Use of Ion-Selective Electrodes for Blood-Electrolyte Analysis. Recommendations for Nomenclature, Definitions and Conventions

International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)

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This paper will familiarize the reader with the terms used to describe the behavior of ion-selective electrodes, particularly in relation to their use in clinical chemistry for determination of blood electrolyte cations. It serves as an introduction to a series of papers dealing with important cations in blood, namely calcium, sodium, and potassium. The detailed relationships between the ion activity determined by means of ion-selective electrode potentiometry in undiluted specimens, and the total substance concentration measured by flame atomic-emission spectrometry are described by flow chart and equations. Adoption of a convention for reporting results is recommended.

The Working Group on Selective Electrodes has taken into account recent revisions of IUPAC recommendations on nomenclature and selectivity coefficient determinations for ion-selective electrodes, and benefited from the experience of a member of the WG, who was also involved in the IUPAC discussions. Nomenclature for determined quantities follows previous IUPAC/IFCC joint recommendations.

1. Introduction

The purpose of this document is to define the commonly used quantities in blood electrolyte analysis using ion-selective electrodes and to give algorithms for derived quantities. Use of these algorithms, especially for calculations performed within instruments, will lead to data that are more comparable between laboratories. The names, units, and symbols are consistent with current IUPAC and IFCC recommendations (1–3). Details relating to the determination of particular ions


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may be found in other documents, pH (4) sodium and potassium (5) and calcium (6).

2. Definitions

2.1 Ion-selective electrode (ISE)
Electrochemical sensor responding to ion X, usually consisting of a membrane in the form of a disc, or other suitable shape, of special material attached to a stem of plastic or glass, complete with internal reference electrode (2.8) and (optionally) an internal filling solution (2.9).

2.2 ISE cell
Electrochemical cell consisting of an ion-selective electrode and a reference electrode (2.3), both in contact with the sample solution, all at the same temperature.

Generally, this consists of two reference electrodes, internal and external, and the membrane. However, besides this conventional arrangement with solution contact on both sides of the membrane, there are all-solid-state and coated-wire types (2.8). Conventionally, the cell is written as

| Outer reference electrode | test solution | membrane | internal reference electrode |

or in more detail, for example, as

\[
\text{Hg} | \text{Hg}_2\text{Cl}_2 | \text{KCl (conc.)} | \text{membrane} | \text{inner reference electrode} \]

\[
| \text{soln.} | \text{AgCl} | \text{Ag or Ag} | \text{AgCl} |
\]

On each side of the ion-selective membrane (M), an electrical potential difference develops across the membrane/solution boundary. The inner side of the ion-selective membrane is in contact with an inner reference solution of constant ion activity and hence develops a constant potential. At the other side, on the external surface of the membrane, the electrical potential difference varies linearly with the logarithm of ion activity of the sample solution X only. The two electrodes are connected to a voltmeter.

2.3 Reference electrode
External electrode system, intended to give a virtually invariant potential, and comprising an inner element, usually calomel (Hg|Hg$_2$Cl$_2$) or silver-silver chloride (Ag|AgCl), a chamber containing the appropriate filling solution (commonly concentrated potassium chloride) and a device for forming the liquid junction, e.g., ceramic plug, fritted disc, or ground glass sleeve.

2.4 Liquid junction
Any junction between two electrolyte solutions of different composition. Across such a junction there arises a potential difference, called the liquid junction potential.

In the ISE cell, there is a junction between the solution...
tion X and the filling solution of the reference electrode. If a bridge solution (2.7) is used, there is a constant liquid junction potential between it and the reference electrode-filling solution, and a variable one between it and solution X. The presence of protein in the sample can affect the liquid junction potential, but the extent is not quantifiable and its effect has been disputed.

2.5 Residual liquid junction potential (rljp) error
Error arising from breakdown in the assumption that the liquid junction potential remains constant on change of solution.

The error can be estimated by the use of the Henderson equation (7–9). The rljp error introduces an uncertainty, probably of the order of 1 mV.

2.6 Filling solution (of an external reference electrode)
Solution containing the anion to which the reference electrode of the ISE cell is reversible, e.g., chloride for silver-silver chloride electrode.

In the absence of a bridge solution (2.7), a high concentration of filling solution, comprising cations and anions of almost equal mobility, is employed as a means of maintaining the liquid junction potential small and approximately constant on substitution of test solution for standard solution(s).

2.7 Bridge solution
Solution of high concentration of inert salt, preferably comprising cations and anions of equal mobility, optionally interposed between the reference electrode filling (2.6) and both the test and standard solution. An equitransferent sodium formate bridge solution has been suggested as appropriate for measurements on blood samples (9).

2.8 Inner reference electrode (of an ion-selective electrode)
Electrode, e.g., silver-silver chloride, electrically connected to the shielded input cable of the voltmeter, and in contact with the internal filling solution. In all-solid-state electrodes, the material of the reference electrode is deposited directly on to the membrane.

2.9 Inner reference solution (of an ion-selective electrode)
Aqueous electrolyte solution, which may be gelled, containing a fixed concentration of ion X and a fixed concentration of the ion, to which the inner reference electrode is reversible, e.g., chloride ion in the case of silver-silver chloride electrodes.

2.10 Classification of ion-selective electrodes
2.10.1 Crystalline electrodes may be homogeneous or heterogeneous.
(a) Homogeneous membrane electrodes are ion-selective electrodes in which the membrane is a crystalline material prepared from either a single compound or a homogeneous mixture of compounds (i.e., Ag₂S, AgI + Ag₂S).
(b) Heterogeneous membrane electrodes are formed when an active substance or mixture of active substances, is mixed with an inert matrix, such as silicone rubber or polyvinyl chloride (PVC), to form the sensing membrane.

2.10.2 Noncrystalline electrodes. In these electrodes, the ion-selective membrane consists of a matrix containing an ion-exchanger (see (a) and (b) below) and is usually interposed between two aqueous solutions. The matrix may be porous (e.g., cellulose ester) or nonporous (e.g., glass or inert polymeric material such as PVC).

Fig. 3 The different sodium fractions in blood plasma.
2.14 Sensitivity

g, of the ion-selective electrode is defined as:

\[ g = \frac{dE}{d \log a_x} \]

where \( E \) is the cell potential and \( a_x \) is the relative molal activity of the ion.

**Theoretical Nernstian sensitivity**, \( g_o \), of an ideal ion-selective electrode at 37°C is

\[ g_o = \frac{RT}{z_F} \ln 10 = \frac{0.0615}{z_x} \text{J} \text{C}^{-1} \text{mol}^{-1} = \frac{0.0615}{z_x} \text{V} \]  \[ \text{1a} \]

where

- \( R \) is the gas constant (8.3144 J K\(^{-1}\) mol\(^{-1}\))
- \( T \) is the absolute temperature (K)
- \( F \) is the Faraday constant (96485 C mol\(^{-1}\))

The number \( z_x \) in the denominator corresponds to the charge of the ion.

**Relative sensitivity**, \( S \), of the ion-selective electrode is defined as:

\[ S = \frac{g}{g_o} = \frac{dE}{d \log a_x} \cdot \frac{z_F}{RT \ln 10} \]  \[ \text{1b} \]

It can be calculated using the equation above. However, to prepare a solution containing a known activity of an ion is strictly impossible as it requires the activity coefficient of that ion. Single ion activity coefficients cannot be assigned without recourse to a nonthermodynamic assumption which, while arbitrary, is judged by its ‘reasonableness’. The assumption made is either on the basis of division of the mean ionic coefficient or by recourse to the Debye-Hückel theory or the Pitzer treatment of electrolyte solutions (10). In practice, an ion-selective electrode can be calibrated with solutions of known concentration or activity depending on which parameter is to be determined in the samples.

The relative sensitivity (\( S \)) of an ion-selective electrode should not deviate by more than ±0.02 or ±0.05 from the theoretical value (1.00).

2.15 Limit of detection

Practical limit of detection is defined as the concentration (or activity) of X at the point of intersection of the two extrapolated linear mid-range and final low concentration segments of the calibration curve, as shown in Fig.1.

Since many factors affect the detection limit, the experimental conditions must be reported, i.e., composition of the solution, the previous usage and preconditioning of the electrode, stirring rate, etc.

2.16 Interfering substance

Any species, other than the ion being measured, e.g., ions, proteins, surfactants whose presence in the sample solution affects the accuracy of measurements.

Proteins can adsorb on to ion-selective electrode membrane surfaces causing, with some membranes, a shift of 0.3–0.5 mV, but shifts of 10–15 mV have been reported. Proteins are removable with some difficulty by frequent washing, or by the use of enzyme (pepsin) cleaning solutions, which often also contain a nonionic detergent and a bactericide.
2.17 Practical response time

Length of time that elapses between the instant at which an ion-selective electrode and a reference electrode are brought into contact with a sample solution (or the instant at which the concentration of the ion of interest is changed) and the first instant at which the slope of $E$ against $t$ becomes equal to 0.1mV/min.

The experimental conditions used should be stated, i.e., the stirring or flow rate, the ionic concentration and composition of solution for which the response time is measured, the ion concentration and composition of the solution to which the electrode was exposed prior to this measurement, the previous usage and preconditioning of the electrode, and the temperature. This definition is preferred for, unlike previously defined response times, such as the time to reach a certain percentage, e.g., 95%, of the steady state value, it does not require prior knowledge of that steady state or 'infinite time' reading.

2.18 Drift

Slow nonrandom change with time in the potential difference of the ion-selective reference electrode cell containing a solution of constant composition and temperature.

The determination of drift is carried out by rectilinear curve fitting on a data set collected in a specified period of time in a solution of constant composition, concentration, and temperature. The slope of the $E$ versus $t$ line is called drift. Random fluctuations about the line defines the standard deviation of the potential difference data.

2.19 Electrode memory

Memory effects occur if, after the concentration has been changed and restored to its original value, a different value of potential difference is observed. The reproducibility of the electrode will consequently be poor. The systematic error is generally in the direction of the concentration of the solution in which the electrode was previously immersed.

2.20 Suspension or Pallmann effect

This effect occurs (11,12) when ion-selective electrodes are used in concentrated, spacefilled suspensions and the external reference electrode remains in the supernatant (suspension-free) solution.

The measured ion activity in the suspension differs from the value in the supernatant by the interfacial potential difference and corresponds to a higher value. The effect nearly disappears when the external reference electrode is placed in the same region as the ion-selective electrode. There are some changes in the liquid junction potential differences of the external reference electrode between suspension and supernatant.

2.21 Selectivity coefficient

Potentiometric selectivity coefficient, $k_{xy}$, defines the ability of an ion-selective electrode to distinguish the primary ion from other ions in the same solution.

The selectivity coefficient is evaluated by means of the ion-selective electrode potential response in mixed solutions of the primary ion, $X$, and interfering ion, $Y$. 

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**Fig. 4** The different calcium fractions in blood plasma.
(or, less desirably, in separate solutions). The activities of the primary ion, X, and the interfering ion, Y, at which \( k_{x,y} \) is determined should always be specified. The value of \( k_{x,y} \) is defined by the Nikolsky-Eisenman equation (2.22) The smaller the value of \( k_{x,y} \) the greater is the electrode’s preference for the primary ion, X.

*Note.* The terms, selectivity constant and selectivity factor, have been used instead of selectivity coefficient. Use of the term selectivity coefficient is recommended and the fixed interference method for its evaluation.

2.22 Modified Nernst equation, Nikolsky-Eisenman equation,

for ion-selective electrodes and definition of \( k_{x,y} \). If the potential difference between an ion-selective electrode (predominantly responsive to species X) and a suitable reference electrode is measured in mixed solutions containing ions Y and Z, for example, then, the following empirical equation is often obeyed

\[
E = \text{constant} + \frac{RT}{z_xF} \ln [a_x + k_{x,y} (a_y)^{z_y/z_x} + k_{x,z} (a_z)^{z_z/z_x} \ldots] \tag{2a}
\]

where \( a_x \) ist the activity of the ion, X; \( a_y \) and \( a_z \) are the activities of the interfering ions, Y and Z, respectively; \( z_x \) is an integer with sign and magnitude corresponding to the charge of the primary ion, X; \( z_y \) and \( z_z \) are integers with the same sign as \( z_x \) and magnitude corresponding to the charges on the interfering ions, Y and Z, respectively.

The ‘constant’ term includes the ‘standard potential’ of the ion-selective electrode, the reference electrode potential, and the liquid junction potential.

2.23 Methods for determining selectivity coefficient, \( k_{x,y} \).

2.23.1 Fixed interference method. Potential difference of the ISE cell measured with solutions of constant activity of interference, \( a_{Bj} \) and varying activity of the primary ion. The values obtained are plotted versus the activity of primary ion, \( a_A \). The intersection of the extrapolation of the linear portions of this plot will indicate the value of \( a_A \), which is to be used to calculate \( k_{x,y} \) from

\[
k_{x,y} = \left( \frac{a_B}{a_B} \right)^{z_x/z_B} \tag{2b}
\]

2.23.2 Separate solution method. The potential difference of an ISE cell is measured with each of two separate solutions, one containing ion A at activity \( a_A \) (but no B), the other containing ion B at activity \( a_B \) at the same activity, i.e., \( a_B = a_A \) (but no A). If the measured values are \( E_A \) and \( E_B \), respectively, then \( k_{x,y} \) can be calculated from

\[
\frac{\log k_{x,y}}{(E_A-E_B)z_xF/(RT \ln 10) + (1-2z_B/2B) \log a_A} \tag{3}
\]

In this document, the general term ‘plasma’ refers so plasma and the forms in which it is sampled to determine its sodium or potassium concentration, namely, the plasma phase of anticoagulated whole blood, plasma separated from blood cells, or serum.

This method can only be used when the ISE exhibits the same response (slope), preferably Nernstian, to both ions A and B.

3. Relationship between ion activity and total substance concentration of ion in plasma*.

(For definitions of terms used see (1))

The relationship between the activity measured by ISE in undiluted specimens and the total substance concentration measured by flame atomic emission spectrometry may be expressed in a mathematical form as shown in the flow chart (see Fig.2). The substance concentration of total M in specimen (\( c_{m\text{spec}} \)) may be converted to the molality of total M by dividing the mass concentration of water (\( \rho_{H_2O} \)) in the specimen:

\[
m_{m} = \frac{c_{m\text{spec}}}{\rho_{H_2O}} \tag{4}
\]

To obtain the molality of free \( M^+ \) (\( m_{m^+} \)), the sum of the molalities of a number \( (j = 1,2,3 \ldots) \) of species \( (B_j^{-}) \) bound to \( M^+ \) (e.g., ion-protein bound MPr, MCO \( \text{__} \), etc. [MB \( \text{__} \)]) must be subtracted (see Fig.3 for Na\( ^+ \) and Fig.4 for Ca\( ^{2+} \)).

\[
m_{m^+} = m_{m^+} - \sum_{j=1}^{n} \rho_{MBj} m_{MBj}^{-} \tag{5}
\]

or, introducing the equilibrium constants of these complexes:

\[
K'_{MBj} = \frac{m_{MBj}}{m_{m^+} m_{MBj}^{-}} \tag{6}
\]

The equation may be written:

\[
m_{m^+} = m_{m^+} \left( 1 + \sum_{j=1}^{n} K'_{MBj} \right) \tag{7}
\]

The molality of free \( M^+ \) may be converted to the active molality \( \gamma_{m,M^+} \) by multiplying by the (molal) activity coefficient \( (\gamma_{m,M^+}) \),

\[
m_{m^+} = \gamma_{m,M^+} m_{m^+} \tag{9}
\]

The active molality may be converted to the relative (molal) activity by dividing by \( m_0 \) = 1mol/kg

\[
a_{m,M^+} = \frac{m_{m^+}}{m_0} \tag{10}
\]

Combining and rearranging equations (4, 8, 9, 10) results in the relationship:

\[
c_{m} = \frac{a_{m,M^+} \rho_{H_2O} \left( 1 + \sum_{j=1}^{n} K'_{MBj} \right) m_0 \gamma_{m,M^+}}{m_{m} \gamma_{m,M^+}} \tag{11}
\]

with which one can calculate the substance concentration of total M on the basis of the (molal) activity of \( M^+ \) or vice versa. The factor

\[
\rho_{H_2O} \left( 1 + \sum_{j=1}^{n} K_{MBj} \right) m_{MBj}^{-} \tag{12}
\]

by which the ISE measured \( a_{M^+} \) is related to \( c_{m} \) may be called the ISE adjustment factor and the result the adjusted activity instead of total substance concentration. For sodium in normal plasma, we may apply the follow-
ing values: mass concentration of water = 0.933 kg/l; bound Na is ~2%, which means

\[ \sum_{i} K'_{MBj} m_{Bj} = 0.02; \text{ and } \gamma_{Na} = 0.747. \]

From equation [10] can also be derived equation [13]

\[ c_{MB} = \bar{c}_{MB}, p_{H,O}(1 + \sum_{j=1}^{n} K'_{MBj} c_{Bj})/(\gamma_{M} \cdot p_{H,O}) \]  

where \( \bar{c}_{MB} \) is the active substance concentration of ion \( M^+ \) and \( p_{H,O} \) is mass concentration water in pure water. In this case, the term

\[ p_{H,O}(1 + \sum_{j=1}^{n} K'_{MBj} c_{Bj})/(\gamma_{M} \cdot p_{H,O}) \]

may be called the adjustment factor by which the active substance concentration of \( M^+ \) is related to \( c_{MB} \) and the result is the concentration of ionized, or free \( M^+ \), instead of the total substance concentration.

These relations are indicated as a flow chart in Fig.2. Relative values of \( M^+ \) etc. are given for Na + in Fig. 3 and for Ca²⁺ in Fig.4.

4. Determination

4.1 Calibration

Although the electrochemical cell responds to changes in the activity of ions, the cell is calibrated in terms of concentration. The activity is equal to the concentration multiplied by the (molar) activity coefficient (\( \gamma \)). Hence, the composition of the calibration solutions is chosen such that the activity coefficient of the ion is assumed to be identical, both in calibration solutions and normal plasma, i.e., plasma of healthy human subjects. The cell is calibrated by means of the primary calibration (standard) solutions.

4.2 Plasma water mass concentration

This quantity is preferably determined by weighing plasma before, and its residue after, drying in air to a state in which water cannot be demonstrated in the dried residue, by the Karl Fischer reagent (13).

4.3 Standard plasma specimens

are defined as having mass concentration of plasma water of 0.93 ± 0.005 kg/l, plasma pH of 7.40 ± 0.05, and concentrations of albumin, total protein, cholesterol, and triglycerides within the reference range for healthy subjects.

4.4 Conventions for reporting results

In principle, ion measurements could be reported as substance concentration (mol/l), molality (mol/kg), or as activity. To avoid a proliferation of units, the convention is adopted to report ion measurements as substance concentration (mol/l). It is emphasized that there is a choice of convention and the term ion concentration may refer to ions in the plasma water or in the entire volume of plasma. The concentration of ions in the standard plasma will be lower by conversion factor of ~0.93 (see Fig.2).

4.5 Routine methods

Most routine methods are based on secondary calibration solutions. These are aqueous solutions to which values of ion concentration have been assigned by means using the primary calibration solutions (14). Routine methods often produce highly precise, but not necessarily accurate values because of variations in electrode systems, liquid/liquid junctions, calibration and measurement procedures. In order to monitor the inaccuracy of routine results, it is necessary to employ quality control solutions, e.g., plasma samples or simulated materials for which the value has been established with the reference method.

References

