ION-SELECTIVE SENSORS FOR CLINICAL ANALYSIS

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Abstract Ionophores suitable for ion-selective electrodes may be used in a variety of optode systems as well. Such optodes that ideally measure products or ratios of ion activities may be used in main frame clinical analyzers for an assay of ion concentrations after dilution of the specimen with an adequate pH buffer. Such optodes have been described for assays of Cl^- , Na^+ , K^+ and total Ca^{2+}

Key words ion-selective electrodes, optodes, optrodes, clinical

INTRODUCTION

Chemical sensors for ions have to meet several strict requirements simultaneously to be used as integrated devices in clinical chemical analyzers. The most critical parameters are: selectivity, sensitivity, life time, response time and ruggedness against matrix effects. At present potentiometric sensors (ion-selective electrodes (ISEs)) are predominantly in use. They are based on glass membranes (for H_3O^+ and Na^+) on classical ion exchanger membranes (for Cl^-) or on ionophore based membranes (Li^+ , Na^+ , K^+ , Ca^{2+} , Mg^{2+}) [1,2]

Potentiometric sensors (ISEs)

Membrane-active ion-complexing and -transporting agents such as mobile, lipophilic ion carriers (ionophores), especially electrically neutral ones, have been widely used in ion-selective membrane electrodes for potentiometric determinations of cation or anion activities. The first representatives have been macrocyclic molecules but nonmacrocyclic ionophores may exhibit even superior membrane properties. They induce a membrane potential response which is related to the equilibrium distribution of the selected ions between the membrane and the contacting aqueous solutions. Ideally, such membrane electrode half-cells therefore respond reversibly to single-ion activities according to the Nernst equation (Fig. 1) [3,4] It was shown for valinomycin-containing plasticized PVC membranes that only a small percentage of the carriers is usually complexed. Furthermore, the concentration of cation/carrier complexes in such membranes appears to be roughly constant and predetermined by the content of anionic sites R^- existing in the polymeric material (see e.g. E. Pungor, R.P. Buck et al. ref. 22 to 26 in [3])

Potentiometric sensors based on neutral carriers have been described which perfectly meet the requirements mentioned for an extracellular measurement of Na^+ , K^+ and Ca^{2+} Although there were many efforts to design ionophores for Mg^{2+} [5] and Li^+ [6] there still remains significant interference by Ca^{2+} and Na^+ respectively which has to be taken care of by measuring these ions simultaneously and correcting for their contribution to the sensor signal. ISE membrane technology progressed so far, that electrically symmetric membranes with adequate selectivity, sufficient ruggedness against

Fig. 1 Neutral carrier based potentiometric sensor (ISE)

S: neutral carrier for sample cation M^+ ,
 R^- : anionic site, X^- : sample anion

matrix effects and theoretical response may be obtained. Such membranes have been used successfully for measurements of Na^+ and K^+ in undiluted sera without any previous sensor calibration (so called *ab initio* measurement) [7,8]. This technology offers a new tool for standardization and quality control.

Optical sensors (optodes, optrodes)

Recently we introduced a concept for an optical assay of ion activities which is based on chromoionophores and fluoroionophores in bulk membranes [3,9]. These chromo- and fluoroionophores drastically change their optical properties in the ultraviolet and visible region upon complexation. For any sensor system using an ionophore-based bulk membrane it must a priori be taken into account that sample dependent variations of complex formation in the electroneutral membrane phase can be realized only by the following two processes:

- 1) Simultaneous coextraction of cations and anions or extraction of electrically neutral ionogenic species from the sample into the membrane, and/or
- 2) Simultaneous ion-exchange of cations or of anions (or of corresponding ionogenic species) between the sample and the membrane

Fig. 2 Coextraction optode based on two neutral carriers

S: neutral ionophore for sample anion X^- ,
 P: neutral chromo-/fluoroionophore for H^+

For case 1) an ideally working membrane optode can thus at best respond to products of cation and anion activities in the sample (i.e., to salt activities or activities of neutral ionogenic species), whereas for case 2) the response can at best be related to ratios of cation or anion activities. This is in clear contrast to the response behavior of an analogous ion-selective membrane electrode responding to single ion activities.

Systems realized so far are:

- 1a) Combination of a cation-selective and an anion-selective neutral carrier in a membrane (coextraction; cotransport) of which at least one is a chromo- or fluoroionophore (see e.g. Fig. 2),
- 1b) Combination of charged cation and charged anion selective carriers or combination of a charged carrier with an ion exchanger of opposite charge (see e.g. Fig. 3) optionally combined with a neutral carrier (see e.g. Fig. 4) of which at least one carrier is a chromo- or fluoroionophore (coextraction; cotransport)

Fig. 3 Coextraction optode based on two charged lipophilic species

P^- : deprotonated chromo-/fluoroionophore for H^+

R^+ : exchanger for sample anions X^-

SENSOR RESPONSE $\log(a_{X^-} \cdot a_{H^+})$

Fig. 4 Coextraction optode based on two charged, lipophilic species and a neutral ionophore

R^+ : cationic site, S: neutral carrier for sample anion X^- , P^- : deprotonated chromo-/fluoroionophore for H^+

SENSOR RESPONSE : $\log(a_{X^-} \cdot a_{H^+})$

- 2a) Combination of a cation-selective neutral carrier with a negatively charged ligand or cation exchanger in a membrane (and vice versa for anion-exchanger systems) of which at least one is a chromo- or fluoroionophore (see e.g. Fig. 5, ion exchange, countertransport)
- 2b) Combination of two different cation-selective neutral carriers in a membrane with negatively charged sites (and vice versa for anion-exchanger systems) of which carriers at least one is a chromo- or fluoroionophore (see Fig. 6, ion exchange; countertransport)

The response of the optodes of type 1 and 2 (absorbance/ fluorescence vs the logarithm of ratios or products of activities) is sigmoidal. In contrast to ISEs, the linear response range is short and typically covers only 1 to 2 orders of magnitude in activity [3,9,10]. The range may however been shifted on the activity scale by several orders of magnitude depending on the stability constants of ionophores and chromo-/fluoroionophores as well as on the pH of the sample solution [3,9,10]

Although the sensor response is given by ratios or products of activities (Fig. 2-6), ideally single ion activity changes may be determined e.g. by fixing one of the involved activities utilizing buffer systems. If the specimen pretreatment by means of buffers keeps the ionic strength of the final sample sufficiently constant, assays of analyte concentrations become possible. This concept has been used for a clinically relevant assay of Na^+ [11], K^+ [12], Cl^- [13] and total Ca^{2+} [14] by diluting serum samples with adequate pH buffers. In the optode sensor systems realized so far, the selectivity exhibited by the ionophores used is comparable to the selectivity induced in ISE membranes.

Since both anion and cation concentrations can be measured optically in diluted serum samples with the technology described here, we believe to offer a possibility to eliminate electrochemical sensors in some main frame analysers and to replace them by an optical read-out. This will bring an optical read-out for all analytes including the electrolytes.

Fig. 5 Ion exchange optode based on a neutral and a charged carrier

S: neutral carrier for sample cation M^+
 P^- : deprotonated chromo-/fluoroionophore for H^+

SENSOR RESPONSE : $\log \left(\frac{a_{\text{M}^+}}{a_{\text{H}^+}} \right)$

Fig. 6 Ion exchange optode based on two neutral carriers and a lipophilic charged species

S: neutral carrier for sample cation M^+

P: deprotonated chromo-/fluoroionophore for H^+

R^- : anionic site

SENSOR RESPONSE $\log\left(\frac{a_{M^+}}{a_{H^+}}\right)$

EXPERIMENTAL

All measurements on optodes have been made in the transmission mode as described earlier [15,16]. Of course [3,9,10] fluorescence studies are an alternative as corroborated by Wolfbeis et al. on some of our optode systems [17]. For measurements on ion-selective electrodes see [18-20] and references in there.

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REFERENCES

1. M.E. Collison and M.E. Meyerhoff, *Anal.Chem.*, **62**, 425A (1990)
2. J. Wang, *Electroanalytical Techniques in Clinical Chemistry and Laboratory Medicine*, VCH, Weinheim, 1988.
3. W.E. Morf, K. Seiler, B. Lehmann, Ch. Behringer, K. Hartman and W. Simon, *Pure Appl. Chem.*, **61**, 1613 (1989)
4. D. Ammann, W.E. Morf, P. Anker, P.C. Meier, E. Pretsch and W. Simon, *Ion-Sel. Electrode Rev.*, **5**, 3 (1983)
5. U.E. Spichiger, R. Eugster, E. Haase, G. Rumpf, P. Gehrig, A. Schmid, B. Rusterholz and W. Simon, *Critical parameters and optimisation of a magnesium-selective liquid membrane electrode for application on human blood serum*, *Fresenius J. Anal. Chem.*, in press (1991)
6. R. Kataký, P.E. Nicholson, D. Parker and A.K. Covington, *Analyst*, **116**, 135 (1991)
7. H.W. Bühler, G.G. Rumpf, L.F.J. Dürselen, U.E. Spichiger and W. Simon, *Methodology and Clinical Applications of Ion-Selective Electrodes*, Eds. A.H.J. Maas, B.M. Buckley, A. Manzoni, R.F. Moran, O. Siggaard-Andersen and R. Sprokholz, Elinkwijk, Utrecht, 1989, p 315.
8. G.G. Rumpf, L.F.J. Dürselen, H.W. Bühler and W. Simon, *Contemporary Electroanalytical Chemistry*, Eds. A. Ivaska, A. Lewenstam, R. Sara, Plenum Press, New York, 1990, p 305.

9. W.E. Morf, K. Seiler, B. Lehmann, Ch. Behringer, S. Tan, K. Hartman, P.R. Sørensen and W. Simon, *Ion-Selective Electrodes*, 5, Ed. E. Pungor Pergamon Press, Oxford, New York, Akadémiai Kiadó, Budapest, 1989, p 115.
10. W.E. Morf, K. Seiler, P.R. Sørensen and W. Simon, *Ion-Selective Electrodes*, 5, Ed. E. Pungor, Pergamon Press, Oxford, New York; Akadémiai Kiadó, Budapest, 1989, p 141.
11. K. Seiler, K. Wang, E. Bakker, W.E. Morf, B. Rusterholz, U.E. Spichiger and W. Simon, *Characterization of Sodium-Selective Optode Membranes Based on Neutral Ionophores and Assay of Sodium in Plasma*, *Clin. Chem.*, in press (1991)
12. K. Wang, K. Seiler, W.E. Morf, U.E. Spichiger, W. Simon, E. Lindner and E. Pungor, *Anal. Sci.*, 6, 715 (1990).
13. S. Tan, P.C. Hauser, K. Wang, K. Fluri, K. Seiler, B. Rusterholz, G. Suter, M. Krüttli, U.E. Spichiger and W. Simon, *Anal. Chim. Acta* (1991) in press.
14. K. Seiler, R. Eugster, W.E. Morf, K. Wang, M. Czösz, B. Rusterholz, W. Simon and U.E. Spichiger, *Fresenius J. Anal. Chem.*, 337, 109 (1990)
15. W.E. Morf, K. Seiler, B. Rusterholz and W. Simon, *Anal. Chem.* 62, 738 (1990)
16. S.S.S. Tan, P.C. Hauser, N.A. Chaniotakis, G. Suter and W. Simon, *Chimia*, 43, 257 (1989)
17. O.S. Wolfbeis. *Proceedings of the International Congress on Optical Science & Engineering*. SPIE Publications Bellingham, Washington Vol. 1510 (1991)
18. U. Oesch, D. Ammann and W. Simon, *Clin. Chem.*, 32, 1448 (1986)
19. T.P. Byrne, *Select. Elect. Rev.*, 10, 107 (1988)
20. J. Koryta, *Anal. Chim. Acta*, 233, 1 (1990)