Chapter 9
The Basics of the Routine Analysis with ISEs

9.1 Reference Electrodes

It is not possible to measure a single electrode potential. Therefore, all electrode potentials are measured against some reference electrode (RE), and the respective EMFs are used for further processing. The primary RE which is supposed to have zero potential at all temperatures is the so-called *standard hydrogen electrode*. This is a piece of platinum with rough surface, immersed into acidic solution, and gaseous hydrogen is bubbling through the solution. Platinum works as catalyst for hydrogen oxidation reaction which takes place on the platinum surface:

$$H_2{}^{Pt} \leftrightarrow 2H^{+,aq} + 2e^-$$

The respective potential depends on the pH and also on the hydrogen partial pressure:

$$\varphi = \varphi^0 - \frac{RT}{F}\mathrm{pH} - \frac{RT}{2F}\ln p_{H_2} \tag{9.1}$$

Thus, the primary reference for all potentiometric measurements is the potential of the hydrogen gas electrode immersed into solution with pH = 0, at pressure of 1 atm.

In practice, this electrode is inconvenient and therefore replaced in routine measurements with a suitable secondary RE. Earlier, calomel electrode, Hg/Hg_2Cl_2 in saturated KCl, was often in use. However, given its toxicity, mercury must be avoided, if possible. Therefore, most frequently used is silver chloride electrode (Ag/AgCl) immersed into 3 M KCl solution. This solution is connected with sample or calibrator via salt bridge. In the so-called *single-junction* REs, the bridge is filled with the same solution as that in the RE, for example, 3 M KCl. If the contamination of the sample with K^+ and/or Cl^- ions is of no importance for the results, a *single-junction RE* schematically shown in Fig. 9.1 is a suitable choice.

In many cases, the contamination of samples with K^+ and Cl^- ions from the salt bridge may bias the results. Then use of the so-called *double-junction* RE is preferable. A scheme of a double-junction RE is shown in Fig. 9.2. This kind of

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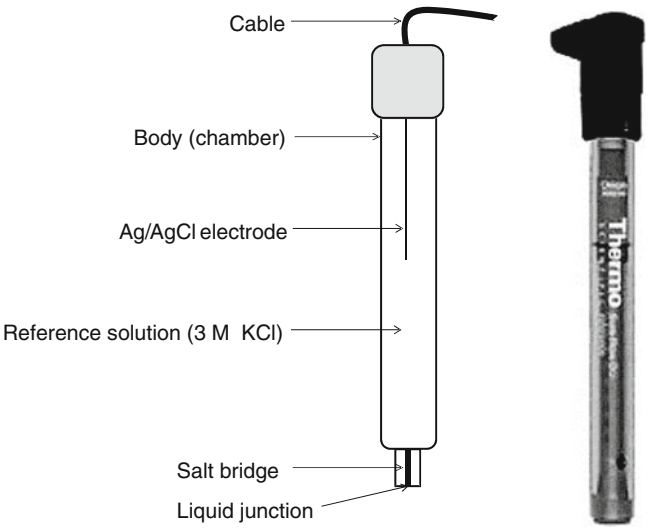

Fig. 9.1 Schematic design of a single-junction reference electrode (*left*) and Thermo-Fisher sure-flow reference electrode (*right*)

REs consist of two bodies one inside another one, making two chambers. The inner chamber is equipped with an electrode, most commonly—Ag/AgCl and filled with 3 M KCl. The inner reference solution is connected with the outer chamber via inner salt bridge made of a suitable porous material. The outer chamber is filled with electrolyte consisting of ions different from those to be measured. In this way, it is possible to avoid the errors caused by the contamination of sample or calibrator with ions leaking from the reference chamber. The most common choice for the electrolyte in the outer chamber is 1 M $LiCH_3COO$. Thus, there are two liquid junctions between KCl and LiOAc and between LiOAc and sample solution. Besides silver chloride RE, a large variety of REs are known. Detailed description of various REs is presented in classical book by Ives and Janz [1] and in Cammann's book [2].

There are reports on solid-contact REs without liquid junction, for detailed review see [3]. However, this technology is in early stage of development and will not be discussed here.

9.2 Instrumentation for the Measurements with ISEs

In principal, measurements of EMF require a voltmeter. However, ordinary voltmeters are not suitable for ISEs. This is because ion-selective electrodes, especially those with glass membranes have high electrical resistance, up to 10^8 Ohm. Therefore, measurements with ISEs require pH- or ionometers: high-precision

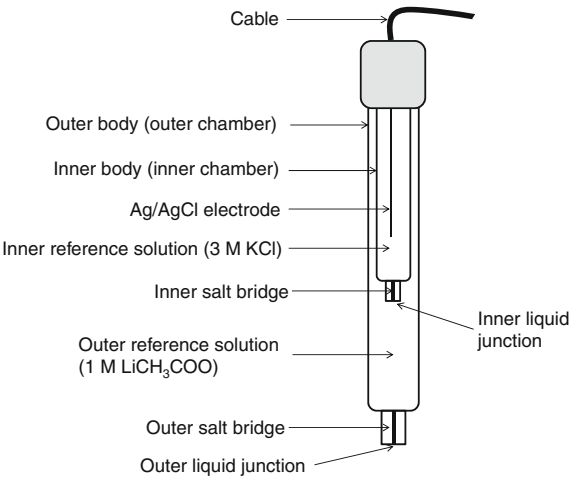

Fig. 9.2 Schematic design of a double-junction reference electrode

voltmeters with high input impedance. To elucidate the need of high input impedance, let us discuss a fictitious circuit shown in Fig. 9.3. Let us assume that the measuring instrument is an ideal voltmeter, that is, with infinite resistance, while the resistance of the real (non-ideal) instrument equals R_{input} and is connected in parallel to the ideal voltmeter and to the galvanic cell. The signal registered by the instrument equals the potential drop on R_{input} that is, $U_{\text{measured}} = IR_{\text{input}}$. On the other hand, E_{gc}—the EMF of the galvanic cell, is the sole source of I—the current in the circuit. The current flows through R_{input} and R_{gc}, the latter is the own resistance of the cell. These resistances are connected in series, so current is $I = E_{\text{gc}} / \left(R_{\text{input}} + R_{\text{gc}}\right)$. This means that the measured signal relates to the EMF as follows:

$$U_{\text{measured}} = E_{\text{gc}} R_{\text{input}} / \left(R_{\text{input}} + R_{\text{gc}}\right) \tag{9.2}$$

In other words, the signal registered by the instrument approaches the EMF if $R_{\text{input}} \gg R_{\text{gc}}$. In turn, the resistance of the cell is determined by the resistance of the ISE membrane because all other parts of the cell are low-resistant.

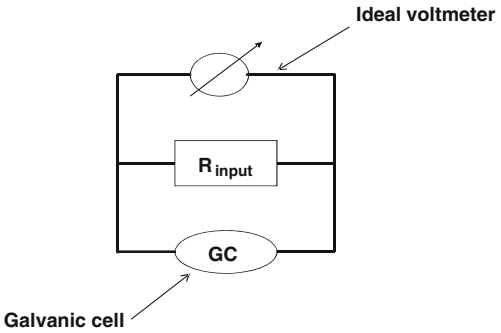

Fig. 9.3 Fictitious electrical circuit with a galvanic cell and an ideal voltmeter

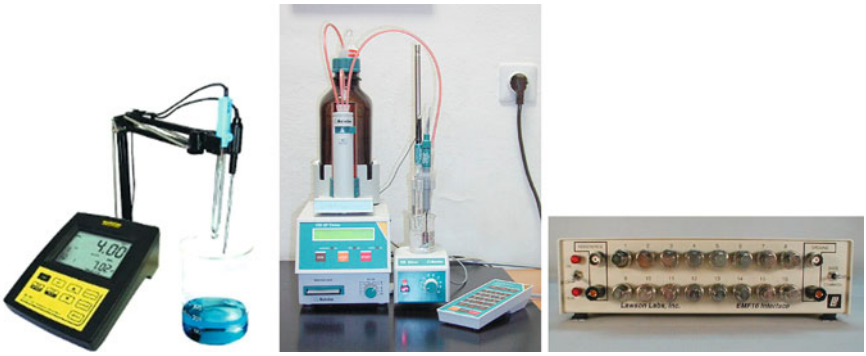

Fig. 9.4 Some examples of modern measuring devices. *Left* ordinary pH meter with a glass pH electrode and a reference electrode; *middle* titrator with electrodes to control the titration process; *right* multichannel EMF measuring station: the potentials of up to 16 ISEs can be measured simultaneously

The deviation of the registered signal from the EMF depends on the $R_{\text{input}}/R_{\text{gc}}$ ratio. If this ratio equals 10^2, the measured signal is about 1 % less than the EMF; if it is 10^3, then the loss equals 0.1 %, etc.

Having high input resistance is necessary also for another reason: to maintain low currents during measurements, so that electrodes are not polarized. Modern pH- and ionometers (see Fig. 9.4 for some examples) have input impedances of 10^{14} Ohm and higher, and therefore, the registered signal equals the EMF of the cell, and electrodes are not polarized because of the measurement.

9.3 Direct Potentiometry with ISEs, Calibrators, and Buffer Solutions

The direct potentiometry assumes measurements of the EMF when the ISE and RE are in contact with non-perturbed (native) sample. Respectively, the interpretation of the data relies on the calibration curve plotted against the activity of the analyte ion. The EMF readings therefore deliver information on the activity of the analyte, not on its concentration. Sometimes, this is very advantageous. For instance, calcium ion activity is of higher importance for diagnostic purposes than the total calcium concentration [4]. In most practical applications, however, the users wish to know the free analyte ion concentration or the total content of the analyte rather than the activity. The relation between the concentration and the activity of species is given by the Debye–Hückel theory (see Sect. 2.5). This allows for the calculations of ion activities in calibrators (standard solutions) prepared by the user or supplied by the ISE manufacturer. The critical issue here is the knowledge of the ionic strength of the calibrator. Since the composition of an artificial solution is, obviously, known, the ionic strength is also known and can be calculated with

Table 9.1 Kjelland parameter values (according to [9])

Ion	a_{Kjel}
H^+	9
Li^+	6
Rb^+, Cs^+, NH_4^+, Tl^+, Ag^+	2.5
K^+, Cl^-, Br^-, I^-, CN^-, NO_2^-, NO_3^-	3
OH^-, F^-, SCN^-, NCO^-, HS^-, ClO_3^-, ClO_4^-, BrO_3^-, IO_4^-, MnO_4^-	3.5
Na^+, $CdCl^+$, ClO_2^-, IO_3^-, HCO_3^-, $H_2PO_4^-$, HSO_3^-, $H_2AsO_4^-$	4.5
Hg_2^{2+}, SO_4^{2-}, $S_2O_3^{2-}$, SeO_4^{2-}, CrO_4^{2-}, HPO_4^{2-}	4
Pb^{2+}, CO_3^{2-}, SO_3^{2-}, MoO_4^{2-}	4.5
Sr^{2+}, Ba^{2+}, Cd^{2+}, Hg^{2+}, S^{2-}, WO_4^{2-}, $Fe(CN)_6^{4-}$	5
Ca^{2+}, Cu^{2+}, Zn^{2+}, Sn^{2+}, Mn^{2+}, Fe^{2+}, Ni^{2+}, Co^{2+}	6
Mg^{2+}, Be^{2+}	8
PO_4^{3-}, $Fe(CN)_6^{3-}$	4
Al^{3+}, Fe^{3+}, Cr^{3+}, La^{3+}, Ce^{3+}	9
$HCOO^-$, $Citrate^-$	3.5
$Acetate^-$, $Cl\text{-}acetate^-$, $(CH_3)_4N^+$, $(C_2H_5)_2NH_2^+$, $Citrate^{2-}$	4.5
$Cl_3\text{-}acetate^-$, $(C_2H_5)_3NH^+$, $Citrate^{3-}$	5

Eq. (2.42) except of weak electrolytes present in the solution. Thus, the activity of the analyte ion in calibrators is known, and this allows for plotting the calibration curve. The values of a_{Kjel}, the Kjelland parameters needed for these calculations, are summarized in Table 9.1.

The ionic strength of samples is, typically, not known although it sometimes can be estimated by, for example, the conductivity measurements of the samples. This is why the inverse task, the calculation of the concentration on the basis of the activity data obtained from the ISE, is not executable.

The practical approach here is as follows. Since samples to be analyzed, normally, belong to certain type—blood, blood serum, or some industrial solutions, etc., the so-called typical ionic strength of each particular type of samples is approximately known. Calibrators, therefore, must mimic this particular type of samples having the same ionic strength. Then the activity coefficients are the same in all calibrators and in samples of this particular type. The target analyte content in calibrators must cover the whole possible range of the respective concentrations. Since activity coefficients are constant, the calibration curve can be plotted against log of concentration, and the EMF readings in samples directly show the sought free analyte ion content. As most simple example, below are presented calibrators for the measurements of potassium in blood serum with K^+-ISE. These are three mixed solutions, all having the same ionic strength: $J = 150$ mM. Solution #1 contains 2 mM of KCl and 148 mM NaCl, #2 is 4.5 mM KCl + 145.5 mM NaCl, and #3 is 10 mM KCl + 140 mM NaCl. The K^+ ion concentrations in these simple calibrators cover the physiologically relevant range, and the span of the values is enough for the calibration of the ISE.

Ion activity coefficients can be calculated using equations below [Eqs. (2.42) and (2.44) are presented here for the reader convenience]:

$$J = \frac{1}{2}\sum_{k=1}^{n} C_k z_k{}^2 \quad \log \gamma_I = -\frac{A z_I{}^2 \sqrt{J}}{1 + a_{\mathrm{Kjel}} B \sqrt{J}} + 0.1 z_I J$$

The adjustment of the ionic strength is a common approach in measurements with ISEs. Total ionic strength adjustment buffer (TISAB) was invented first for measurements of F^- [5]. The buffer contains acetic acid, NaCl, sodium citrate, and water. By adding NaOH, the pH is adjusted at 5.5—to prevent interference from hydroxyl ions. The ionic strength of the buffer is high enough, so samples diluted 1:1 with the buffer have a constant ionic strength. Furthermore, fluoride bound with iron and aluminum gets free and forms citric complexes. Therefore, the fraction of the free F^- anions is always the same. In this way, it is possible to calibrate the ISE and then measure total fluoride in samples. Various modifications of TISAB are described in [6].

There is a large number of pH buffers: traditional buffers based on mixtures of weak inorganic or organic acids and NaOH: citrate, acetate, phosphate, borate buffers. The pH of biological samples is normally adjusted at pH 7.4 with phosphate buffer saline, containing 137 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4 \cdot 2H_2O$, and 2.0 mM KH_2PO_4. Morpholine acids are also widely used to prepare buffers for clinical and biological studies.

Metal buffers for calibrators of ISEs selective to heavy metals in industrial and environmental applications are typically based on EDTA, NTA, and other similar agents.

Buffers are commercially available and their handling is described in manuals, so we do not go into further details discussing buffers here.

9.4 Standard Addition Methods, Potentiometric Titration with ISEs

Standard addition, standard dilution, and titration methods are thoroughly discussed by Cammann [2] and by Koryta and Stulik [7]. The basics of these methods obviously remain the same in spite of the progress in the ISE technology. Therefore, here, we discuss these issues only briefly.

Addition/dilution and titration methods are advantageous because allow for circumventing the problems of the unknown activity coefficients and even of partial complexation of the analyte. Titration methods, especially the Gran method [8], deliver more accurate results than the direct potentiometry. On the other hand, only the direct potentiometry allows for the continuous monitoring without perturbation of the sample composition.

Let us assume that we have an unknown sample: γ the activity coefficient of the analyte is not known, furthermore, only β fraction of the analyte is present as free ions. Thus, the free target ion activity in the sample is $a = C^{\mathrm{ionized}}\gamma = C^{\mathrm{total}}\gamma\beta$. The respective EMF value is

$$E_1 = E^0 + S\log a^{\text{native}} = E^0 + S\log C^{\text{total}} + S\log\gamma + S\log\beta \tag{9.3}$$

Now, we add ΔC^{total}—a known total quantity of the target ions (as a suitable electrolyte). The EMF value is as follows:

$$E_2 = E^0 + S\log a^{\text{processed}} = E^0 + S\log\left(C^{\text{total}} + \Delta C^{\text{total}}\right) + S\log\gamma + S\log\beta \tag{9.4}$$

Now, for the total concentration of the analyte in the native sample, we have

$$C^{\text{total}} = \Delta C^{\text{total}} \Big/ \left(10^{\frac{E_2 - E_1}{S}} - 1\right) \tag{9.5}$$

The critical issue in this procedure is the constancy of γ and β values. Therefore, the added quantity of the analyte must be small. On the other hand, a too small addition causes a too small effect in the EMF, so that the accuracy of the result is low. Normally, it is necessary to make a few trials and find ΔC^{total} value which gives the EMF effect of about 10 mV.

There are situations when we do not know whether the calibration parameters in an unknown sample are the same as in standard solutions. For whatever reason, E^0 may be shifted, and S may be sub- or super-Nernstian. Under these circumstances, the double-addition method helps. This method, however, suggests $C^{\text{ionized}} = C^{\text{total}}$.[1] The EMF when the ISE and the RE are immersed into the native sample is

$$E_1 = E^0 + S\log\gamma + S\log C \tag{9.6}$$

Here, E^0, S are not necessarily the same as in simple standards and therefore considered unknown. After addition of the ΔC of the analyte, the EMF is

$$E_2 = E^0 + S\log\gamma + S\log(C + \Delta C) \tag{9.7}$$

The second addition of the same ΔC results in:

$$E_3 = E^0 + S\log\gamma + S\log(C + 2\Delta C) \tag{9.8}$$

Now, we have equation which does not contain the unknown E^0, S values:

$$\frac{E_3 - E_1}{E_2 - E_1} = \frac{\log\frac{C+2\Delta C}{C}}{\log\frac{C+\Delta C}{C}} \tag{9.9}$$

This equation can be solved by iteration procedure; the most efficient is the bisection method.

The advantage of the potentiometric titration is low sensitivity to the ISE calibration parameters. Example of potentiometric titration curves is shown in Fig. 9.5 The curves refer to titration of analyte A with titrant B, and the AB

[1] Cammann [2] describes the double-addition method for solutions with $C^{\text{ionized}} = \beta C^{\text{total}}$. It is, however, doubtful whether β remains constant when more than one addition is made.

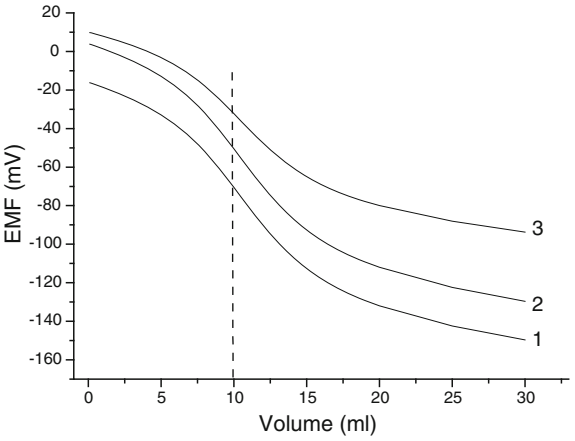

Fig. 9.5 Titration curves calculated for ISEs with different E^0 (mV) and S (mV/log(a)): 100, 58 (*curve* 1); 120, 58 (*curve* 2); and 100, 45 (*curve* 3)

complex formation constant is 10^4 M^{-1}. The analyte concentration is 0.01 M, and the concentration of the titrant is 0.001 M. Thus, the equivalent volume is 10 ml. The calibration parameters of the ISE used for the titration are shown in the figure capture. One can see that the location of the inflexion point (equivalent point) is the same whatever the standard potential and whatever the ISE slope. However, sub-Nernstian slope results in worsened accuracy.

The Gran titration method [8] is based on the Nernst equation rewritten as

$$E/S = E^0/S + \log a_I \tag{9.10}$$

This immediately results in linear equation:

$$10^{E/S} = 10^{E^0/S} + a_I = \text{Const} + a_I \tag{9.11}$$

Figure 9.6 illustrates the method. The data refer to the titration procedure shown in Fig. 9.5, curve 1. The EMF values are shifted 200 mV up to prevent

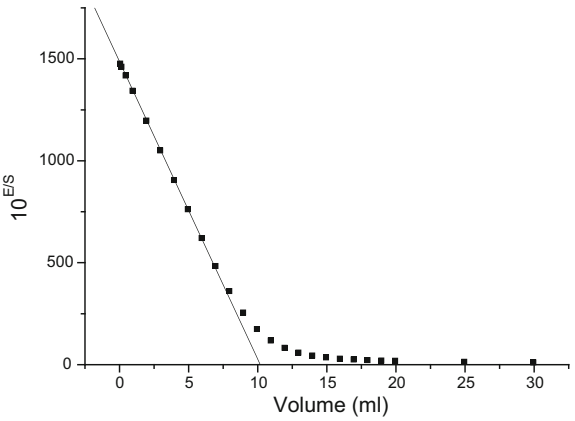

Fig. 9.6 Gran plot of the titration procedure shown in Fig. 9.5, *curve* 1

negative values under logarithm. One can see that the linearity is excellent in the beginning of the titration procedure, and the extrapolation to $10^{E/S} = 0$ does give the equivalence point.

Thus, the Gran method secures better accuracy of the results than the search for the inflection point in the ordinary titration curve. Furthermore, the $10^{E/S}$ data in the linear part of the Gran plot refer to relatively high concentrations of the analyte—far from the detection limit of the ISE. This is in contrast with use of the ordinary titration curve: close and after the equivalence point, the curve can be distorted because the analyte concentration is below the ISE detection limit.

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