Study of Physiologically Required Selectivity Coefficients of Potentiometric Sensors in Clinical Assays

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Abstract: Required selectivity coefficients of ion-selective electrodes (ISEs) in physiological analysis are studied based on the conventional Nicolski-Eisenman equation and a newly proposed consistent approach with the worst allowable error (1%). The physiologically required selectivity coefficients from both methods are compared in this work. The revised consistent approach gives out a more reasonable description of selectivity even in case of the unequally charged primary and interfering ions. An Mg^{2+}-selective electrode using ionophore ETH 5504 is tested to measure the selectivity of free Mg^{2+} against the interfering ions in physiological levels. Obviously the required selectivity coefficients derived from the consistent approach shows the more accurate description of selectivity than the conventional one. For evaluating the applicability of ISEs in physiological solution, the consistent required selectivity coefficients provide the reasonable criteria especially regarding the primary and interfering ions of different charges. [Life Science Journal. 2005;2(1):40−45] (ISSN: 1097−8135).

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1 Introduction

Potentiometric ion-selective electrodes (ISEs) based on neutral inonphore are widely used in clinical assays and also integrated in diagnostic analyzers[1−3]. The selectivity pattern of any ion-selective electrode is clearly the most important character that often reflects whether a sensor may be reliably employed in analysing the target electrolyte over other coexisting interfering ions. One important application of ISE is detecting electrolyte ion activities in whole blood or serum in which a small concentration range of electrolyte extremely mandates the measuring accuracy. The ISE should achieve a sufficient selectivity pattern of the primary ion over coexisting interfering ions (secondary ions) to meet the physiological requirement of worst allowable error of 1%[4]. Conventionally, the required selectivity coefficients for physiological analysis are derived from the well-known semi-empirical Nicolsky-Eisenman (N-E) equation[5,6]:

$$E = E_i^0 + \frac{RT}{z_i F} \ln(a_i + K_{ij}^{pot} a_j^{z_i/z_j}) \qquad (1)$$

where E is the measured electromotive force (EMF) of the electrode in the sample, all constant potential contributions are included in E_i^0; K_{ij}^{pot} is the selectivity coefficient; z_i and z_j are the charges of primary ion I and interfering ion J; a_i and a_j are the sample activities of I and J; and R, T and F have their usual meanings. It is well known that the selectivity coefficient is, in theory, a constant parameter for a particular electrode, irrespective of the charges of primary and interfering ions since K_{ij}^{pot} is a function of E^0 values respectively for two ions of interest.

Although the N-E equation is, with some exceptions[7,8], accurate for ions of the same charge, the selectivity coefficient of ISEs derived from this equation may lead to an inconsistent description for unequally charged primary ion and interfering ion. For instance, taking primary ion as interfering ion and vice versa does not give the same predicted electrode potential[9]. In fact, when the monovalent and divalent ions considered as primary and interfering ions, this discrepancy can amount to 8 mV or more than 30% activity variation. Such discrepancy of selectivity is a serious limitation of N-E equation that may lead to wrong practical predictions. A lot of discussions on this problem have been conducted and many new modification approaches of N-E equation have also been proposed[8,10,11]. In 1998, a consistent formalism was proposed by Zhang et al[9] with modifying the conventional N-E equation with introduction of consistent selectivity coefficient K_{ij}^{cons}

$$E = E_i^0 + \frac{RT}{F} \ln(a_i^{1/z_i} + K_{ij}^{cons} a_j^{1/z_j}) \qquad (2)$$

where K_{ij}^{cons} is defined as consistent selectivity coefficient and

$$K_{ij}^{cons} = (K_{ij}^{pot})^{1/z_i} \qquad (3)$$

With this modification, the selectivity coefficient K_{ij}^{cons} is completely independent of the charge number of the ions involved.

In measuring ion activity in intercellular fluid samples, ion-selective electrode should specifically respond to the activity of target ion with a sufficient discrimination over the co-existing interfering ions. The minimum selectivity requirement of ISEs determines the applica-

bility of certain ISEs and the accuracy of assays. The physiologically required selectivity coefficients are calculated assuming an allowable error of 1% (0.01) in the worst case of a maximum quantity of interfering ion and a minimum quantity of primary ion within physiological range.

Traditionally, the required selectivity coefficients are calculated based on the conventional Nicolsky-Eisenman equation, assuming an allowable error of 1% (0.01) in the worst case of a maximum quantity of interfering ion and a minimum quantity of primary ion within the physiological range by the following equation[12]:

$$(K_{ij}^{pot})_{required} \leqslant 0.01 \cdot \left(\frac{a_{i,min}}{a_{j,max}^{z_i/z_j}}\right) \qquad (4)$$

or

$$\log(K_{ij}^{pot})_{required} \leqslant \log(0.01 \cdot \frac{a_{i,min}}{a_{j,max}^{z_i/z_j}}) \qquad (5)$$

When electrode is calibrated by physiological background solutions, the variations of interfering ion activity around its medium value need to be considered. The difference between the activity of the calibrator $a_{j,cal}$ and the unknown solution a_j, $(a_{j,cal}^{z_i/z_j} - a_j^{z_i/z_j})$, contributes to the actual interference. Then, Eq. (5) can be then changed to:

$$\log(K_{ij}^{pot})_{required} \leqslant \log\left[0.01 \cdot \frac{a_{i,min}}{a_{j,cal}^{z_i/z_j} - a_j^{z_i/z_j}}\right] \qquad (6)$$

Obviously, the required selectivity coefficients in Eq. (5) and (6) are inaccurate in case of unequal charge numbers of the concerned ions ($z_i \neq z_j$), which is originated from the discrepancy of the conventional N-E equation.

In order to get the consistent selectivity criteria for physiological assay with ISEs, the consistent required selectivity coefficients are derived from the newly modified N-E equation for a given allowed relative error, 1% (0.01), in the measurement of physiological solution[2]:

$$\log(K_{ij}^{cons})_{required} \leqslant \log\left[0.01 \cdot \frac{a_{i,min}^{1/z_i}}{a_{j,max}^{1/z_j}}\right] \qquad (7)$$

With the physiological background calibration, the consistent required selectivity coefficient is described as:

$$\log(K_{ij}^{cons})_{required} \leqslant \log\left[0.01 \cdot \frac{a_{i,min}^{1/z_i}}{a_{j,max}^{1/z_j} - a_j^{1/z_j}}\right] \qquad (8)$$

It is shown that Eq. (7) and (8) are obviously different from Eq. (5) and Eq. (6). For primary and interfering ions of different charges ($z_i \neq z_j$), the consistent required selectivity coefficients $\log(K_{ij}^{pot})_{required}$ gives out the reliable criteria to determine the applicabil-

ity of ISEs, which gets rid of the discrepancy from the conventional N-E equation. In this work, an Mg^{2+}-selective electrode with iononphore ETH 5504[13] was used to check the selectivity coefficient in physiological measurements.

2 Experiments

2.1 Reagents

All chemicals were of analytical reagent grade or higher purity. The following chemicals were purchased from Fluka Chemie AG, Buchs, Switzerland: $MgCl_2$, $CaCl_2$, KCl, NaCl, KTpClPB (potassium tetrakis (4-chlorophenyl)borate), PVC (poly(vinyl chloride), high molecular weight) and tetrahydrofuran (THF, distilled prior to use). The Mg^{2+}-selective ionophore ETH 5504 (1,3,5-tris[10-(methyl-7,9-dioxo-6,10-diazadecyl)benzene]) and the plasticizer ETH 8045 (12-(4-ethylphenyl)dodecyl-2-nitrophenyl-ether) were synthesized as described in[2,14]. Doubly distilled water was used throughout.

2.2 Membrane preparation and EMF measurement

The PVC-based Mg^{2+}-ISE membrane was prepared as described elsewhere[13,15] using 1 wt% Mg^{2+}-selective ionophore; 155 mol% (relative to the ionophore) lipophilic anionic sites KTpClPB; 65 wt% plasticizer, ETH 8045; and 33 wt% PVC. The working electrode was assembled by cutting disks of 7 mm diameter out of the membrane (thickness is about 150 μm). The disk was mounted into a Phillips electrode body (ISE – 561, Glasbläserei Möller, Zürich). A solution of 0.1M $MgCl_2$ was used as the internal filling solution for the Mg^{2+}-selective electrode.

After conditioning the electrode in 0.1M $MgCl_2$ for 24 hours, EMF measurements were conducted with following cell: Hg, Hg_2Cl_2 | KCl (satd.) MMKCl (3.0 M) MM sample solution ‖ membrane ‖ internal solution ($MgCl_2$, 0.1 M) | AgCl, Ag.

The calomel reference electrode corresponded to the free-flowing double-junction type[16]. The cell potential was measured using an Apple IIGS (Apple Computer, Cupertino, CA, USA) equipped with a 7150 Digital Multimeter (Solartron Instrumentation, UK). The single-ion molal molar activities in physiological electrolyte situation were calculated using the Pitzer program[17]. The selectivity coefficients were measured based on the IUPAC recommended procedure of separate solution method. All EMF values were corrected for changes in the liquid-junction potential, E_j, using the Henderson formalism[18]. All potentiometric measurements were performed at ambient temperature (~25℃).

2.3 The main cations in physiological solution

In physiological fluids, the main cations are Na^+,

K^+, Ca^{2+} and Mg^{2+} which play important roles in biological functions. In human blood serum, the intercellular concentrations of these cations vary in certain ranges that are listed in Table 1. In calculating the required selectivity coefficients of ISEs for physiological assays, an allowable measuring error of 1.0% is considered with minimum level of a physiological molar activity. The required selectivity coefficients for detecting main inorganic cations in physiological solution are calculated assuming an allowable error of 1.0% at the minimum level of a physiological molar activity of the detecting ion. The sensors are measured with- and without the calibration of physiological ion background (Dc, assuming variation of the background ion molality level around the mean physiological points, Table 1).

The calculation without background calibration (Eq. (5) and Eq. (7)) is based on the maximum level of interfering ion $a_{j,max}$ and the minimum level of primary ion $a_{i,min}$. For obtaining the required selectivity coefficients with the background calibration (Eq. (6) and Eq. (8)), the minimum level of the primary ion $a_{i,min}$ and the medium level of the interfering ion $a_{j,cal}$ is considered for the background calibration.

3 Results and Discussion

3.1 Comparison of methods for calculating the required selectivity coefficients

The required selectivity coefficients of ISE for different primary ions are calculated based on the conventional N-E equation and the modified consistent N-E equation. The obtained required selectivity coefficients with and without the physiological background calibration (Eq. (6) and Eq. (8)) are compared in Table 2.

Table 1. The main cation levels in physiological solution

Cation		min. level	Medium level	max. level
Mg^{2+}	Concentration c (M)	3.0×10^{-4}	4.5×10^{-4}	6.0×10^{-4}
	molar activity a (M)	1.1×10^{-4}	1.6×10^{-4}	2.1×10^{-4}
Ca^{2+}	Concentration c (M)	1.0×10^{-3}	1.15×10^{-3}	1.3×10^{-3}
	molar activity a (M)	3.4×10^{-4}	3.9×10^{-4}	4.4×10^{-4}
K^+	Concentration c (M)	3.3×10^{-3}	4.05×10^{-3}	4.8×10^{-3}
	molar activity a (M)	2.4×10^{-3}	2.9×10^{-3}	3.5×10^{-3}
Na^+	Concentration c (M)	0.134	0.138	0.143
	molar activity a (M)	0.099	0.103	0.106

Table 2. Comparison of the required selectivity coefficients of ISEs for measuring the main inorganic cations in physiological solutions, assuming an allowable error of 1.0% at the lower level of the physiological range of the detecting ion.

Primary ion	Conventional SSM Secondary ion	$(\log K_i^{pot})_{required}$ no calibration	with calibration	Consistent SSM Secondary ion	$(\log K_i^{pot})_{required}$ no calibration	with calibration
Mg^{2+}	Ca^{2+}	-2.4	-1.5	Ca^{2+}	-2.2	-1.0
	K^+	-0.9	-0.3	K^+	-1.4	-0.6
	Na^+	-3.8	-2.6	Na^+	-2.9	-1.4
Ca^{2+}	Mg^{2+}	-1.8	-1.1	Mg^{2+}	-1.9	-0.9
	K^+	-0.6	-0.0	K^+	-1.3	-0.5
	Na^+	-3.5	-2.3	Na^+	-2.8	-1.2
K^+	Mg^{2+}	-2.8	-1.8	Mg^{2+}	-2.8	-1.8
	Ca^{2+}	-2.9	-1.7	Ca^{2+}	-2.9	-1.7
	Na^+	-3.6	-2.1	Na^+	-3.6	-2.1
Na^+	Mg^{2+}	-1.2	-0.2	Mg^{2+}	-1.2	-0.2
	Ca^{2+}	-1.3	-0.1	Ca^{2+}	-1.3	-0.1
	K^+	-0.5	0.3	K^+	-0.5	0.3

Because the modified consistent N-E equation removes the influence from the ion charge-dependence, the consistent required selectivity coefficient $(K_{ij}^{\text{cons}})_{\text{required}}$ reasonably illustrates the preference of the sensor to the primary ion over interfering ions, especially for measuring the unequally charged primary and interfering ions. It can be seen from Table 2 and Table 3 that when monovalent ion (K^+ or Na^+) is considered as the primary ion, two methods give out the equal required selectivity coefficients $(K_{ij}^{\text{cons}})_{\text{required}} = (K_{ij}^{\text{pot}})_{\text{required}}$. However, the consistent coefficient values of $\log(K_{ij}^{\text{cons}})_{\text{required}}$ for divalent primary ions (Mg^{2+} or Ca^{2+}) are significantly different from the conventional ones of $\log(K_{ij}^{\text{pot}})_{\text{required}}$. The logarithm of the required selectivity coefficient for Mg^{2+} over Ca^{2+} changes from -2.4 to -2.2 and the one for Mg^{2+} over K^+ from -0.9 to -1.4. As well, the log $(K_{ij}^{\text{pot}})_{\text{required}}$ for Mg^{2+} over Na^+ decreases from -3.8 to -2.9. Such differences lead to the necessity of applying the consistent formulism of the required selectivity coefficient for divalent primary ions.

3.2 Mg^{2+}-selective electrode and the required selectivity coefficients in physiological analysis

In recent years, studies of developing selective Mg^{2+} potentiometric sensors have been intensively conducted in many groups and the adequate discrimination between Mg^{2+} and Ca^{2+} is still the main target[19-21]. Suzuki et al[22] synthesised a selective Mg^{2+} ionophore K22B5 which logarithm of selectivity coefficient of Mg^{2+} over Ca^{2+} was reported as -2.5 measured with the IUPAC recommended separate solution method (SSM)[23]. The inadequate selectivity against monovalent cations (Na^+ and K^+) of this kind of Mg^{2+} ionophores made the corresponding Mg^{2+} membrane electrodes hard to be applied in physiological assays. Spichiger et al developed an Mg^{2+} selective electrode based on ionophore ETH 5504 which has high selectivity over Ca^{2+}, K^+ and Na^+ while its high lipophilicity prevents ETH 5504 leaching from plasticized PVC membrane. So far, many Mg^{2+} selective microelectrodes based on various Mg^{2+} ionophores have also been reported in measuring the physiological systems[24-28].

In serum samples, the intercellular Mg^{2+} molar activity range ($0.46 \sim 0.66$ M) is much lower that Ca^{2+} level range ($1.01 \sim 1.26$ M) and the discrimination of Mg^{2+} over Ca^{2+} is very critic for accurate measurement of Mg^{2+} in physiological assays. The selectivity coefficients of Mg^{2+}-selective electrode based on ionophore ETH 5504 was determined according to the IUPAC recommended separate solution method (SSM)[23] as well as the newly modified consistent SSM[9].

In order to ensure the selectivity coefficients of Mg^{2+}-ISEs to reflect the "worst case" in physiological situation, the minimum level of Mg^{2+} and the maximum levels of interfering ions were used in calculating the selectivity coefficients. EMF values of the minimum physiological level Mg^{2+} were compared with that of maximum physiological levels of other interfering ions, Ca^{2+}, Na^+ and K^+ separately (Figure 1). Two sets selectivity coefficients for Mg^{2+} − ISE, $\log K_{Mg,J}^{\text{pot}}$ and $\log K_{Mg,J}^{\text{cons}}$, were calculated based on both the conventional N-E method and the modified consistent N-E method. As shown in Figure 2, the selectivity coefficients (SSM) of Mg^{2+}-selective electrode based on both methods were obtained by using electrolyte solutions of standard concentration (0.1 M) and of physiological medium levels of primary and interfering ions (Table 1).

It can be seen from the results in Figure 2 that the selectivity pattern of Mg^{2+}-selective electrode with ETH 5504 were measured based on both approaches of the conventional and the modified consistent approaches. The selectivity of Mg^{2+} against Ca^{2+} reaches $-1.0 \sim -1.8$ with conventional SSM approach and $-1.0 \sim -1.2$ with the consistent method. Both values satisfied the physiologically required selectivity coefficients calculated from Eq. (6) (conventional approach, $\log(K_{ij}^{\text{pot}})_{\text{required}} = -1.5$) and Eq. (8) (consistent approach, $\log(K_{ij}^{\text{cons}})_{\text{required}} = -1.0$) with physiological background calibration. However, for the direct measurements without background calibration, the selectivity still has space to improve. Regarding the interfering monovalent cations of K^+ and Na^+, this Mg^{2+}-ISE showed the adequate selectivity over K^+ even for non-calibration measurement of Mg^{2+} either with conventional method ($\log(K_{ij}^{\text{pot}})_{\text{required}} = -0.9$) and consistent method ($\log(K_{ij}^{\text{cons}})_{\text{required}} = -1.4$). However, the discrimination against Na^+ of this Mg^{2+}-ISE also requires the physiological background calibration so as to satisfy the physiological requirement (with background calibration, $\log(K_{ij}^{\text{pot}})_{\text{required}} = -2.6$ and $\log(K_{ij}^{\text{cons}})_{\text{required}} = -1.4$). According to this comparison, it is obvious that the physiological background calibration is really needed for using the Mg^{2+}-ISE with ionophore ETH 5504 to measure free intercellular Mg^{2+} level. This requirement can predict the necessary enhancing space of selectivity pattern for developing future Mg^{2+}-ISEs in direct measurements in physiological assays.
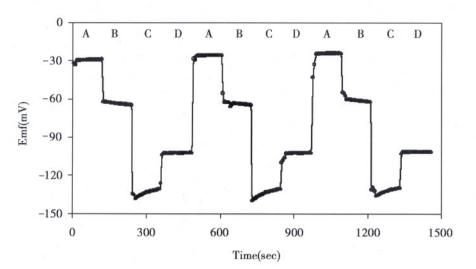
Figure 1. Selectivity measurement in the "worst case" for Mg^{2+}-ISE in physiological electrolyte situation
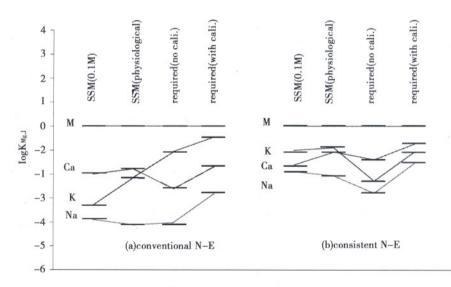A:0.3 mM $MgCl_2$;   B:1.3 mM $CaCl_2$;   C:4.8 mM KCl;   D:140 mM NaCl.

Figure 2. The comparison of the Mg^{2+}-selectivity coefficients with required selectivity coefficients.
(a) Conventional N-E method; (b) Consistent N-E method.

4   Conclusion

In conclusion, the physiologically required selectivity coefficients of ISEs for physiological analysis were studied by comparing the conventional N-E method and the modified consistent N-E method. The modified consistent method eliminates the discrepancies originated from the conventional N-E equation for unequally charged primary and interfering ions. Indeed, the consistent required selectivity coefficients for the divalent primary ion (Mg^{2+} or Ca^{2+}) gives out a more accurate calculation that the conventional one and provides a reasonable criteria for evaluating the applicability of ISEs in physiological assays. However for the monovalent primary ion (K$^+$ or Na$^+$), the required selectivity coefficients calculated with two methods are identical. For comparing the performances of ISE in the physiological solution with the required selectivity pattern, the electrode was checked in the "worst case" of minimum level of primary ion and maximum level of interfering ion in physiological solution. Indeed, the modified consistent required selectivity coefficients are more accurate than the ones derived from the conventional N-E equation, which provides the more reliable criteria for evaluating the applicability of ion-selective electrodes in physiological assays.

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