 \times 10^{-4}$ M.

The activity coefficient of Ca^{2+} in the mixed solution is given by:

$$-\log \gamma = 0.51 \times 2^2 \times \sqrt{(0.103)}/[1 + 3.3 \times 10^7 \times 6 \times 10^{-8}\sqrt{(0.103)}] = 0.400$$

and so $\gamma(Ca^{2+}) = 0.3978$.

Hence, the activity of Ca^{2+} in the mixed solution $= 0.001 \times 0.3978 = 3.978 \times 10^{-4}$ M.

The activity coefficient of Na^+ in the mixed solution is given by:

$$-\log \gamma = 0.51 \times 1^2 \times \sqrt{(0.103)}/[1 + 3.3 \times 10^7 \times 4 \times 10^{-8}\sqrt{(0.103)}] = 0.114\,97$$

and so $\gamma(NaCl) = 0.7674$.

Hence, the activity of NaCl in the mixed solution $= 0.1 \times 0.7674 = 7.674 \times 10^{-2}$ M.

Therefore:

$$k_{Ca^{2+},Na^+} = [a_{Ca^{2+}}(a) \times 10^{(-19.8+20.1)/29.6} - a_{Ca^{2+}}(m)]/a(Na^+)^{n/z}$$

$$= [(7.929 \times 10^{-4} \times 1.0236) - (3.978 \times 10^{-4})]/(7.67 \times 10^{-2})^{2/1}$$

$$(as\ n = 2\ (Ca)\ and\ z = 1\ (Na))$$

$$= 0.000\,413\,8/0.005\,883 = 7.03 \times 10^{-2}$$

which shows that sodium interferes only weakly.

5.1.3 Examples of Ion-Selective Electrodes

5.1.3.1 Glass Membrane Type

The best, indeed almost universally known, example of an ISE is the glass membrane electrode for measuring hydrogen ion concentration or acidity, usually

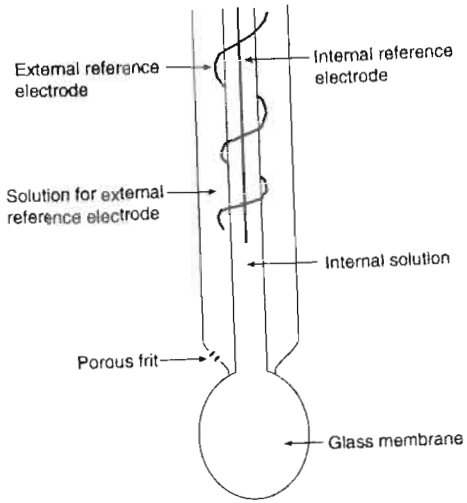

Figure 5.1 Schematic of a combination pH electrode. From Eggins, B. R., *Biosensors: An Introduction*, Copyright 1996. © John Wiley & Sons Limited. Reproduced with permission.

called the pH electrode. The thin glass membrane is highly selective to hydrogen ions over a very wide range of concentrations, with the composition of the glass being critical for this performance. If it is changed, this may make the glass membrane selective to other ions. The usual composition of the glass employed for detecting hydrogen ions is 22% Na_2O, 6% CaO and 72% SiO_2. The basic reaction is as follows:

$$SiO^-Na^+ + H^+ = SiO^-H^+ + Na^+$$

$$E = K + 59.1\ pH$$

A typical pH combination electrode is shown in Figure 5.1. This type incorporates the second reference electrode in a concentric glass tube around the main electrode tube. Contact between this electrode and the test solution is through a small glass frit. The two reference electrodes are normally of the Ag/AgCl type. The hydrogen ion glass electrode is usually called a pH electrode and calibrated in terms of pH rather than hydrogen ion activity, where:

$$pH = -\log a_{H^+}$$

Therefore:

$$E = K + 0.059 \log a_{H^+} = K - pH$$

and so:

$$pH = (K - E)/0.059$$

Other glass membrane ion-selective electrodes are available for measuring Na^+, Li^+, K^+ and Ag^+.

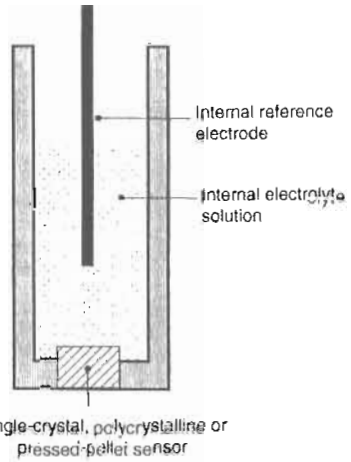

Figure 5.2 Schematic of a solid-state ion-selective electrode. From Eggins, B. R., *Biosensors: An Introduction*, Copyright 1996. © John Wiley & Sons Limited. Reproduced with permission.

5.1.3.2 *Solid-State Type*

The simple schematic in Figure 5.2 exemplifies the general structure for this type of electrode. Such a system normally has a separate reference electrode provided by the operator to dip into the test solution. It may be (but need not be) the same as the internal reference electrode built in by the manufacturer. The solid-state membrane can be a solid crystal, such as LaF_3 in the fluoride electrode, or a pressed pellet of powdered material, such as AgS in sulfide electrodes.

A single crystal of LaF_3 (doped with EuF_3) has been used in the fluoride ISE since 1966.

$$E = K - 59.1 \log a_{F^-}$$

The fluoride electrode is regularly used in water-treatment plants for measuring the fluoride levels in drinking water. However, most solid-state ISEs contain a pressed pellet of powdered material, such as silver sulfide in sulfide and silver electrodes. Examples of this type of ISE include Ag^+, Cl^-, Br^-, SCN^- and S^{2-}.

5.1.3.3 *Liquid Ion-Exchange Membrane Type*

The membrane is made of a hydrophobic material such as plasticized poly(vinyl chloride) (PVC). Absorbed into this membrane is the liquid ion-exchange material, such as valinomycin (for potassium). In order to maintain the concentration level in the membrane, there is a reservoir of the ion-exchange liquid dissolved in an organic solvent. Figure 5.3 shows the details of this type of ISE, including the special reservoir for the ion-exchanger solution, as well as the reference solution

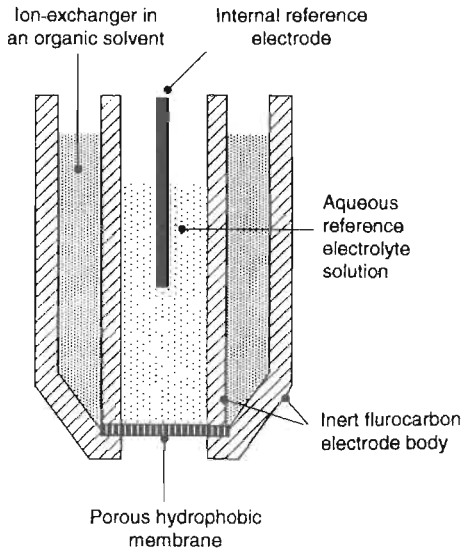

Figure 5.3 Schematic of a liquid ion-exchange membrane ion-selective electrode. From Eggins, B. R., *Biosensors: An Introduction*, Copyright 1996. © John Wiley & Sons Limited. Reproduced with permission.

and internal reference electrode. Some examples of this type of ISE are NO_3^-, Cu^{2+}, Cl^-, BF_4^-, ClO_4^- and K^+. The nitrate electrode is used extensively for the measurement of nitrate in soils and waters.

SAQ 5.2

A fluoride ion electrode is used to measure the fluoride concentration in a cup of tea. When immersed in a mixture of 25 cm^3 of tea and 25 cm^3 of an ionic-strength adjustment buffer, the electrode gave a reading of 98 mV. When 2.0 cm^3 of a 100 ppm fluoride solution was added to this mixture, the reading became 73 mV. Calculate the concentration of fluoride ions in the tea.

5.1.4 Gas Sensors – Gas-Sensing Electrodes

These are mainly based on pH electrodes and can detect gases which in aqueous systems form acidic or basic solutions. Here, a gas-permeable membrane is included in the arrangement, as shown in Figure 5.4. Between the membrane and the hydrogen-selective glass membrane is an internal electrolyte containing material that will form a buffer with the gas material. For example, for the ammonia electrode, ammonium chloride is used, so that an equilibrium is set up as follows:

$$NH_4Cl = NH_4^+ + Cl^-$$

$$NH_3 + H^+ = NH_4^+$$

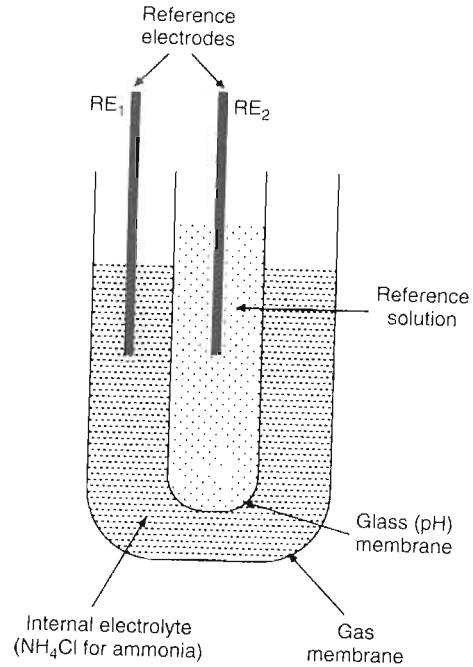

Figure 5.4 Schematic of a gas-permeable membrane electrode. From Eggins, B. R., *Biosensors: An Introduction*, Copyright 1996. © John Wiley & Sons Limited. Reproduced with permission.

so:

$$K = [NH_3][H^+]/[NH_4^+]$$

and therefore:

$$\log [NH_3] = pH + pK_a + \log [NH_4^+]$$

The presence of the high concentration of ammonium chloride keeps the concentration of ammonium ions constant. Hence, the logarithm of the ammonia concentration is directly proportional to the pH of the solution.

Electrodes for SO_2, NO_2, and H_2S are constructed in a similar way.

In biosensors, the most commonly used are H^+, NH_4^+ and NH_3 electrodes, which are all based on the pH principle (see Section 5.2). Occasionally, a CO_2, an I^-, or perhaps an S^{2-} electrode, may be used. Table 5.2 shows a selection of gas-sensing electrodes.

SAQ 5.3

How is selectivity achieved with electrochemical gas sensors?

Table 5.2 Some examples of dissolved-gas sensors

Gas	Inner solution	Sensor
CO_2	$NaHCO_3$	pH glass
SO_2	$NaHSO_3$	pH glass
HF	H^+	F–LaF_3
H_2S	pH 5 buffer	S^{2-}–Ag_2S
HCN	$KAg(CN)_2$	Ag^+–Ag
NH_3	NH_4Cl	pH glass

5.2 Potentiometric Biosensors

These are largely based on specific ion-selective or gas-selective electrodes.

5.2.1 pH-Linked

These are the simplest potentiometric biosensors, and are applicable to any system in which there is a change of pH during the (chemical) reaction. An appropriate enzyme must be immobilized on to the pH electrode to fabricate the sensor. There are many examples of such sensors, three of which are described in the following. The required enzyme is shown in the reaction scheme for each of these. As well as conventional pH electrodes, these types of biosensors are readily adapted for use with field-effect transistors (FETs).

5.2.1.1 Penicillin

$$\text{penicillin} \longrightarrow \text{penicilloate} + H^+$$

5.2.1.2 Glucose

Because gluconic acid is formed as a product, there is a change in the pH and so pH measurements can be used to monitor the reaction:

$$\text{glucose} + O_2 \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2$$

5.2.1.3 Urea

Urea was the first important analyte to be determined with a potentiometric biosensor (by Guilbault and Kuan in 1987). In this system, urea is hydrolysed by the use of the enzyme urease found in jack bean meal, as follows:

$$CO(NH_2)_2 + 2H_2O \xrightarrow{\text{urease}} 2NH_4^+ + CO_3^{2-}$$

The analysis may be carried out in a number of ways. With the aid of a suitable buffer, such as histidine, one can measure the reaction with a pH electrode and pH meter. The enzyme can be immobilized onto the pH electrode by using gelatin and glutaraldehyde. A simpler, although less reliable, method is to use a platinum electrode coated with polypyrrole instead of the standard glass pH electrode. Some other methods for the determination of urea are described below.

5.2.2 Ammonia-Linked

Any reaction in which ammonia is formed as a product can be monitored by using an ammonia-selective electrode.

5.2.2.1 Urea

In the reaction described above, we could use a cationic ammonium-selective electrode, or more commonly, we could make the solution alkaline and determine the liberated ammonia by the use of an ammonia-selective gas electrode. The latter has been the most successful method. Here, the urease is attached to the polypropylene membrane of an ammonia ISE. This has the highest sensitivity and the lowest detection limit (10^{-6} M), and can achieve 20 assays per hour with a relative standard deviation of ±2.5% over a range of 5×10^{-5} to 10^{-2} M.

5.2.2.2 Creatinine

$$\text{creatinine} \xrightarrow{\text{creatinase}} NH_3 + \text{creatine}$$

With the creatinase immobilized on the polypropylene membrane of an ammonia electrode, the latter was stable for 8 months and 200 assays, and had a detection limit of 8×10^{-6} M.

5.2.2.3 Phenylalanine

$$\text{L-phenylalanine} \xrightarrow{\text{phenylalanine ammonia-lyase}} NH_3 + \textit{trans}\text{-cinnamate}$$

This sensor is very highly selective, but has a poor range and slow response.

5.2.2.4 Adenosine

$$\text{adenosine} \xrightarrow{\text{adenine-deaminase}} NH_3 + \text{inosine}$$

In this system, the adenine–deaminase is cross-linked with glutaraldehyde on the ammonia electrode.

5.2.2.5 Aspartame

$$\text{aspartame} \xrightarrow{\text{L-aspartase}} NH_3 + C_6H_5CH_2CH(CO_2H)NHCOCHCHCO_2H$$

5.2.3 Carbon Dioxide-Linked

A few applications are known which involve the use of carbon dioxide gas ISEs in biosensors. These are described in the following.

5.2.3.1 Urea

In the reaction described above in Section 5.2.1.3, we could make the solution acidic and determine the liberated carbon dioxide with a carbon-dioxide-selective gas electrode.

5.2.3.2 Oxalate

The determination of oxalate in urine is important in the diagnosis of hyperoxaluria:

$$\text{oxalate} \xrightarrow{\text{oxalate decarboxylase}} CO_2 + \text{formate}$$

Phosphate and sulfate, which are usually present in urine, inhibit this enzyme, so oxalate oxidase can be used, although this enzyme is also inhibited by some anions:

$$\text{oxalate} \xrightarrow{\text{oxalate oxidase}} 2CO_2 + H_2O_2$$

5.2.3.3 Digoxin

Digoxin is immobilized on polystyrene beads, and a sample of digoxin is added together with a peroxidase-labelled antibody. The complexed peroxidase label is then reacted with pyrogallol and hydrogen peroxide and measured from the liberated carbon dioxide:

$$H_2O_2 + \text{pyrogallol} \xrightarrow{\text{peroxidase}} CO_2$$

SAQ 5.4

Compare the different types of urea biosensors.

5.2.4 Iodine-Selective

5.2.4.1 Glucose

The hydrogen peroxide formed from the reaction of glucose with glucose oxidase can be estimated by using it to oxidize iodide to iodine in the presence

of peroxidase. The remaining iodide is measured by using the iodide-selective electrode:

$$\text{glucose} + O_2 \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + 2I^- + 2H^+ \xrightarrow{\text{PO}} I_2 + 2H_2O$$

The iodide electrode follows the decrease in iodide concentration as it is consumed by the hydrogen peroxide.

5.2.4.2 Phenylalanine

L-Aminooxidase (LAO) and peroxidase (PO) are co-immobilized in a polyacrylamide gel on the surface of an iodide electrode. However, this sensor suffers more interference and selectivity problems than the ammonia-based sensor described above.

$$\text{L-phenylalanine} \xrightarrow{\text{LAO/PO}} H_2O_2$$

$$H_2O_2 + 2I^- + 2H^+ \longrightarrow I_2 + 2H_2O$$

5.2.4.3 Oestradiol

This is a potentiometric immunoassay. Anti-17β-oestradiol is immobilized on a gelatin membrane on the surface of an iodide ion-selective electrode. Peroxidase-labelled antigen and sample antigen are added (as shown in Figure 5.5(a)). The amount of sample antigen is inversely proportional to the labelled antigen, with the latter being determined by adding hydrogen peroxide and iodide, which is converted into iodine in the presence of the peroxidase. The remaining iodide is then determined by the ISE. A calibration graph of emf versus log [oestradiol] is obtained, as shown in Figure 5.5(b).

5.2.5 Silver Sulfide-Linked

5.2.5.1 Cysteine

$$Ag^+ + xR - S^{y-} \longrightarrow Ag(R{-}S)_x^{(1-xy)-}$$

The above reaction provides a direct potentiometric non-enzymatic method of analysis for cysteine, although it is not totally selective:

$$\text{cysteine} \longrightarrow \text{cystine (non-enzymatic)}$$

The following reaction:

$$\text{cysteine} + CN^- \xrightarrow{\beta\text{-cyanoalanine synthesase}} HS^- + \beta\text{-cyanoalanine}$$

is more specific, but in this case the cyanide interferes with the electrode.