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Articles

Potentiometric Estimation of the Stability Constants of Ion−Ionophore Complexes in Ion-Selective Membranes by the Sandwich Membrane Method: Theory, Advantages, and Limitations

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Segmented sandwich membrane method of studying stoichiometry and stability constants of ion−ionophore complexes in ion-selective membranes is considered in detail. The experimental data (reported earlier in Russian) concerning complexes of various ions with valinomycin, with H^+-selective neutral ionophore hexabutyltriamidophosphate, and with anion-binding neutral ionophore *p*-hexyl trifluoroacetylbenzoate is presented in a compact form. Advantages of titration technique in the sandwich membrane method (the presence of an internal criterion of reliability, and the possibility of direct determination of complex stoichiometry coefficients) are specially addressed. Biases of the estimates of the constants caused by ion-pair formation in real membranes and by diffusion potential are analyzed by means of computer simulations. The possibility of revealing two coexisting complexes with different compositions is also discussed.

During the last three decades, a number of ion-selective electrodes (ISEs) with solvent polymeric membranes have been introduced and became a routine measuring tool in many applications.[1−5] Some different viewpoints exist toward the theoretical interpretation of the membrane electrical potential and how the mechanism of charge transfer across membranes affects the selectivity,[6−10] e.g., concerning the significance of diffusion potential and its contribution to the overall membrane effect.[1,11−13] However, a selective complexation of analyte ions by ionophores is commonly recognized as primarily responsible for the selectivity of sensors.

Paradoxically, progress in design and application of ISEs and optodes coexists with insufficient characterization of the ionophores. In the early years of ISE study, some attempts were made to measure complex stability constants in model solutions, mostly in water−ethanol mixtures.[14−17] The data obtained showed only a poor correlation with the potentiometric selectivity. More recently, a number of methods allowing the measurement of complex stability constants in situ have been invented.[18−21]

These methods suffer from two intrinsic drawbacks. First, an additional reference is required. The reference is either a chromoionophore[18] or a pH ionophore,[19,20] which supposedly does not interact with the ion of interest, or it is an ion (e.g., tetrabutylammonium), which supposedly does not interact with the ionophore under study.[21] Since the respective interactions may occur (at least to some extent), the usage of references may bias the results. Second, the complex stoichiometry has to be known or postulated beforehand or can be determined only indirectly by means of an iteration procedure.[21] In many cases, the optimization of a membrane composition relies on a difference of the complexation stoichiometry of the ligand with primary and interfering

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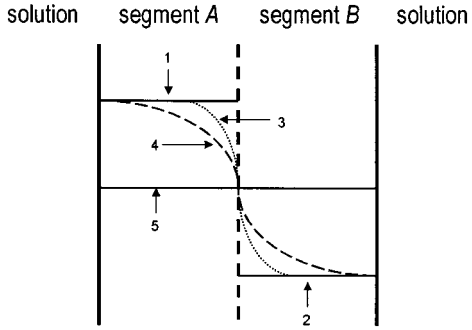

Figure 1. Schematic representation of a segmented sandwich membrane. (1) Initial high level of a neutral ionophore in segment A. (2) Initial low (or zero) level of a neutral ionophore in segment B. (3) Ionophore concentration profile with unaltered initial boundary levels: gives plateau of the EMF. (4) Ionophore concentration profile when diffusion process reaches the boundaries: EMF starts to diminish. (5) Final uniform distribution of the ionophore in the whole sandwich membrane: EMF equals zero.

ions.[1] Therefore, an exact knowledge of the complexes' composition in membranes is of utmost importance.

A different approach to measure complex stability constants in ISE membranes relies on recording electrical potential of segmented sandwich membranes.[22] The sandwich consists of two ordinary membranes attached to one another (see Figure 1). The only difference between the membranes is the ionophore content. An artificial gradient of a neutral ionophore in the segmented (sandwich) membrane dividing two identical aqueous solutions with two identical electrodes immersed (e.g., Ag/AgCl) evokes a nonzero electromotive force (EMF) in the galvanic cell. Initially, the effect was studied "as such".[22−24] Later, it was utilized to reveal the free ionophore fraction in membranes[25] and, finally, to measure stability constants of ion-to-ionophore complexes.[26−31]

Unfortunately, most of these works was published in Russian and remained almost unknown except in the former USSR. Only recently, has the method been reevaluated and successfully applied for measurements of stability constants of a number of complexes in real ISE membranes.[32,33]

This work brings together the experimental results obtained earlier,[23,26−31] and focuses on the advantages and limitations of the sandwich membrane method. Attention is paid to the importance of getting a linear domain in the sandwich membrane potential curve for the correct treatment of the data. It is also shown that the slope of the linear part of the EMF curve gives the complex stoichiometry coefficient directly. In this respect, the titration technique[26−31] discussed here is preferable to the one-sandwich technique.[32,33]

Possible biases of the estimates of the constants caused by ion-pair formation in real membranes (for any association degree) and by diffusion potential are studied by means of computer simulations.

THEORY

Basically, the EMF of a totally symmetric galvanic cell is equal to zero, and any nonzero EMF requires some asymmetry of the cell. Normally, in the case of ISEs, the asymmetry is due to a difference between the compositions of aqueous solutions on two opposite sides of the membrane. A special case addressed in this paper is an artificial asymmetry of the ISE membrane itself.

In analytical practice, one of the solutions (internal) is kept constant while another one (external) is varied. The ISE membrane is assumed uniform, which is true in most of the practically relevant cases. In practical applications, a nonuniform distribution of an ionophore in a membrane gets established when primary ions are replaced by interferences to a large extent (provided primary and interfering ions differ significantly in their affinity to the ionophore). Also, a nonuniform distribution of an ionophore appears if the electrolyte in the external solution is lipophilic or concentrated. Then a coextraction of the electrolyte may cause a strong consumption of an ionophore on the external side of the membrane. In such a case, a gradient of the ionophore appears in the initially uniform membrane, and the measured signal deviates from Nernstian behavior. For cation-selective ISEs, this phenomenon is called "anion interference".[10] In principle, the deviations provide some information upon complexation of ions by the ionophore. For instance, the position of the maximum of the calibration curve depends on the value of the extraction constant.[10,34] The extraction constant of an electrolyte comprises a multiple from the electrolyte distribution coefficient and the ion−ionophore complexation constant.[10] To get the latter separately, the cell should be asymmetric only with respect to the ionophore distribution.

In principle, an EMF signal caused by uneven distribution of a mobile ionophore species across a sandwich membrane is intrinsically unsteady. Initially, there are two flat concentration levels of the ionophore in two respective segments of the sandwich (see Figure 1), horizontal lines 1 and 2. Diffusion of the ionophore from A, the segment with a higher concentration, to B, the segment with a lower concentration, changes the initial steplike profile of the ionophore distribution. The measured EMF is steady (giving a "plateau") when boundary conditions on both sides of the sandwich membrane remain unaltered by the diffusion (see Figure 1, curve 3). When the diffusion front reaches the membrane boundaries (curve 4 in Figure 1), the EMF starts to decrease. Diffusion of the ionophore eventually levels its distribution (horizontal line 5 in Figure 1) and reduces the EMF to zero. A typical example of the respective kinetic curves obtained first by Stefanova and Suglobova[23] is presented in Figure 2.

As one can see from Figure 2, the plateau time gets increased with a decrease of the gradient of the ionophore in the membrane (except for curve 1, which refers to a very low initial concentration

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of valinomycin). When the segments' geometry and contact area are well defined, it is possible to obtain the diffusion coefficients of the ionophore in the membrane from the kinetic curve. Data obtained for valinomycin in PVC membranes plasticized with dibutyl phthalate,[35] $D \cong 10^{-8}$ cm^2/s, agree well with the values obtained by radiotracers,[36] by chronopotentiometry,[37] and by spectropotentiometry.[38] In this way, the potentiometric effect from nonequilibrium distribution of an ionophore allows obtaining data on species transportation across ISE membranes. However, in this paper, we will concentrate on studying the equilibrium parameters: complex formation constants. From now on (in the text and in the figures), by EMF we will mean only the "plateau" values.

For the interpretation of the potentiometric effect from a nonuniform distribution of a neutral ionophore in an ISE membrane, we will use a model proposed earlier.[39]

The model describes a membrane with L neutral ionophore and R^- mobile sites, dividing two electrolyte solutions with I^+ monovalent primary ions, J^+ monovalent interference, and X^- common anion. The model assumes that the electrolytes distribute between aqueous phase and membrane phase, and an interfacial equilibrium with respect of I^+, J^+, and X^- free ions is established. In the membrane, the cations form a number of various IL_n^+ and JL_n^+ complexes with the neutral ionophore. The stoichiometry coefficient n varies from 0 (free cations) to some integer k. Cationic species can be partly associated with the anions: R^- sites and X^- anions (coextracted from the aqueous electrolyte). In the model, the boundary potential is expressed through interfacial equilibrium regarding I^+ free ions. Fluxes of all charged species are taken into consideration to obtain the diffusion component of the overall membrane potential. The latter is expressed by the equation below:[39]

$$E_m = -\frac{RT}{F}(1-2\tau)\left[\ln\frac{a_I^{in} + K_{IJ}^{in} a_J^{in}}{a_I^{ex} + K_{IJ}^{ex} a_J^{ex}} + \ln\frac{\sum_{n=0}^{k}(C_L^{in})^m K_{ILn}}{\sum_{n=0}^{k}(C_L^{ex})^m K_{ILn}}\right] - 2\frac{RT}{F}\tau \ln\frac{a_I^{in}/C_I^{in} + a_J^{in}/C_J^{in}}{a_I^{ex}/C_I^{ex} + a_J^{ex}/C_J^{ex}} \quad (1)$$

where

$$K_{IJ} = \frac{k_J}{k_I}\frac{\sum_{n=0}^{k}(C_L)^n K_{JLn}}{\sum_{n=0}^{k}(C_L)^n K_{ILn}} \quad (2)$$

Here, C_I (C_J) denotes the concentration of I^+ (J^+) free ions in the membrane, and a_I (a_J) is their activity in aqueous solution. The superscripts ex and in denote the external and the internal sides of the membrane. In eq 1, $\tau = U_-/(U_+ + U_-)$ is the relative mobility of anions in the membrane. This parameter appears in the equation because, concerning the species mobilities, Morf's assumption on two classes of ions, all cationic species and all anionic species, is utilized.[10]

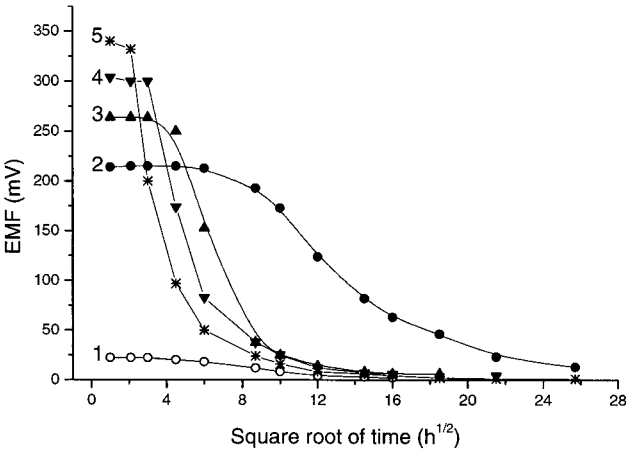

Figure 2. Kinetics of sandwich membrane potential: valinomycin in DBP.[23] C_{Val}^{tot} (mM): (1) 0.022, (2) 2.24, (3) 11.2, (4) 28.0, and (5) 270.

In eqs 1 and 2, C_L is the concentration of free ionophore molecules, K_{IL_n} and K_{JL_n} are the cumulative stability constants of complexes, and k_I and k_J − are the ionic distribution coefficients of I^+ and J^+. Their ratio is thermodynamically determined and equals the respective ratio of k_{JX} and k_{IX} electrolyte distribution coefficients between aqueous and organic phases. Equation 1 is valid for systems with ideal Donnan exclusion and with a coextraction as well. The eq 1 describes Nernstian response to I^+, and the deviations from Nernstian behavior caused by J^+, by X^-, and by possible gradients of L in the membrane. A special feature of the eq 1 is its validity for any association degree of electrolytes in the membrane. For limiting cases of full dissociation and strong association, and no neutral ionophore in the membrane, eq 1 can be reduced to respective limiting equations of Sandblom, Eisenman, and Walker theory[40] as described elsewhere.[39]

The first logarithmic term in the eq 1 is Nikolsky-like with a selectivity coefficient given by eq 2. The second term in the eq 1 represents the contribution from possible nonuniform distribution of the neutral ionophore. The effect, however, is partly reflected also by the first term: if $C_L^{in} \neq C_L^{ex}$, then K_{IJ} selectivity coefficients are different on different sides of the same membrane. In case of water-soluble neutral ionophores, distributed asymmetrically in the system, eqs 1 and 2 describe the potentiometric effect from neutral ion-binding species present in aqueous solutions. Therefore, the equations can be used to rationalize the direct potentiometric response to nonionic species, e.g., phenols and nonionic surfactants,[41−45] and an unusual selectivity exhibited by some membranes in the presence of nonionic surfactants.[46]

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Since concentrations of free species in the membrane are unknown, eqs 1 and 2 do not give an explicit description of the membrane potential. Therefore, the membrane/solution system has to be simulated numerically, as was done earlier.[12,39,47,48] The input parameters for the simulation are the above-mentioned a_I, a_J, τ, k_I, and k_J and sets of K_{IL_n}, K_{JL_n}- for n from 1 to k ($K_{IL_0} = K_{JL_0} = 1$). Other input parameters are the association constants K_{IL_nR} and K_{JL_nR}, and also C_L^{tot} and C_R^{tot}—the total concentrations of the ionophore and sites in the membrane.

The results of the simulations of selectivity as dependent on the membrane composition have been presented elsewhere.[31,39] Here, we will concentrate on the potentiometric effect from a nonuniform distribution of a neutral ionophore in a segmented sandwich membrane in contact with two identical pure solutions of electrolyte IX. In the segments, the total content of a neutral ionophore is different, while the total content of ionic additive is the same.

The sandwich membrane method of measurements of ion−ionophore complex stability constants, as described originally,[26−29] relies on a consideration of a simplified case when I^+ ions form only one sort of IL_n^+ complex with the ionophore, and these complexes are predominating (no free I^+ ions). In the membrane, R^- does not associate with IL_n^+. Under these assumptions, eq 1 gets very much reduced:

$$E_m = -\frac{RT}{F}\ln\left(\frac{1 + (C_L^{in})^n K_{IL_n}}{1 + (C_L^{ex})^n K_{IL_n}}\right) \quad (3)$$

If on one (internal) side $C_L^{in,tot} = 0$, while $(C_L^{ex})^n K_{IL_n} \gg 1$, we can obtain from (3)

$$E_m = n\frac{RT}{F}\ln C_L^{ex} + n\frac{RT}{F}\ln K_{IL_n} \quad (4)$$

According to eq 4, the EMF is linearly dependent on the free ionophore concentration in the external segment of the sandwich. If n stoichiometry coefficient is known, the free ionophore concentration can be calculated as $C_L^{ex} = C_L^{ex,tot} - nC_R^{tot}$. In membranes with large excess of neutral ionophore over sites, $C_L^{ex,tot} \gg C_R^{tot}$, and therefore, the concentration of the free ionophore approaches the total concentration, $C_L^{ex} \approx C_L^{ex,tot}$. Thus, a domain of the plot EMF versus $C_L^{ex,tot}$ has to appear, where EMF linearly depends on log $C_L^{ex,tot}$. The respective slope nRT/F gives n, the stoichiometry coefficient of the complex. In this way, variation of the ionophore concentration in a wide range (titration technique) allows one to obtain the stoichiometry of the complex without a priori knowledge. Extrapolation of the straight line to ln $C_L^{ex,tot} = 0$ provides information on the complex formation constant.

The presence of a linear domain in the EMF plot provides an internal criterion of the reliability of the sought quantities: stoichiometry coefficient and formation constant of the complex. Only in the linear domain, is one sort of complex predominating, whereas nonlinearity of the curve indicates a simultaneous presence of complexes with different compositions. Therefore, only the EMF values measured in the linear domain allow correct calculations by means of limiting eq 4 or is analogues proposed by Bakker.[32,33] For correct measurements of complex stability constants from EMF values obtained from a nonlinear part of the curve, fitting by eq 1 is needed.

EXPERIMENTAL SECTION

All the membranes consisted of poly(vinyl chloride) (PVC) S-66 (high molecular weight) from Ohtalen (St. Petersburg, Russia) and solvent plasticizers dibutyl phthalate (DBP) or dioctyl phthalate (DOP), obtained from Reahim (Moscow, Russia) and purified by vacuum rectification. Valinomycin was synthesized in Shemyakin Institute for Bio-Organic Chemistry (Moscow, Russia). Hydrogen ion-binding ionophore hexabutyltriamidophosphate (HBTAP), tetrahydrofuran (THF), and cyclohexanone (CH) were obtained from Reahim. Anion-binding neutral ligand *p*-hexyl trifluoroacetylbenzoate (HFAB) was synthesized in St. Petersburg University by Dr. V. S. Karavan, who invented this particular trifluoroacetophenone.[49] Sodium tetraphenylborate (NaTPB) was from Sigma. Tetraphenylborates of potassium and ammonium (KTPB, NH_4TPB) were obtained from aqueous solutions of NaTPB by precipitation with KCl or NH_4Cl. Potassium tetrakis(*p*-chlorophenyl)borate (KClTPB) and tetradecylammonium bromide (TDABr) were synthesized in St. Petersburg University according to known methods.[50,51] Conversion of TDABr to TDAClO_4 was performed by sequential equilibration with 0.1 M aqueous $NaClO_4$. Other tetradecylammonium salts were synthesized as described elsewhere.[52] The ratio of PVC to plasticizer was 1:3 (traditional for St. Petersburg University). Ionophore concentrations varied in wide ranges. Valinomycin membranes have been studied with and without anionic additives. Hydrogen-selective membranes with HBTAP always contained 1 mM KClTPB (molar concentrations here and below refer to the plasticizer: PVC was considered as inert matrix). Anion exchanger TDABr was always added to membranes with HFAB.

The membrane cocktails were prepared by mixing appropriate amounts of PVC, a plasticizer, an ionic additive, and an ionophore and dissolving them in freshly distilled THF. The cocktails were poured into glass Petri dishes and closed with filter paper to make THF evaporation slow. After 1−2 days, membranes with a thickness of 0.7 mm and with smooth and flat surfaces were obtained.

Disks with diameters of 14 mm were cut from master membranes and glued to PVC tubes with lengths of 4 cm. The glue was PVC solution in CH.

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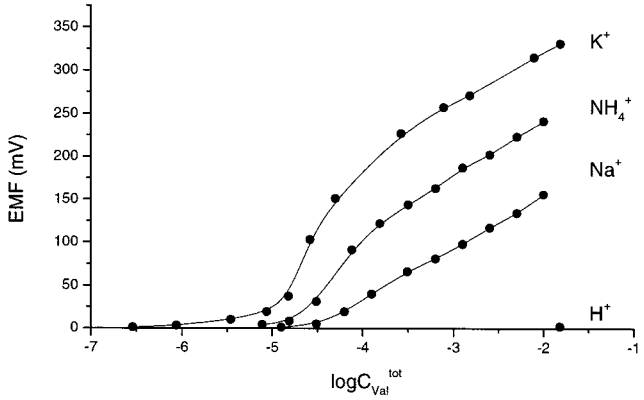

Figure 3. Sandwich membrane potential for valinomycin membranes with 7×10^{-5} M respective tetraphenylborate additive (plasticizer, DBP), equilibrated with 0.05 M chlorides.[27,28]

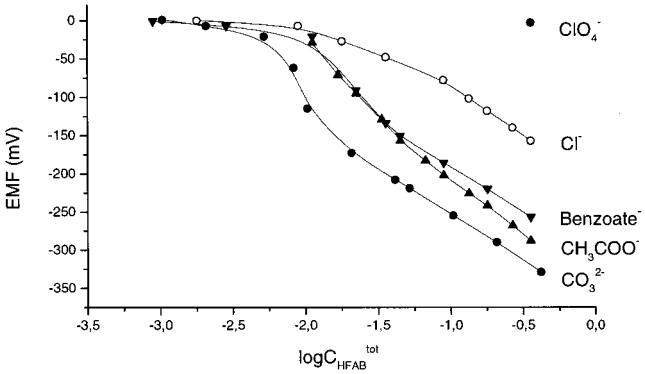

Figure 4. Sandwich membrane potential of HFAB membranes with 5×10^{-3} M $(TDA)_2CO_3$ (plasticizer, DBP) equilibrated with 0.05 M $NaHCO_3$ and with 0.02 M TDAX equilibrated with 0.02 M for $NaClO_4$, NaCl, sodium benzoate, and $NaCH_3COO$.[26,28]

The ISEs were filled with appropriate electrolyte solutions, normally 0.01−0.05 M, and soaked in the same solutions for 3−5 days. After that, the ISEs were checked by ordinary calibration, refilled with fresh portions of the same electrolyte, and kept for additional soaking under the same conditions for 1−2 weeks. Then, E_a asymmetry potentials were measured, and ISEs with E_a less than ±2 mV were wiped with filter paper and used for sandwich membrane experiments. When cation-selective membranes were studied, the internal solution was a respective chloride and an Ag|AgCl electrode was the internal reference. For anion-selective membranes, saturated Ag|AgCl electrodes connected with the internal solution by a flexible narrow bridge were utilized.

Sandwich membranes were obtained by attaching two ISEs (membrane to membrane) with special mechanical fixers. In the sandwiches, one of the membranes always contained the same low concentration of a neutral ionophore (or no ionophore), and in the other membrane, the concentration of the ionophore varied in a wide range. The time delay between attaching the membranes and recording the first EMF values did not exceed 5 min. The EMF was recorded for several days, sometimes for weeks.

Each given experiment was repeated 3−5 times. The standard deviations of the EMF values were ±5−10 mV for membranes without ionic additives and ±2−4 mV for membranes with additives.

RESULTS AND DISCUSSION

Valinomycin-based sandwich membranes with DBP as a plasticizer, and doped with respective tetra(phenyl)borates, KTPB, NaTPB, and NH_4TPB (always 7×10^{-5} M), were studied after equilibration with KCl, NaCl, and NH_4Cl.[27,28] The measured EMFs versus concentration of valinomycin are presented in Figure 3. The curves can be divided into three domains. In the first domain, only a small fraction of ions is complexed by valinomycin since TPB^- is present in excess. Therefore, the slope is very low. The second domain refers to a super-Nernstian response to total concentration of the ionophore. It happens when C_L^{tot} is close to C_R^{tot} and, respectively, $dC_L/dC_L^{tot} > 1$ and $dC_I/dC_L^{tot} < -1$, as in a titration procedure. The third domain is linear with a slope of 55−59 mV, indicating 1:1 complexation. The values of complex stability constants were obtained from the linear domain of the curve: log K_{KV} = 7.5, log K_{NaV} = 4.4, and log K_{NH_4V} = 5.7. Selectivity coefficients of membranes without neutral ionophore provide information upon the ratios of ionic distribution coefficients: k_J/k_I. The respective data for membranes plasticized with alkyl phthalates[53] are $\log K_{KNa}^{pot} = -0.9$ and $\log K_{KNH_4}^{pot} = -0.5$. Combinations of these values, $\log((k_{Na}/k_K)(K_{NaV}/K_{KV})) = -4.0$ and $\log((k_{NH_4}/k_K)(K_{NH_4V}/K_{KV})) = -2.3$, agree well with known experimental selectivity coefficients. There was no potentiometric effect recorded in the case of HCl, indicating the lack of complexation of H^+ by valinomycin.[28]

Complexation of H^+ by neutral ionophore HBTAP in membranes plasticized with DOP has been studied in the same way. Formation of complexes $H[HBTAP]_2^+$ with log K_{HL_2} = 8.5 has been demonstrated.[30]

Anion complexation by HFAB was studied in membranes equilibrated with $NaHCO_3$, $NaCH_3COO$, sodium benzoate, NaCl, and $NaClO_4$.[26,28] Being a strong Lewis acid, HFAB is more selective to anions of high basisity than *p*-butyltrifluoroacetylbenzene, which was used in early carbonate electrodes.[54] Carbonate membranes were doped with 5×10^{-3} M $(TDA)_2CO_3$. Other anion-selective membranes included respective tetradecylammonium salts in 2×10^{-2} M concentration. The results obtained are presented in Figure 4. In 0.05 M $NaHCO_3$ solutions, the EMF curve was linear in the log C_{HFAB} range from −1.6 to −0.4, with the slope −117 mV and the intercept point −370 mV. These data were interpreted as an indication of the presence of complexes $CO_3[HFAB]_4^{2-}$ with log K_{CO_3HFAB} = 12.8.[26] However, if one assumes that the complexed anion is actually HCO_3^-, the same data suggest a 1:2 complexation (which is more reliable[55]) with log $K_{HCO_3}^{HFAB}$ = 6.4. Double-Nernstian slopes obtained for monovalent anions were interpreted as indicating complexes $X[HFAB]_2^-$. The data obtained suggest no complexation of ClO_4^- by HFAB. The lack of complexation of H^+ by valinomycin and that of ClO_4^- by HFAB is important in view of the two-ionophore method.[19,20] The summary of the results of studies[26−28,30] is presented in Table 1.

The sensitivity of the valinomycin sandwich membrane potential to the ionic additive content in membranes is shown in Figure 5. The figure presents experimental data[27] (scattering) and results

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Table 1. Stoichiometry and Complex Stability Constants Obtained by Sandwich Membrane Method (Summary of Data[26−28,31])

L ionophore	plasticizer	I^+ (X^-) ion	complex	log K_{IL_n} (log K_{XL_n})	ref
valinomycin	DBP	K^+	IL^+	7.5	27, 28
	DBP	NH_4^+	IL^+	5.7	28
	DBP	Na^+	IL^+	4.4	28
	DBP	H^+			28
HBTAP	DOP	H^+	IL_2^+	8.5	31
HFAB	DBP	CO_3^{2-}	XL_4^-	12.8	26, 28
	DBP	CH_3COO^-	XL_2^-	5.9	28
	DBP	benzoate$^-$	XL_2^-	5.3	28
	DBP	Cl^-	XL_2^-	3.3	28
	DBP	ClO_4^-			28

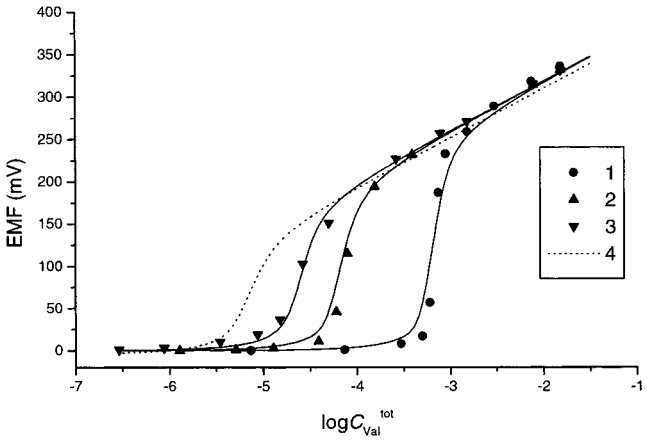

Figure 5. Sandwich membrane potential of valinomycin-based membranes doped with KTPB and equilibrated with 0.05 M KCl: experimental data[27] (scattering) and simulation by eq 1 (lines). (1) 7×10^{-4} M KTPB, (2) 7×10^{-5} M KTPB, (3) 7×10^{-6} M KTPB, and (4) simulation results for 7×10^{-6} M KTPB without a correction for impurities. For input parameters of simulations, see Table 2.

of newly performed simulations by means of eq 1 (lines). The input parameters of the simulations are summarized in Table 2. The data obtained from membranes with relatively high content of KTPB, 7×10^{-4} and 7×10^{-5} M, suggest a kind of inflection point, where $C_{Val}^{tot} \approx C_{KTPB}^{tot}$. This seems natural, since the curves reflect a titration of potassium with valinomycin. In this respect, curve 3 obtained from a membrane with lower content of the additive—7×10^{-6} M KTPB—looks shifted: it should be located similar to the simulated curve 4. Most probably, the shift is caused by the presence of some anionic impurities. Assuming that the inflection point gives the total amount of all kind of anionic sites, we estimated the concentration of the impurities as 1.8×10^{-5} m. This estimate is close to the known value of 6.3×10^{-5} M,[56] bearing in mind different manufacturers of PVC and other membrane components, and the ratio PVC to plasticizer, 1:3. The curve simulated for $C_R^{tot} = 2.5 \times 10^{-5}$ M—the sum of KTPB and the impurities—fits the experimental data satisfactorily. For higher content of KTPB, correction for impurities does not change the results significantly. Results presented in Figure 5 prove that eq 1 ensures a correct description of sandwich membrane potential in a wide range of ionophore concentration.

EMF of galvanic cells with sandwich membranes does not give direct information on ion pairing in membranes. However, the association between complexed ions and lipophilic additive may alter the measured effects. Two limiting cases, full dissociation and strong association of complexed ions with sites, were thoroughly discussed earlier.[32] It was shown that the ion−ionophore complex formation constant in strongly associated membranes gets biased by the square root of the ratio of the ion-pair formation constants for complexed and free cations.

Numeric simulation aided with eq 1 allows an estimation of the bias caused by association in membranes in a general way, for any degree of ion pairing. In the simulations, it was assumed that ions form IL^+ complexes in the membrane and $K_{IL_1} = 10^7$. The C_L^{tot} ionophore content in one of the two layers was 10^{-6} M, while in the other layer, C_L^{tot} was varied from 10^{-6} to 10^{-2} M. The C_R^{tot} total additive concentration was 10^{-4} M in both attached layers. The diffusion coefficients of cationic and anionic species were varied, producing $\tau = 0.25$, $\tau = 0.50$, and $\tau = 0.75$. The whole sets of the input parameters are given in Table 2. The results of the simulations are presented in Figure 6. The data simulated by general eq 1 were treated by simplified eq 4, and the results of this treatment are presented in Table 3.

It can be seen clearly that the association alters the EMF versus C_L^{tot} curves (Figure 6A), and the complex formation constant obtained experimentally will be underestimated. The logarithmic error is 0.2−0.7. These results are consistent with theoretical estimations and experimental data reported earlier.[32] Basically, complexation of I^+ ions by an ionophore changes the free content of I^+ ions and, therefore, also the boundary potential drop. Thus, the ratio of I^+ free ion concentrations on the two sides

Table 2. Input Parameters Used for Simulations by the eq 1, $k_i = 1 \times 10^{-5}$

simulated system, figure	set no.	K_{IL_1}	K_{IL_2}	K_{IR}	K_{IL_1R}	K_{IL_2R}	τ	C_R^{tot}	curve
valinomycin + KTPB in DBP, Figure 5	1	3×10^7	0	10^3	10^3	0	0.50	7×10^{-4}	1
	2	3×10^7	0	10^3	10^3	0	0.50	7×10^{-5}	2
	3	3×10^7	0	10^3	10^3	0	0.50	2.5×10^{-5}	3
	4	3×10^7	0	10^3	10^3	0	0.50	7×10^{-6}	4
dissociated and partly associated membranes, Figure 6	5	10^7	0	0	0	0	any	10^{-4}	1, 6
	6	10^7	0	10^5	10^3	0	0.75	10^{-4}	2, 5
	7	10^7	0	10^5	10^3	0	0.50	10^{-4}	3, 6
	8	10^7	0	10^5	10^3	0	0.25	10^{-4}	4, 7
membranes with coexisting IL^+ and IL_2^+ complexes, Figure 7	9	10^7	0	10^5	10^4	0	0.50	10^{-4}	1
	10	10^7	10^9	10^5	10^4	10^3	0.50	10^{-4}	2
	11	10^7	10^{10}	10^5	10^4	10^3	0.50	10^{-4}	3
	12	10^7	10^{11}	10^5	10^4	10^3	0.50	10^{-4}	4
	13	10^7	10^{10}	10^5	10^4	10^3	0.50	10^{-5}	5

Table 3. Complexes and Their Stability Constants Revealed by Processing Simulated EMF versus C_L^{tot} Curves by Means of Limiting Eq 4[a]

simulated system, figure	set no.	curve	complexes assumed	log K_{IL_n} assumed $n=1$	log K_{IL_n} assumed $n=2$	complexes revealed	log K_{IL_n} revealed $n=1$	log K_{IL_n} revealed $n=2$
dissociated and partly associated membranes, Figure 6	5	1, 6	IL^+	7.00		IL^+	7.00	
	6	2, 5	IL^+	7.00		IL^+	6.61	
	7	3, 6	IL^+	7.00		IL^+	6.32	
	8	4, 7	IL^+	7.00		IL^+	6.85	
membranes with coexisting IL^+ and IL_2^+ complexes, Figure 7	9	1	IL^+	7.00		IL^+	6.53	
	10	2	IL^+, IL_2^+	7.00	9.00	IL^+	6.20	
	11	3	IL^+, IL_2^+	7.00	10.00	IL_2^+		9.90
	12	4	IL^+, IL_2^+	7.00	11.00	IL_2^+		10.3
	13	5	IL^+, IL_2^+	7.00	10.00	IL^+, IL_2^+	6.30	10.2

[a] For the input parameters see Table 2.

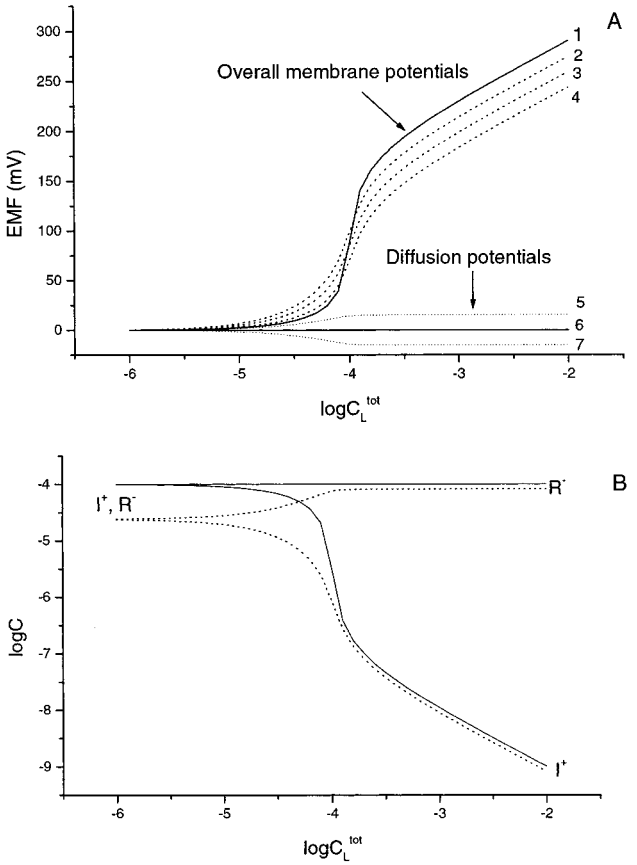

Figure 6. Simulated data on dissociated and associated sandwich membranes: (A) overall membrane potentials and diffusion potentials in dissociated (solid) and associated (dot) membranes; (B) ions and sites concentrations in dissociated (solid) and associated (dot) membranes. Curve 1, dissociated membrane; curves 2 and 5, partly associated membrane with $\tau = 0.75$; curves 3 and 6, $\tau = 0.50$; curves 4 and 7, $\tau = 0.25$. For input parameters of simulations, see Table 2.

of the sandwich membrane is most critical for the potential. In our simulations, the ion-pairing constant for the complexes was assumed substantially lower than for the free ions (see Table 2). Therefore, at high values of C_L^{tot}, the C_I gets very close to the C_I values in the dissociated membrane (although, the curves do not coincide). However, at low content of the ionophore, most of I^+ ions are associated. Thus, the net change of C_I along the increase of C_L^{tot} is significantly smaller than in the dissociated membrane.

In the frames of our model, the mobilities of all cationic species are assumed the same. Therefore, in simulated dissociated membranes (and in associated membranes with $K_{IR} = K_{IL_nR}$), diffusion potential does not exist. In associated membranes with $K_{IR} \neq K_{IL_nR}$, diffusion potential appears. Numeric simulations by eq 1 allow separate calculations of boundary and diffusion contributions to overall measured EMF. One can see that boundary potentials give the major effect; however, the input from the species diffusion also is significant. If the sites are more mobile than ions and complexes, the diffusion potential is positive and partly compensates the bias caused by ion pairing. If the sites are less mobile, the bias gets even bigger (see Figure 6A and Table 2).

These results correlate with the data obtained from membranes doped with lipophilic background electrolyte, which swamps out the diffusion potential. The reported differences between the signals from sandwiches with and without the background electrolyte are 15 mV,[29] and up to 17 mV.[32] Of coarse, these differences only partly originate from diffusion potential, reflecting also changes in dissociation degrees due to different ionic strength.

In principle, sandwich membrane potential allows resolving different complexes formed by the same ionophore, e.g., IL^+ and IL_2^+. The results of respective numerical simulations are presented in Figure 7 and in Table 3. For the first formation constant, it was always assumed $K_{IL_1} = 10^7$. Curves 1−4 have been calculated for $C_R^{tot} = 10^{-4}$ M (see Table 2 for the whole sets of parameters). Curve 1 refers to membrane without IL_2^+ complexes: $K_{IL_2} = 0$. Curve 2 was calculated for $K_{IL_2} = 10^9$; one can see that the curve is very similar to curve 1. Thus, it is hardly possible to resolve IL_2^+ complexes with relatively low K_{IL_2} experimentally. Curves 3 and 4 obtained for $K_{IL_2} = 10^{10}$ and $K_{IL_2} = 10^{11}$ clearly indicate 1:2 complexation, while IL^+ complexes would not be revealed experimentally.

However, usage of low site concentration, e.g., $C_R^{tot} = 10^{-5}$ M, favors resolving both complexes: IL^+ with $K_{IL_1} = 10^7$ and IL_2^+ with $K_{IL_2} = 10^{10}$. Two domains of the EMF versus C_L^{tot} curve were used to estimate the stoichiometry of complexation and the respective constants: domain from A to B and domain from C to D (see curve 5 in Figure 7). In the first domain, the slope was $S = 64$ mV, suggesting 1:1 complexation, and the respective intercept point gave log $K_{IL_1} = 6.3$. The constant is underestimated because of ion pairing (see above) and because of the slightly

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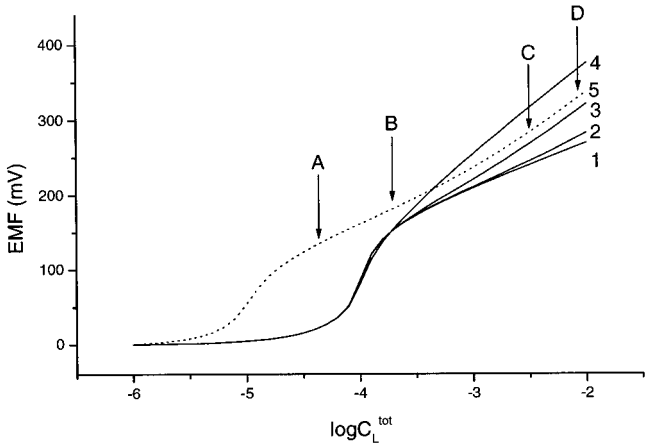

Figure 7. Sandwich membrane potential in systems where complexes IL^+ and IL_2^+ coexist. First formation constant $K_{IL_1} = 10^7$. Curves 1−4: total content of sites $C_R^{tot} = 10^{-4}$ M. Cumulative constant K_{IL_2} values: curve 1, 0; curve 2, 10^9; curve 3, 10^{10}; curve 4, 10^{11}; curve 5, $C_R^{tot} = 10^{-5}$ M, $K_{IL_2} = 10^{10}$. For input parameters of simulations, see Table 2.

super-Nernstian slope in the domain. The slope is super-Nernstian due to the impact from IL_2^+ complexes. In the second domain, the slope was $S = 108$ mV, indicating 1:2 complexation. For the cumulative constant, $\log K_{IL_2} = 10.2$ was obtained. The cumulative constant get slightly overestimated because of the sub-Nernstian slope in the domain used: $S = 108$ mV instead of $S = 116$ mV for pure 1:2 complexes.

CONCLUSIONS

As we stated in the Theory section, a nonzero EMF is always caused by some asymmetry of the galvanic cell under study. In principle, the theoretical description of systems asymmetric with respect to solutions and with respect to membranes is the same. The ordinary asymmetry with respect to solutions allows measurements of free ion activities and, therefore, also measurements of ion complexation in solutions (if total concentrations are known). The asymmetry with respect to membrane composition allows study of the interactions in membranes.

The sandwich membrane method is a powerful tool for studying complexation in real membranes. An advantage of the method, in particular, when the ionophore concentration is varied in a wide range (titration technique), is the ability to obtain the complex formation constant together with the stoichiometry of complexation. Another important advantage is the presence of the internal criterion of reliability: appearance of a linear domain in the sandwich potential curve.

According to computer simulations, it is also possible to resolve a number of complexes formed by the same ionophore. Thus, the titration technique is more informative and also more robust than measurements with only one sandwich.[32,33]

The disadvantage of the method comes from the bias in the measured stability constants of complexes, caused by ion pairing. However, the ion-pairing constants of R^- sites with complexes of different ions (for the same neutral ionophore) should be similar since the ions are normally hidden within complexes. Therefore, the ratios of formation constants obtained for different ions should be less sensitive to ion pairing than the constants themselves and can be used to try correlations between complexation in membranes and the potentiometric selectivity of ISEs.

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