Ionic Liquid-based Reference Electrodes for Miniaturized Ion Sensors: What Can Go Wrong?

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Abstract

Ionic liquid-based reference electrodes, especially those containing 1-octyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide (MeOctIm TFSI), are increasingly used in potentiometric measurements. They replace the conventional salt bridges and establish the reference potential through local partitioning of the ions of the ionic liquid between the reference membrane and the sample solution. Unless the electrochemical cell and the measurement protocol are designed appropriately, these ions can interfere with the response of ion-selective electrodes (ISEs) with polymeric membranes. This work characterizes the effect of MeOctIm TFSI on the response of K^+, Na^+, and Cl^- ISEs with polymeric membranes. The leaching of MeOctIm TFSI from the reference membrane to the sample solution was monitored with UV-Vis spectroscopy. The concentration of MeOctIm TFSI in the aqueous phase increased gradually and plateaued at approximately 1.0 mM. Concentrations as low as 100 μM of MeOctIm TFSI caused large changes to the *emf* (50–150 mV) of K^+, Na^+, and Cl^- ISEs. The presence of 10 μM of $TFSI^-$ was enough to cause Donnan failure of K^+ ISEs with valinomycin as ionophore (that is, co-extraction of K^+ and $TFSI^-$ into the sensing membrane). Use of less lipophilic anions such as tetrafluoroborate (BF_4^-) or triflate (OTf^-) postponed the onset of Donnan failure of cation-selective ISEs to higher concentrations of the anion, but decreased the stability in the reference potential and lifetime of the reference electrode. These results imply that although MeOctIm TFSI-based reference electrodes provide sample-independent and stable electrical potentials, they should be used with caution for measurements with polymeric-membrane ISEs, due to strong interference of both $MeOctIm^+$ and $TFSI^-$ with measured values of *emf.*

Keywords: Reference Electrode, Ionic Liquid Salt Bridge, Potentiometry, Ion-selective Electrode, Liquid Junction Potential, Ion Sensing

Introduction

Interest in hydrophobic, room-temperature, ionic liquids (ILs) such as 1-octyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide (MeOctIm TFSI) for fabrication of reference electrodes is growing [1-14]. The interface of a hydrophobic IL (either in the form of a pure liquid, or dissolved in a polymeric supporting membrane) and an aqueous sample exhibits a stable and sample-independent electrical potential [1-5, 12]. This interfacial electrical potential is established by continuous local partitioning of the cation and anion of the IL from the reference membrane (the IL phase) to the aqueous phase, as demonstrated in extensive work by Kakiuchi and co-workers [1-5].

The IL-based reference electrodes have two advantages over conventional reference electrodes that are based on the $AgCl/Ag/Cl^{-}_{(aq)}$ redox system. (i) The electrical potential across the interface of the IL and the aqueous phase is stable over a range of concentrations of electrolytes and a range of different ionic strengths in the aqueous phase. A reference membrane that contains an IL can therefore directly contact the sample solution without the need for a salt bridge. This feature eliminates the glass or ceramic junctions that are employed in conventional reference electrodes to separate the reference and sample solutions (and thus eliminates complications caused by clogging of the junction or electrostatic screening of ions at the junction), and it does not require computational correction of the liquid junction potential [1, 3, 4, 15-19]. (ii) The IL can be incorporated into the polymeric support membranes, which can be deposited on conductive substrates to give solid-contact reference electrodes [7, 8, 10, 12, 20, 21]. These electrodes do not require maintenance of a reference solution and thus are easy to store [7, 8, 10, 20]. Moreover, IL-based reference electrodes can be fabricated in smaller sizes and with lower costs than conventional reference electrodes, and they are also more convenient to use [7-9]. In particular, IL-polymeric membranes can be incorporated in paper-based or thread-based devices as needed for portable and low-cost electrochemical sensing systems [7, 8, 20, 22].

These advantages make IL-based reference electrodes an appealing choice for potentiometric measurements, which rely heavily on the stability and sample-independence of the electrical potential of the reference electrode [23-27]. The biggest limitation of IL-based reference electrodes is that they introduce two new hydrophobic ions—the cation and anion of the IL—into the sample solution, and these species may cause strong interference to the response of ISEs with polymeric receptor-doped membranes (because these ISEs are selective towards hydrophobic ions). Kakiuchi et al., and more recently Lindner et al., warned users about this possible interference as a limitation of IL-based reference electrodes [2, 14]; however, this interference has not been quantitively discussed prior to the work we describe here.

Our prior experience with use of miniaturized IL-based reference electrodes for measurements of blood electrolytes (K^+, Na^+, and Cl^-) with miniaturized ISEs prompted us to study the compatibility of these reference electrodes with ISEs with polymeric membranes [28]. In prior work, to measure K^+, we used a thread-based reference electrode (containing a MeOctIm TFSI-doped reference membrane), and a thread-based K^+ ISE (containing valinomycin as ionophore) [28]. When we performed such measurements in large volumes of sample (~50 mL), the K^+ ISEs exhibited Nernstian behavior, and we could measure K^+ in blood serum accurately (< 5% error) [28]. The compact design of thread-based ISEs allowed us to decrease the volume of sample to 200 μL; however, at this volume of sample, the K^+ ISEs (i) behaved super-Nernstian (slope of *emf* vs. log concentration of K^+> 100 mV/decade), (ii) showed large drifts in their *emf* (> 1 mV/min), and (iii) and (in some measurements) no longer exhibited an increase in their *emf* upon increasing the concentration of K^+ in the solution [28].

To explain the aforementioned effects and provide a guide for successful application of IL-based reference electrodes in potentiometric measurements, this work characterizes the time-dependent leaching of MeOctIm TFSI from the polymeric membrane (the reference membrane) and shows that this leaching can produce concentrations of MeOctIm $TFSI_{(aq)}$ as high as 1 mM in the sample solution. We also quantified the effect of $MeOctIm^+$ and $TFSI^-$ on the response of K^+, Na^+, and Cl^- polymeric-membrane ISEs.

We hope that this work helps to avoid unexpected errors when IL-based reference electrodes are used for measurements with polymeric-membrane ISEs, particularly in miniaturized analytical systems.

EXPERIMENTAL SECTION

Materials: Valinomycin (potassium ionophore I), bis[(benzo-15-crown-5)-15-ylmethyl] pimelate (potassium ionophore II), 4-tert-butylcalix[4]arene-tetraacetic acid tetraethyl ester (sodium ionophore X), 4,5-dimethyl-3,6-dioctyloxy-o-phenylene-bis(mercurytrifluoroacetate) (chloride ionophore II), potassium tetrakis(4-chlorophenyl)borate (KTClPB), sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB), and tridodecylmethylammonium chloride (TDDMACl) were purchased from Sigma-Aldrich. The supporting information (SI) lists the suppliers of other materials used in this work.

Membrane Compositions: We fabricated the ISEs and reference membranes according to protocols established in the literature [24, 29]. All the sensing membranes had a molar ratio of ionophore to ionic site of 2:1. The SI describes the composition and fabrication of ion-selective membranes. The reference membranes contained 900 mg of *o*-nitrophenyl octyl ether (*o*-NPOE), 450 mg of poly(vinyl chloride) (PVC), and 150 mg of ionic liquid (MeOctIm TFSI, DodecMeIm TFSI, MeOctIm OTf, and MeOctIm BF_4).

The SI lists details of the measurements with and fabrication of electrodes.

Results and Discussion

Leaching of the ionic liquid from reference membranes: MeOctIm TFSI has been the most commonly used IL for the fabrication of reference electrodes [1-4, 6-8, 10, 12, 13]. To fabricate reference electrodes, this IL was dissolved in a polymeric support (e.g., PVC film plasticized *o*-NPOE) to form a self-supporting membrane [2-4, 7, 8, 20]. To quantify leaching of MeOctIm TFSI from such membranes, we monitored the UV absorbance of $MeOctIm^+$ at 216 nm (Fig. S1, $TFSI^-$ is not UV active).

We placed the IL-containing membrane (80 mg; circular, with a 1-cm diameter) in a cuvette containing 2 mL of deionized water, and monitored the UV absorbance at 216 nm for six hours. (The membrane was floating on top of the solution and did not obstruct the light path; we did not stir or shake the cuvette during the measurement.) Fig. 1 shows the gradual increase in the UV absorbance, which was due to continuous leaching of MeOctIm TFSI from the membrane into the aqueous solution. The concentration of MeOctIm TFSI in the aqueous solution (after six hours of contact with the membrane) reached 0.96 mM. This experiment demonstrates that using this IL-based reference electrode for a potentiometric measurement would inevitably expose an ISE to micro- or millimolar concentrations of MeOctIm TFSI. (The solubility of this compound in water determines the maximum concentration.) The exact concentration of MeOctIm TFSI to which the ISEs will be exposed depends on the volume of the sample, the volume and composition of the reference membrane, and the duration of the experiment.

Effect of MeOctIm TFSI on the ISEs: Both the cation and the anion of MeOctIm TFSI are hydrophobic and thus can interfere with the performance of ISEs that have a polymeric ion-selective membrane. To demonstrate how the exposure to MeOctIm TFSI can affect ISEs, we fabricated three K^+ ISEs (one with an ionophore-free ISM, and two with ISMs containing different K^+ ionophores), and two Cl^- ISEs (an ionophore-doped polymeric membrane ISE and a AgCl-coated Ag wire). We placed these ISEs in an aqueous solution of 10 mM KCl, monitored their *emf* (relative to a commercial reference electrode), adjusted the solution to 100 µM of MeOctIm TFSI by adding the pure IL, and again monitored their *emf*. Fig. 2A shows that concentrations as low as 100 µM of MeOctIm TFSI can substantially alter the *emf* of the ISEs (10–125 mV), and can cause an error up to two orders of magnitude in the measurement of K^+ concentration with these ISEs.

Initially, we expected that addition of MeOctIm TFSI to the 10 mM-KCl aqueous solution would decrease the *emf* of Cl^--selective ISE with polymeric membrane (due to an anionic response to $TFSI^-$) and would increase the *emf* of the K^+-selective ISEs (due to a cationic response to $MeOctIm^+$). Indeed, the Cl^- ISE showed a large decrease in its *emf* (≈140 mV) upon addition of 100 µM MeOctIm TFSI. This *emf* change can cause an error of more than a factor of 100 in the determination of Cl^- with this ISE. The Ag/AgCl-based Cl^- ISE was not affected by addition of MeOctIm TFSI to the sample solution.

Unexpectedly, the *emf* of K^+ ISEs decreased drastically (instead of increasing) upon addition of MeOctIm TFSI. Importantly, the effect of the MeOctIm TFSI on the *emf* of the K^+ ISEs was strongly dependent on the ionophore that was used in the ISM. The ISE containing the ionophore with the strongest binding to K^+ experienced the most interference from the IL. (Table 1 lists the binding constant of the ionophores to K^+ in the ISM.) This effect is caused by co-extraction of K^+ and $TFSI^-$ into the sensing membrane (the so-called Donnan failure), which theory predicts to be a problem of increasing concern the stronger the ionophore binds the target ion [30, 31]. To study this effect quantitatively, we looked at the effect of $MeOctIm^+$ and $TFSI^-$ separately.

Effect of $MeOctIm^+$ on the ISEs: We studied the selectivity of the K^+ ISEs for K^+ over $MeOctIm^+$ to identify the extent to which $MeOctIm^+$ can interfere with the detection of K^+. We measured the selectivity coefficients ($K^{Pot}_{K^+,\ MeOctIm^+}$) according to the separate solution method (comparing the emf of the ISE in a solution of K^+ to the emf response to a pure solution of $MeOctIm^+$), which is an established protocol for quantifying the selectivity of ISEs [32]. The SI lists the details of the measurements, and Table 1 lists the thus determined $K^{Pot}_{K^+,\ MeOctIm^+}$ values. The ionophore-free ion-exchanger ISE responds with a selectivity of 400:1 towards $MeOctIm^+$ over K^+ ($logK^{Pot}_{K^+,\ MeOctIm} = 2.62$). This high selectivity for $MeOctIm^+$ explains why the *emf* of the ionophore-free membrane increased by 15 mV when 100 μM of MeOctIm TFSI was added to the 10 mM KCl solution (Fig. 2A). As expected, the interference of $MeOctIm^+$ on the response of the ionophore-based ISEs was much smaller than the ionophore-free ISE. The ISE with K^+-Ionophore I was more selective to K^+ than $MeOctIm^+$ ($logK^{Pot}_{K^+,\ MeOctIm} = -1.85$), and the ISE with K^+-Ionophore II had only a small preference for $MeOctIm^+$ over K^+ ($logK^{Pot}_{K^+,\ MeOctIm} = 0.19$).

$MeOctIm^+$ did not influence the response of Cl^- ISEs (both with polymeric sensing membrane and the Ag/AgCl based ISE) to Cl^-, and the ISEs responded similarly to KCl and to MeOctIm Cl.

Effect of $TFSI^-$ on the ISEs: $TFSI^-$ strongly interfered with the response of the Cl^- ISE with a polymeric sensing membrane, with a preference for $TFSI^-$ over Cl^- of several orders of magnitude ($\log K_{Cl^-,\ TFSI}^{Pot} = 3.78$). We rationalize that this strong interference is explained by the high hydrophobicity of $TFSI^-$, evident from the much stronger response of an ISE with an ionophore-free anion-selective membrane to $TFSI^-$ than Cl^- (Fig. 3A, $logK_{Cl^-,\ TFSI}^{Pot} = 8.88$). $TFSI^-$ did not affect the response of the Ag/AgCl-based Cl^- ISE to Cl^-.

Fig. 4A shows the *emf* of the K^+ ISEs in KCl solutions with different concentrations. As expected, the *emf* increases linearly with the logarithm of the concentration of KCl. We repeated this experiment in KTFSI solutions (Fig. 4B); the response of the ionophore-free ISE to KTFSI was similar to its response to KCl (a cationic response to K^+). The ionophore-doped ISEs showed a cationic response to KTFSI up to a threshold concentration; beyond this concentration, the ISEs responded anionically (that is, the response slope became negative). Similar responses are well known, and thus we assume that this effect is caused by spontaneous co-extraction of K^+ and $TFSI^-$ into the sensing membrane of the ISE [30]. The upper limit of detection of ISEs is limited by co-extraction of the primary ion and a counter ion into the sensing membrane. This coextraction is referred to as the Donnan failure of the ISEs [24, 30, 33-36]. Parameters such as the formation constant of the complex between the ionophore and primary ion, and the hydrophobicity of the counter ion, affect the threshold concentration at which Donnan failure starts [24, 30, 33-35].

A hydrophobic anion such as TFSI$^-$ causes Donnan failure of the K$^+$ ISE (K$^+$ Ionophore I) at concentrations as low as 10 μM. When leaching of MeOctIm TFSI from the reference membrane to the sample solution builds up a 10-μM MeOctIm TFSI concentration in the aqueous phase, the TFSI$^-$ causes co-extraction of K$^+$ and TFSI$^-$ into the sensing membrane (an event that causes the ISE to no longer show a Nernstian response to K$^+$). Fig. 4B confirms that the onset of Donnan failure for the K$^+$ ISEs depends on the type of ionophore in the ISM, as it is predicted by theory [31].

To demonstrate quantitatively the effect of hydrophobicity of the counter ion of K$^+$ on the onset of Donnan failure, we performed numerical simulations (in MATLAB) as reported previously for Donnan failure of ionophore-based ISEs [31]. We first derive the equation we used for the numerical simulations; we note that this derivation has been published previously [31], and is stated here (i) to facilitate better understanding of the experimental results, and (ii) to provide guidance for the design of IL-based reference electrodes. The response of the K$^+$ ISEs with a polymeric membrane can be described by Eq. 1 [24, 31, 37, 38].

$$E = E^{\circ} + \frac{RT}{nF}\ln\frac{a_{\mathrm{K^+,aq}}}{C_{\mathrm{K^+,ISM}}} \qquad \text{(Eq. 1)}$$

Here, $a_{\mathrm{K^+,aq}}$ is the activity of K$^+$ in the sample, $C_{\mathrm{K^+,ISM}}$ is the concentration of K$^+$ in the sensing membrane, *R* is the universal gas constant, *T* is temperature, *n* is the charge of the ion, *F* is the Faraday constant, and E° is a constant term that includes all sample-independent interfacial potentials and coefficients [24, 31, 37, 38]. Because the ionic site (TClPB$^-$) and ionophore (L) are highly lipophilic, we assume that they remain in the ISM, and their concentrations in the ISM are constant [31]. Assuming electroneutrality for the bulk of the ISM and conservation of mass, we obtain Eq. 2 and 3, where X$^-$ is the counter anion of K$^+$ in the sample, L is the ionophore in the ISM, KL^+ is the complex of ionophore and K^+ in the ISM (assuming the ionophore is not charged and that there is a 1:1 complexation ratio), $C_{\mathrm{TClPB,ISM}}$ is the concentration of ionic site in the ISM, $C_{\mathrm{X^-,ISM}}$ is the concentration of X^- in the ISM, $C_{\mathrm{t,L,ISM}}$ is the total concentration of ionophore in the ISM, and $C_{\mathrm{L,ISM}}$ is the concentration of free ionophore in the ISM.

$$C_{\mathrm{X^-,ISM}} + C_{\mathrm{TClPB,ISM}} = C_{\mathrm{K^+,ISM}} + C_{\mathrm{KL^+,ISM}} \qquad \text{(Eq. 2)}$$

$$C_{\mathrm{t,L,ISM}} = C_{\mathrm{L,ISM}} + C_{\mathrm{KL^+,ISM}} \qquad \text{(Eq. 3)}$$

Eq. 4 defines the partition coefficient (P) of KX between the aqueous phase and the ISM. We assume $C_{\mathrm{K^+,aq}}$ is equal to $C_{\mathrm{X^-,aq}}$, and substitute $C_{\mathrm{X^-,aq}}$ by $C_{\mathrm{K^+,aq}}$ in Eq. 4 to get Eq. 5, which describes the concentration of $C_{\mathrm{X^-,ISM}}$.

$$\frac{C_{\mathrm{K^+,ISM}} \cdot C_{\mathrm{X^-,ISM}}}{C_{\mathrm{K^+,aq}} \cdot C_{\mathrm{X^-,aq}}} = P \qquad \text{(Eq. 4)}$$

$$C_{\mathrm{X^-,ISM}} = P\frac{C^2_{\mathrm{K^+,aq}}}{C_{\mathrm{K^+,ISM}}} \qquad \text{(Eq. 5)}$$

Substituting $C_{\mathrm{KL^+,ISM}}$ from Eq. 6 (the formation constant, K_f, of the K^+-ionophore complex in the ISM) and $C_{\mathrm{X^-,ISM}}$ from Eq. 5 into Eq. 2 gives Eq. 7.

$$\frac{C_{\mathrm{KL^+,ISM}}}{C_{\mathrm{L,ISM}} \cdot C_{\mathrm{K^+,ISM}}} = K_f \qquad \text{(Eq. 6)}$$

$$P\frac{C^2_{\mathrm{K^+,aq}}}{C_{\mathrm{K^+,ISM}}} + C_{\mathrm{TClPB,ISM}} = C_{\mathrm{K^+,ISM}} + K_f\, C_{\mathrm{L,ISM}} \,.\, C_{\mathrm{K^+,ISM}} \qquad \text{(Eq. 7)}$$

By rearranging Eqs. 3 and 6 to eliminate $C_{\mathrm{KL^+,ISM}}$, $C_{\mathrm{L,ISM}}$ can be calculated as a function of $C_{\mathrm{t,L,ISM}}$, $C_{\mathrm{K^+,ISM}}$, and K_f (Eq. 9). Substituting $C_{\mathrm{L,ISM}}$ into Eq. 7 gives Eq. 9, which is a third-order polynomial equation that correlates $C_{\mathrm{K^+,ISM}}$ to $C_{\mathrm{K^+,aq}}$, K_f, $C_{\mathrm{TClPB,ISM}}$, $C_{\mathrm{t,L,ISM}}$, and P. Solving Eq.

9 gives three answers, but only one always provides a positive value for $C_{\mathrm{K^+,ISM}}$. By substituting this answer in Eq. 1, we numerically calculated the *emf* at different values of K_f, $C_{\mathrm{TClPB,ISM}}$, $C_{\mathrm{t,L,ISM}}$, and P.

$$C_{\mathrm{L,ISM}} = \frac{C_{\mathrm{t,L,ISM}}}{1+ K_f \, . \, C_{\mathrm{K^+,ISM}}} \qquad \text{(Eq. 8)}$$

$$K_f\ C^3_{\mathrm{K^+,ISM}} + \left(1 + K_f C_{\mathrm{t,L,ISM}} - K_f C_{\mathrm{TClPB,ISM}}\right) C^2_{\mathrm{K^+,ISM}} - \left(K_f P C^2_{\mathrm{K^+,aq}} + C_{\mathrm{TClPB,ISM}}\right) C_{\mathrm{K^+,ISM}} - P C^2_{\mathrm{K^+,aq}} = 0 \qquad \text{(Eq. 9)}$$

Fig. S4 shows that as long as there are no significant changes in the concentration of K^+ in the sensing membrane, the response of the ISE will be Nernstian and cationic to K^+ (that is, the *emf* will increase with the increase in the concentration of KX in the sample solution). A thorough explanation of changes in the concentrations of K^+ and X^- in the sensing membrane in the Donnan Failure region is available [31]. In brief, as the concentration of KX (aq) increases, the transfer of KX from the aqueous phase to the sensing membrane increases to the point that this transfer significantly changes the concentration of K^+ in the sensing membrane [31]. As long as there is free ionophore in the sensing membrane, the energy of transfer of KX from the aqueous phase to the sensing membrane is controlled by P and K_f. Once all the ionophore in the sensing membrane forms a complex with K^+, the transfer of KX from the aqueous phase to the sensing membrane will no longer proceed as favorably, and the concentration of X^- in the sensing membrane stays approximately constant [31]. In this region, KL^+ acts as an ionic site for X^-, and the ISE responds anionically (that is, the *emf* decreases as a result of the increasing concentration of KX in the solution of sample). Increasing the concentration of KX in the aqueous phase even further causes a second phase of significant co-transfer of K^+ and X^- into the sensing membrane, and the concentration of X^- in the sensing membrane will no longer stay constant. In this region, the *emf* of the ISE will not change upon changing the concentration of KX in the aqueous phase (because the ratio of $\frac{a_{K^+,aq}}{c_{K^+,ISM}}$ will not change further) [31].

Fig. 5 shows that increasing the K_f and P lowers the onset of Donnan failure of the ISE. The experimental results confirm this effect; the ISE with K^+-Ionophore I had a lower onset of Donnan failure than the ISE with K^+-Ionophore II. The K^+-Ionophore I binds more strongly to K^+ in the ISM than K^+-Ionophore II [39]. Using an anion that is more hydrophilic than $TFSI^-$ should increase the onset of Donnan failure, and thus decrease the interference of the ionic liquid on the response of cation-selective ISEs. The anions trifluoromethylsulfonate (OTf^-) and BF_4^- are commonly used in ionic liquids [40, 41] and are much less hydrophobic than $TFSI^-$, as evident from the weaker response of anion-selective ionophore-free ISE to these anions (Fig. 3A). The onset of Donnan failure for K^+ and Na^+ ISEs indeed occurred at OTf^- and BF_4^- concentrations that were at least two orders of magnitude higher than for $TFSI^-$ (Fig. 6). Consequently, to take advantage of the lower interference of OTf^- and BF_4^- on ISEs demonstrated by these experiments, we also prepared reference electrodes with membranes doped with OTf^- and BF_4^- salts.

We tested the performance of a reference electrodes with membranes containing the two ionic liquids MeOctIm OTf and MeOctIm BF_4 and recorded the *emf* of the electrodes while changing the concentration of KCl in the sample through successive dilutions. Fig. 3B shows that the MeOctIm OTf- and MeOctIm BF_4-based reference electrodes suffered from larger changes (by a factor of five) in their *emf* than did the MeOctIm TFSI-based electrodes. This difference can be explained by the fact that the solubility of MeOctIm OTf and MeOctIm BF_4 in the aqueous phase is higher than that of MeOctIm TFSI. Significant amounts of MeOctIm OTf and

MeOctIm BF_4 are, therefore, lost to the aqueous phase upon each dilution. This loss results in significant a decrease in the concentration of the ionic liquid in the ISM $(C_{IL,ISM})$, that is, rapid depletion of the reference membrane from the IL. Once the reference membrane is depleted of the IL, the reference potential is ill-defined and affected by the impurities in the reference membrane. MeOctIm OTf and MeOctIm BF_4 are soluble in an aqueous solution in concentrations as high as 37 and 114 mM, whereas MeOctIm TFSI is only soluble in water up to 1.7 mM (values measured in this study).

Fig. 1 shows the time-dependent leaching of ILs from the reference membrane (containing 10 wt.% IL, 30 wt.% PVC, and 60 wt.% *o*-NPOE) monitored by UV absorbance. It confirms that MeOctIm OTf and MeOctIm BF_4 build up a higher concentration in the aqueous phase than MeOctIm TFSI, where the concentrations of IL in the aqueous solution reached 7.6 mM (for MeOctIm BF_4) and 4.6 mM (for MeOctIm OTf) six hours after the IL-containing reference membrane (80 mg) was brought in contact with the aqueous solution (2 mL). While use of MeOctIm OTf and MeOctIm BF_4 would not be suitable for fabrication of IL-based reference electrodes for long-term measurements or for reusable-electrode applications (due to too rapid loss of the IL from the reference membrane to the aqueous solution), they could be an alternative to MeOctIm TFSI for fabrication of single-use point-of-care reference electrodes. Tuning the structure of the ionic liquid or the matrix of the polymeric support can lower the rate of leaching of the IL from the reference membrane and lower its concentration in the aqueous phase, while maintaining a stable and sample-independent reference potential. Fig. 1 shows that increasing the lipophilicity of the cation of the IL (changing from MeOctIm to DodecMeIm) lowers the time-dependent extent of leaching and maintains a sample-independent reference potential (Fig. 3B). The emphasis of this manuscript is to raise awareness of the limited compatibility of IL-based reference electrodes and polymeric-membrane ISEs; a recent study by Lindner et al. thoroughly discusses parameters affecting the potential stability of IL-based reference electrodes [14].

Evaluating the effect of the cation and anion of the IL that is used in the IL-based reference electrode on the *emf* of the ISE is crucial for avoiding unexpected errors when these electrodes are used together for a potentiometric measurement. While the possibility of interference of the IL on the response of the ISE seems quite obvious, it could easily be overlooked in the design of IL-based reference electrodes, which is particularly problematic if measurements are performed in small volumes of sample. To demonstrate this point, we show the *emf* of a K^+ ISE (ionophore: valinomycin) measured relative to a MeOctIm TFSI-based reference electrode in solutions with different concentrations of KCl (Fig. 7). At a sample volume of 10 mL, the ISE had a Nernstian slope and behaved as theoretically expected. When the sample volume was decreased to 1 mL, the ISE no longer showed a Nernstian response due to significant interference from MeOctIm TFSI. When the measurement is performed in small volumes of sample, the concentration of the IL in the bulk will build up more rapidly than with large volumes, and, therefore, the interference of the IL on the response of the ISE will be more significant.

Conclusions

Even though IL-based reference electrodes offer several advantages over conventional reference electrodes (which contact the sample through a liquid junction), their utility for potentiometric measurements with polymeric-membrane-based ISEs has limitations. Continuous leaching of the hydrophobic IL from the reference membrane of these electrodes to the sample solution will expose the ISE to an increasing concentration of the hydrophobic components of the IL. Characterization of MeOctIm TFSI, which has been most often used in IL-based reference electrodes, showed that this IL can build up to millimolar concentrations in the aqueous phase, and can strongly interfere with the response of K^+, Na^+, and Cl^- ISEs with polymeric membranes.

Because the IL can leach out of the reference membrane over the course of the measurement, its concentration in the sample solution depends on the duration of the experiment, the volume and dimensions of the sample, and the parameters affecting mass transfer in the sample (e.g., stirring). IL-based reference electrodes should be used with caution in conjunction with polymeric-membrane ISEs. The leaching profile of the IL from the reference electrode to the sample solution should be investigated in each specific type of measurement, to ensure that the cation and anion of the IL do not interfere with the measurement of the target ion. The degree of interference will depend on the ionophore that is employed in the sensing membrane. We found that $MeOctIm^+$ was only sensed by the cation-selective ISEs, and did not interfere with the response of the Cl^- ISE. On the other hand , $TFSI^-$ interfered with the performance of both cation-selective ISEs (by causing early Donnan failure of the ISE) and anion-selective ISEs. Use of anions that are more hydrophilic than $TFSI^-$ (e.g., triflate or tetrafluoroborate) delayed the onset of Donnan failure of cation-selective ISEs to higher concentrations, but decreased the stability in the potential and lifetime of the IL-based reference electrode.

We recommend the following considerations to those who intend to use the IL-based reference electrodes for potentiometric measurements. (i) The IL-based reference electrodes are compatible (and most suitable for measurements) with ISEs that do not comprise a polymeric membrane, such as pH glass electrodes and silver halide-based ISEs. (ii) The IL-based reference electrodes are not compatible with polymeric-membrane ISEs, and exposure of the ISE to the ionic liquid in the measuring solution should be minimized. This minimization can be achieved, for example, by using samples of sufficiently large sample volumes or by using flowing samples. (iii) In measurements with small volumes of sample, the IL-based reference electrodes should be separated from the polymeric membrane-based ISE by a salt bridge comprising hydrophilic ions to avoid direct contact of the ISE to the IL and subsequent errors. While this third option does not take advantage of the characteristic IL/sample interface, it can be useful in particular to paper- or thread-based devices for its ease of fabrication.

We also recommend that in addition to demonstrating a reference potential that is stable and sample-independent, future articles that describe reference electrodes for potentiometric measurements should investigate compatibility of the reference electrode and the ISEs.

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Table 1. Formation constant of K^+ and Na^+ ionophore complexes in ISMs [39] and the selectivity of these ISEs over $MeOctIm^+$ (measured in this work).

Ionophore	$logK_f$	$logK_{A^+,\ MeOctIm}^{Pot}$
K^+-Ionophore-Free	---	2.62
K^+-Ionophore I	11.63 [39]	-1.85
K^+-Ionophore II	10.22 [39]	0.19
Na^+-Ionophore X	10.27 [39]	1.72

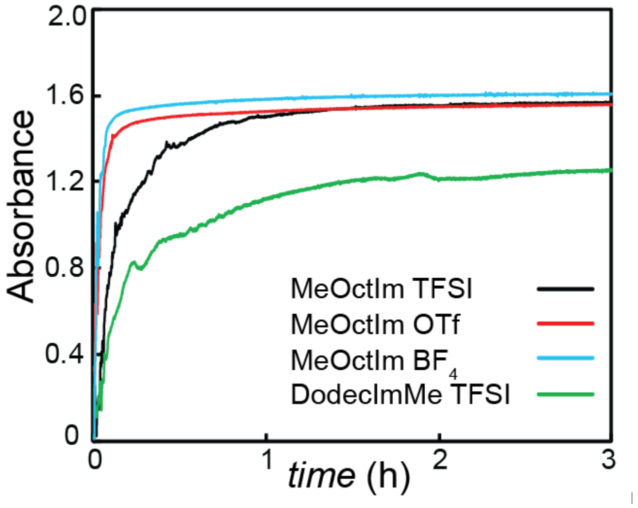

Fig. 1. Time-dependent leaching of the ionic liquid from reference membranes containing the ionic liquids MeOctIm TFSI, MeOctIm OTf, MeOctIm BF_4, and DodecMeIm TFSI into an aqueous solution containing 10 mM KCl. The plateaus correspond to 0.96 mM MeOctIm TFSI, 7.58 mM MeOctIm BF_4, 4.57 mM MeOctIm OTf, and 0.38 mM DodecMeIm TFSI.

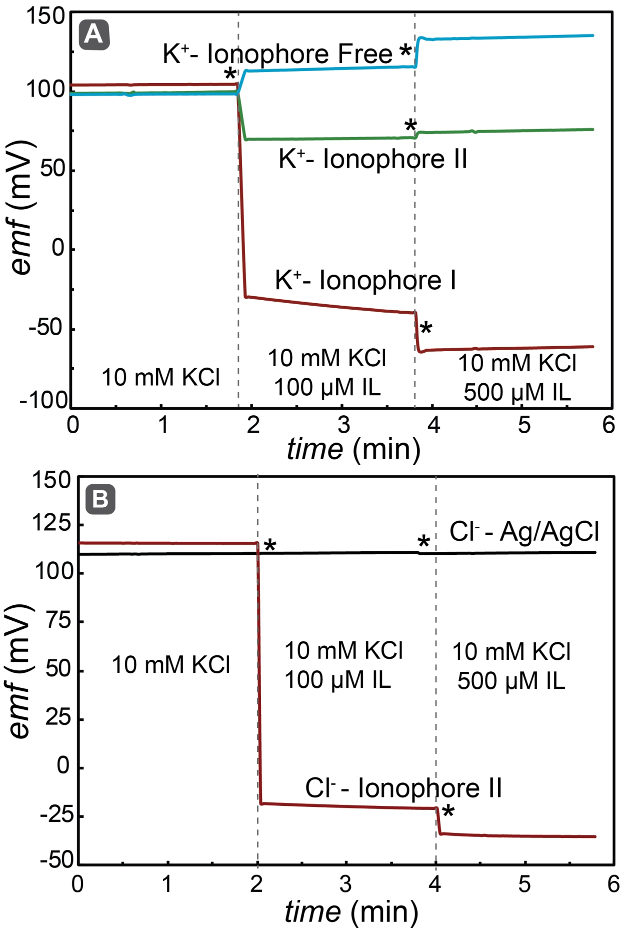

Fig. 2. The *emf* of cation- and anion-selective ISEs (panels A and B, respectively) in 10 mM KCl. Dashed lines and stars show spiking of 100 and 500 µM of MeOctIm TFSI into 10 mM KCl and highlight the corresponding *emf* changes.

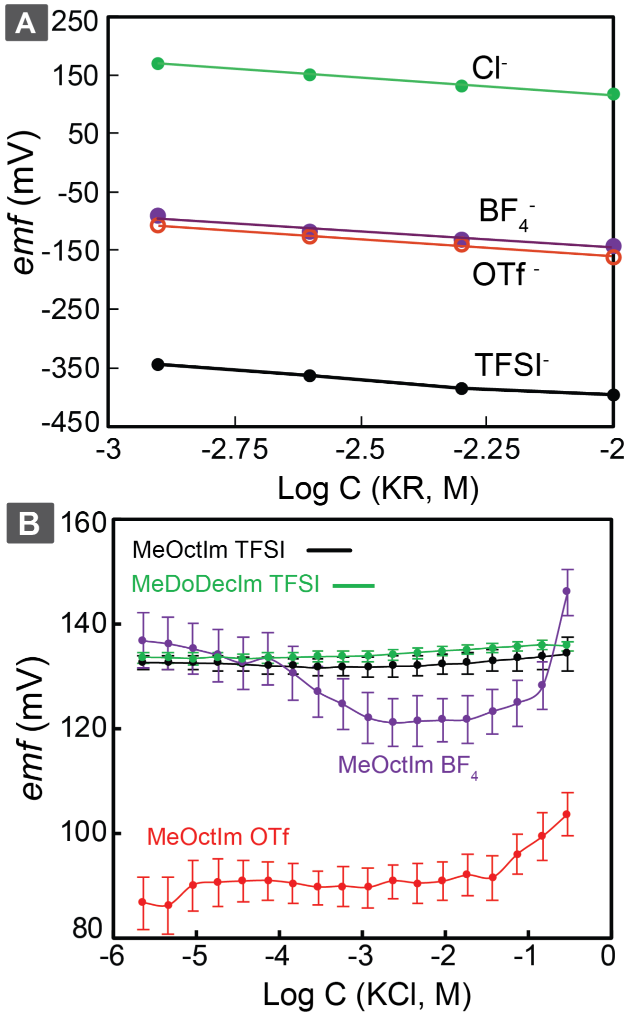

Fig. 3. Panel A shows the response of an ionophore-free anion-selective ISE to Cl^-, BF_4^-, OTf^-, and TFSI. Panel B shows the changes in the electrical potential of the IL-based reference electrodes upon changing the concentration of KCl in the measuring solution.

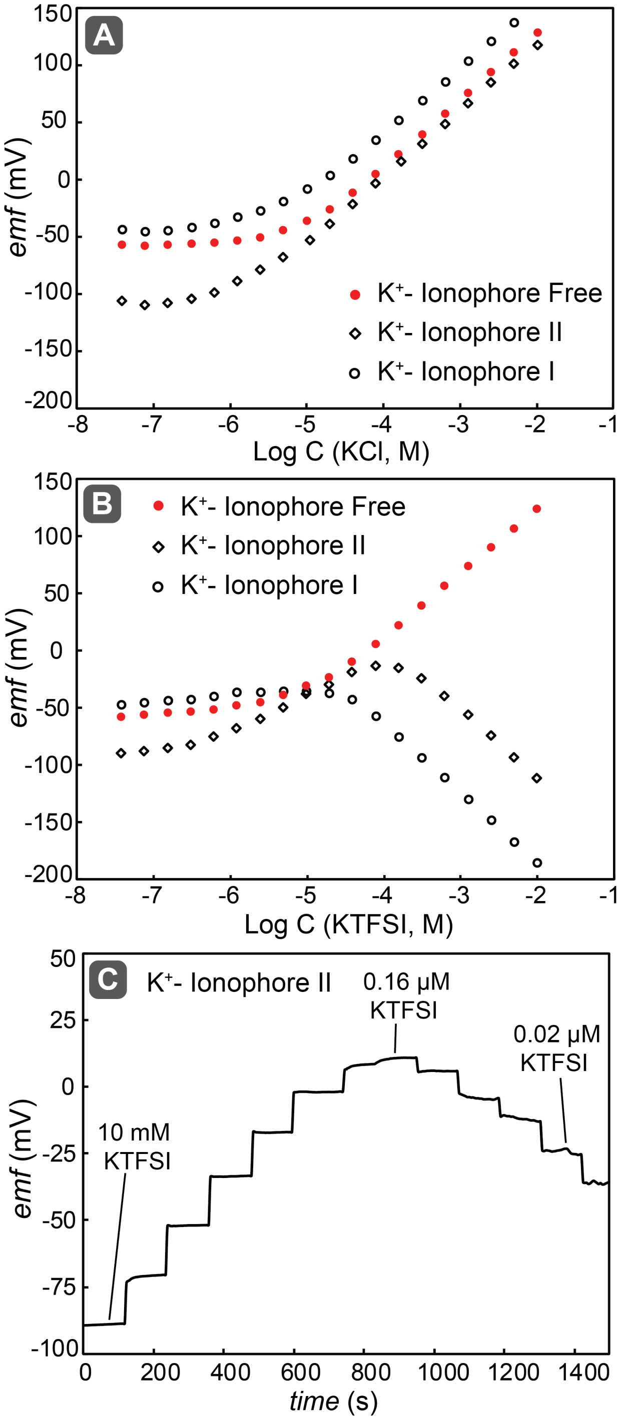

Fig. 4. Response of K^+ ISEs (with an ionophore-free sensing membrane or containing K^+-Ionophore I or K^+-Ionophore II) to KCl (A) and to KTFSI (B). C shows part of the *emf* of ISE with

K^+-Ionophore II vs. time. Calibration curves were measured by step-wise dilution of 10 mM KTFSI or KCl solutions in deionized water.

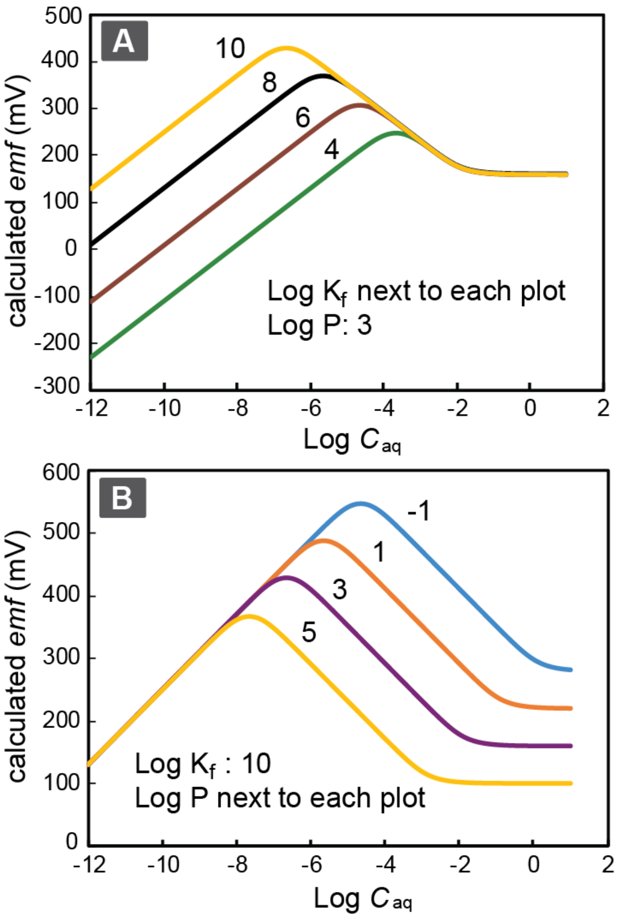

Fig. 5. The *emf* calculated for a K^+ ISE with the specified values of K_f (A) and P (B), and values of $C_{TClPB,ISM}$ of 0.001 M, $C_{t,L,ISM}$ of 0.002 M, and E° of 0 mV. (P is the partition coefficient of K^+X^- between the aqueous phase and the ISM, and K_f is the formation constant of the K^+ complex of the ionophore in the ISM.)

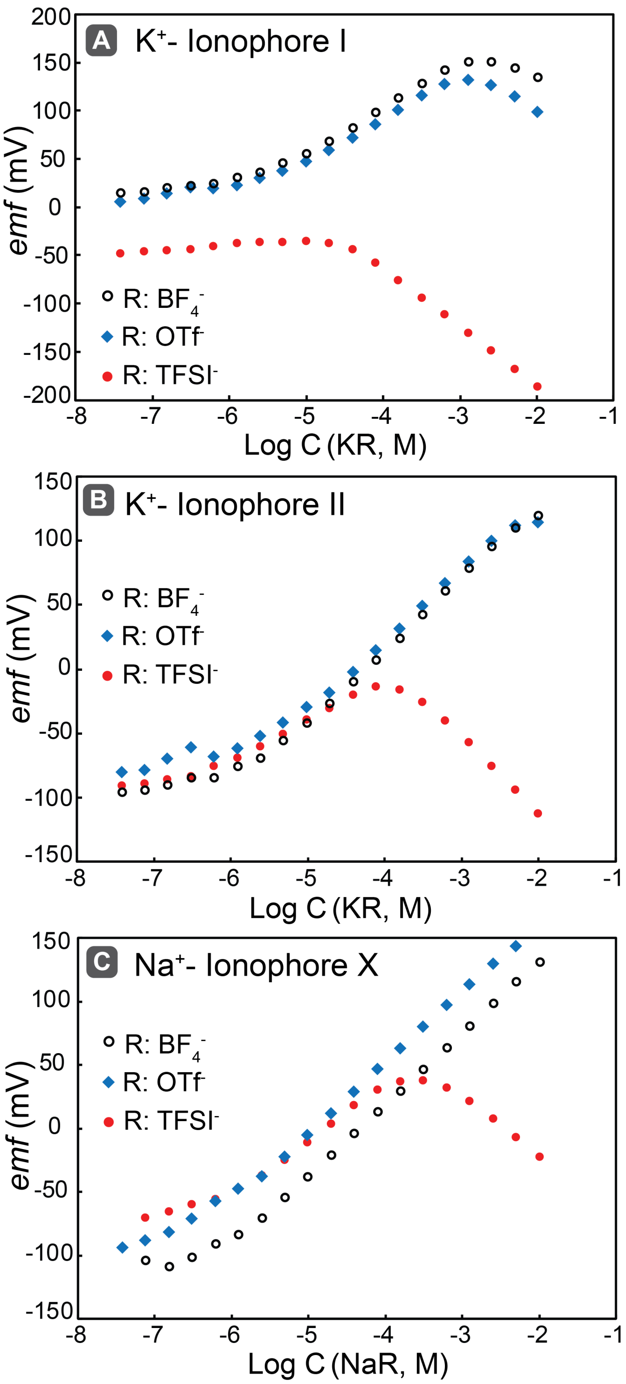

Fig. 6. Donnan failure of K^+ and Na^+ ISEs in presence of anions (R^-: OTf^-, BF_4^-, and $TFSI^-$). The Experimental Section details the names of the ionophores. Panels A, B, and C show data for K^+-Ionophore I, K^+-Ionophore II, and Na^+-Ionophore X, respectively.

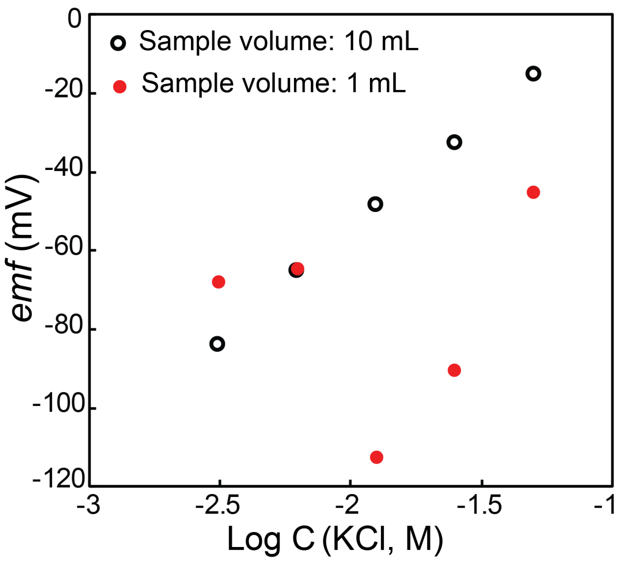

Fig. 7. The emf of a K^+ ISE (K^+ Ionophore I) measured relative to a MeOctIm TFSI-based reference electrode in aqueous solutions of KCl. The volume of sample is specified in the figure. The concentration of KCl was changed by successive dilutions. MeOctIm TFSI interferes with the response of the ISE when the volume of sample is low.