ANALYTICAL SCIENCES FEBRUARY 1998, VOL. 14

Multilayer Potassium Sensor Based on Solid-State Coextraction

Christian KRAUSE, Tobias WERNER† and Otto S. WOLFBEIS

University of Regensburg, Institute of Analytical Chemistry, Chemo and Biosensors, 93040 Regensburg, Germany

A highly selective solid state multilayer sensor is presented which enables fluorescence optical determination of potassium ions. It is based on co-extraction using valinomycin along with a fluorescent anion, both contained in different layers. The sensor membrane is composed of two layers of highly different lipophilicity. The lipophilic phase contains the carrier valinomycin whilst the hydrophilic phase contains the anionic fluorophore sulforhodamin B. On exposure to potassium ions, the lipophilic phase becomes fluorescent after coextraction of both potassium ions and the anionic fluorophore. To prevent leaching of the dye, an additional blocking layer (nafion) was spread on the sensor. The dynamic range is from 0.1 to 50 mmol/l K^+ with very low cross-sensitivity to pH and ionic strength. The response times vary from 1 to 2 h.

Keywords Potassium, optical solid state sensor, valinomycin, coextraction

Potassium analysis in blood is of tremendous clinical interest. Conventional analytical methods like flame spectroscopy are time consuming and require skilled personnel.[1] The use of potassium electrodes, based on plasticized PVC membranes containing valinomycin is most common[2], however potassium optical sensors ("optodes") have gained growing interest due to the ease of handling and the availability of disposable test strips.[3]

Practically all optodes for alkali ions are based on molecular recognition. This is accomplished *via* natural ionophores like valinomycin or by artificial ionophores such as crown ethers.[4–6] Crown ethers can be coupled to chromophores and the resulting chromo-ionophores undergo a change in their optical properties on complexation of the analyte.[7,8] A range of such probes has been developed, including (a), protic dyes that change their pK_a significantly due to specific interaction with the analyte[9–14], (b), probes that undergo internal charge transfer;[7,15] and (c), probes based on photo-induced electron transfer.[16,17] Unfortunately, none of these chromo-ionophores can be used in a test strip or sensor application for detection of potassium in clinically relevant concentration ranges which are 1 to 10 mmol/l for K^+ and 120 – 150 mmol/l for Na^+. This is due to the cross-sensitivity to pH in the case of protic dyes and the cross sensitivity to other alkali ions in the case of non-protic dyes. Hence, some of the approaches require buffered and diluted solutions, while others have inadequate sensitivity and selectivity.[18–23]

Valinomycin shows extremely high selectivity for potassium over other alkali ions, along with sufficiently high sensitivity.[24] Except for optical rotation, it does not change its optical properties due to ion complexation. Therefore, the detection must be indirect. Three approaches can be distinguished, all based on lipohilic membranes containing valinomycin. The need of electroneutrality during extraction of K^+ requires either an ion exchange or the coextraction of an anion. If this is energetically unfavorable, a potential is created at the interface. Ion exchange[25,26], potential sensitive dye membranes[27–29] as well as coextraction[30,31] have been applied to potassium sensing.

Coextraction-based ion selective membranes (ISM) make use of a potassium carrier in a hydrophobic membrane. Potassium is extracted into the hydrophobic membrane along with a lipophilic anion in order to maintain electroneutrality. If a colored anion is coextracted, potassium ion can be measured *via* the absorbance or fluorescence of the anion. This concept was introduced by Nakahara.[31] Charlton introduced a dry chemistry scheme by using plasticized PVC instead of a liquid lipophilic phase.[30] This concept still needed the addition of one reagent. In this paper we report on the first completely solid-state sensor for potassium based on co-extraction.

Experimental

Chemicals and solutions

Analytical grade solvents, poly(vinyl chloride) (PVC high molecular weight), dioctyl sebacate (DOS), valinomycin, sulforhodamin B monosodium salt (SRB) and Nafion 117 were obtained from Fluka (Buchs, Switzerland). Polyester foil (*Mylar*, 125 μm) was from Goodfellow (Cambridge Ltd., UK). Phosphate buffers were used throughout. All buffer compositions were

† To whom correspondence should be addressed.

adjusted to constant ionic strength with NaCl according to the literature.[33] The total buffer concentration (*i.e.* $[HPO_4^{2-}] + [H_2PO_4^-]$) was 20 mmol/l. Buffers and inorganic salts were of analytical grade (Merck, Darmstadt, Germany). Water was double distilled.

Apparatus

All optical measurements were performed on a Aminco AB2 fluorometer (SLM Aminco, Rochester, NY). The membranes were placed in a self-made flow-through cell and fluorescence was measured in an arrangement shown in Fig.1. The flow-through cell was machined from Teflon and had a volume of about 900 µl. A Minipuls 3 pump (from Gilson, Villiers-le-Bel, France) was used, the typical flow was about 1 ml/min. All tubes consisted of silicone. pH was measured using a WTW 638 pH meter (WTW GmbH, Weilheim, Germany). Experiments were performed at 22±1˚C.

Membrane preparation

Membranes were prepared by spreading cocktails (see below) onto polyester foil using a home-made knife coating device to obtain a defined layer thickness on the transparent polyester foil. A first cocktail was prepared by dissolving 60 mg PVC, 180 mg DOS and 0.25 mg valinomycin ($b_{(valinomycin)}$=6 mmol/kg matrix material) in 3 ml THF. From the quantity applied, the resulting layer thickness was calculated to be 1.5 µm.

A second cocktail was prepared by dissolving 70 mg of a polyurethane based hydrogel[32] and 250 µg SRB in a mixture of 600 µl of ethanol and 100 µl of water. After addition of 70 mg of carbon black and 1 h of ultrasonication, the cocktail was diluted with 400 µl of water and ultrasonicated for another hour. The resulting dry layer was calculated to have a thickness of about 5 µm.

An ethanol solution containing 5% Nafion 117 was diluted forty-fold with water and immediately used. The layer thickness was calculated to be less then 0.1 µm. Each layer was allowed to dry for at least 3 h before the next layer was added. Prolonged drying times had no effects on the results. Each membrane was conditioned in potassium-free buffer (ionic strength 140 mmol/l; pH 7.4) for at least 12 h before use. All time traces and $K_{1/2}$ values (which is the point of inflection in the response curve) are for freshly conditioned membranes. All measurements were carried out at room temperature.

Calculation of membrane parameters

$K_{1/2}$ values were extracted from the point of inflection of calibration curves using a Boltzmann fit. They were averaged from data of at least three different membranes. Errors are given as standard deviations. Membrane thickness was calculated by assuming density of all cocktail components to be 1 kg/l.

Results

Sensing mechanism

Plasticized PVC layers containing valinomycin are capable of co-extracting potassium along with a lipophilic anion from an aqueous phase into an organic (polymeric) phase[30] according to

$$K^+_{aq} + V_{org} + A^-_{aq} \rightleftharpoons [KV]^+_{org} + A^-_{org} \quad (1)$$

where V is a lipophilic, neutral cation carrier (valinomycin), and A^- is the most lipophilic anion contained in the aqueous phase.

If the anion A^- is fluorescent, the potassium concentration can be measured *via* the increase in the fluorescence of the PVC layer.[30] In order to obtain a solid state device (with no need to work with an additional reagent) we have placed the anion (SRB) in a black hydrophilic layer (III in Fig. 2) on top of the PVC layer (II in Fig. 2). A Nafion blocking layer (IV in Fig. 2) was spread on top to prevent leaching of the anionic SRB while allowing potassium ions to diffuse through the Nafion into the hydrogel. Potassium ions are co-extracted along with SRB anions into the PVC/valinomycin layer. A cross-section of the membranes and the diffusional mechanisms involved are given in Fig. 2.

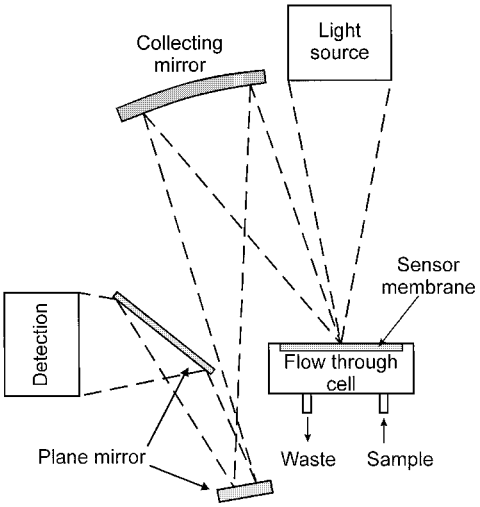

Fig. 1 Schematic view of the optical arrangement.

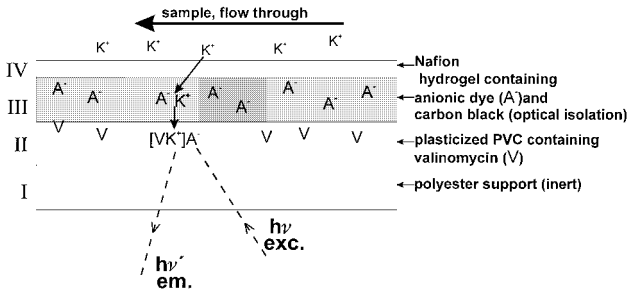

Fig. 2 Cross-section of the solid state composite sensor.

Choice of materials

The hydrophobic layer (II in Fig. 2) was prepared from plasticized PVC. This material is most common for ion-selective sensors. The fraction of plasticizer was kept high (75%w/w) to obtain fast response. Valinomycin is a highly selective and easily accessible potassium carrier and therefore the preferred ionophore.

The fluorophore to be co-extracted along with potassium ions must be anionic and highly mobile in the layer. Sulforhodamin B monosodium salt (SRB) was chosen as the fluorescent anion because it is highly fluorescent and mobile in hydrophilic phases. Furthermore, it does not change its fluorescence properties or solubility with pH between 5.0 and 9.0.

The polymer layer containing the SRB (III in Fig. 2) needs to be ion-permeable and hydrophilic, and the dye is expected to be highly mobile in this phase. Polyurethane-based hydrogels were found to be most suitable polymers in being hydrophilic, soluble in water/ethanol mixtures and adhering well on PVC (which is in contrast to the adhesion of polyacrylamide hydrogels).

Because SRB also fluoresces in polyurethane (thus giving strong background signal), the hydrophilic layer was optically isolated from the sensing PVC layer so that background luminescence of layer III (Fig. 2) is not excited at all. Optical isolation of the hydrophilic membrane was accomplished by blackening it with carbon black. Carbon black is a good optical isolator and can be homogeneously dispersed in the viscous hydrogel cocktail by ultrasonication.

Because SRB is hydrophilic, an additional layer was added to prevent leaching of the dye. Nafion, a high molecular weight and strongly acidic cation exchanger was used. Nafion is known to possess a high permselectivity, which minimizes anion transport through the layer[34,35] and therefore completely blocks leaching of the anionic dye. In contrast, diffusion of cations through nafion is mainly based on ion exchange.

Response

After conditioning the membrane in potassium-free buffer, solutions of constant ionic strength and pH but varying potassium concentrations were pumped through the flow-through cell where the sensing layer was placed. A typical time trace is shown in Fig. 3. The sensing characteristics of such composite membranes can be changed by variation of the composition of the PVC film. According to Eq. (1), an increase in the concentration of valinomycin results in lower potassium concentrations needed for the same signal change to occur. Changing the dye concentration (from 0.6 to 6 mmol/kg matrix material) or thickness of the hydrogel layer did not result in significant differences in the response curves.

The compositions given in the Experimental part proved to be most suitable for detection of potassium concentrations in the clinically relevant 1 to 10 mmol/l range.

The long response times are mostly remarkable. More then 1 h is required to obtain constant signals after changing the potassium concentration. Response times depend on both the potassium concentration and the direction of the change. Kinetic evaluation of the time trace is required in order to obtain quantitative information more rapidly. In principle, the potassium sensing membranes are reversible (Fig. 3) and therefore useful for on-line detection. However, response times on changing from high to low potassium concentration are even slower than vice versa.

Response curves were examined at different pH and ionic strength. Increasing ionic strength leads to higher $K_{1/2}$ values while $K_{1/2}$ decreases with pH. The high standard deviation reflects the poor reproducibility of the films, which at this time are made by hand. Results are summarized in Table 1 and Figs. 4 and 5. The deviation in relative signal change between different membranes do not allow making a statement on the influence of ionic strength or pH on this.

Effect of layer thickness

The thickness of the PVC layer (II in Fig. 2) is

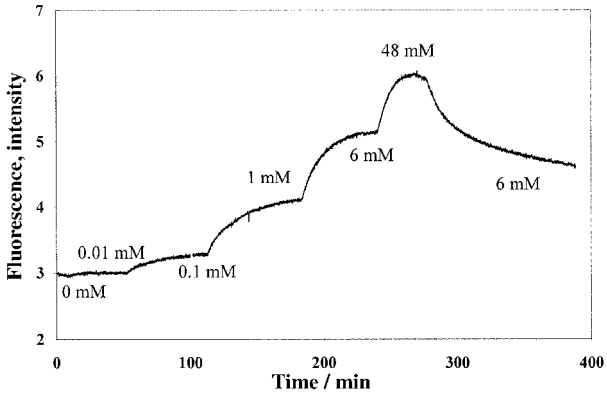

Fig. 3 Typical response curve to various concentrations of potassium chloride (in mmol/l) at constant ionic strength (140 mmol/l) and pH 7.4.

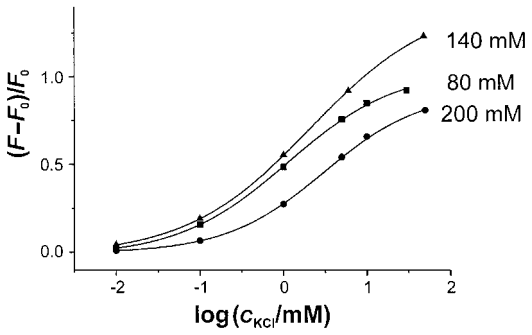

Fig. 4 Normalized calibration curves for composite membranes at varying ionic strength; F_0 is the fluorescence intensity of the membrane exposed to potassium-free buffer, and F the intensity in presence of potassium ions.

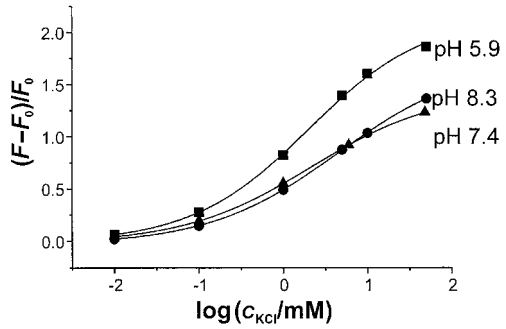

Fig. 5 Normalized calibration curves for composite membranes at varying pH; F_0 is the fluorescence intensity of the membrane exposed to potassium-free buffer, and F the intensity in presence of potassium ions.

supposed to affect the characteristics of response. Membranes with PVC layers of d<1 μm did not respond to potassium ions any longer. On the other side, layers thicker than 5 μm display small relative signal change. Layers of 1.5 μm proved to be adequate. The thickness of the hydrogel layer was varied too, but did not result in significant changes in the sensor characteristics. Nafion layers as thick as 1 μm completely inhibit response to potassium ions, but 0.1 μm layers are adequate to minimize leaching of the dye and still allow diffusion of potassium ions.

Discussion

The sensor presented here is suitable for detection of potassium ions over the clinically important range (1 to 10 mmol/l) and against a high (>100 mmol/l) background of sodium ion. Interferences of pH and ionic strength are low, but not negligible. We assume the Nafion coating to be the main reason for this cross sensitivity. Transport processes through Nafion membranes are mainly based on ion exchange mechanisms[35] and consequently depend on pH and ionic strength. On exposing the membrane to potassium ions, sodium ions leave the membrane and potassium ions will enter. Therefore, the ratio between sodium and potassium in both the sample solution and the Nafion and hydrogel layers determine response. Hence, higher ionic strength (and thus higher sodium concentrations) should lead to larger $K_{1/2}$ values. Indeed, this effect was observed (see Table 1). In our case, leakage of alkali ions and chloride ions in the thin (< 0.1 μm) Nafion layer must be considered as well. This flux depends on ionic strength. Furthermore, hydrogels change their swelling properties due to changes in ionic strength, and may so lead to different response characteristics as well. Another critical parameter is pH. Number and size of leaks in the Nafion layer as well as ion exchange and the flux rate of co/counterion are pH-dependent. Therefore, a decrease in pH leads to a decrease in $K_{1/2}$ (see Table 1).

Table 1 Effect of pH and ionic strength on sensor characteristics

pH	$K_{1/2}$/mmol[a]	Ionic strength	$K_{1/2}$/mmol[a]
5.9	1.5±0.7	80 mmol	1.1±0.3
7.4	2.9±1.14	145 mmol	2.9±1.1
8.3	4.48±1.64	200 mmol	4.4±1.0

a. Data averaged from at least three measurements; errors are standard deviations.

The finding that the dye concentration has no effect on the response curve appears unusual but can be attributed to the fact that the dye concentration in the gel is always in the same order than the amount of dye that is coextracted into the hydrophobic layer. In addition, the contribution of the anionic dye within the hydrogel might be a reason for this result. If the concentration of sulforhodamin B in the gel is increased, dye-gel or dye-carbon black interactions are more likely. In other words, if the anion concentration is elevated, the anionic dye gets adsorbed, whereas the amount of free anionic dye (a player in Eq. (1)) remaining constant.

The unusually long response times are unlikely to be due to the limited diffusion of the potassium/valinomycin/dye ternary complex in the PVC layer. Due to their permselectivity, Nafion membranes allow cation exchange only. Therefore, concentration gradients will not lead to considerable fluxes through the membrane. However, a slow flux is possible in this case due to possible leaks in the extremely thin nafion layer. This flux is supposed to be the rate limiting step.

The solid state co-extraction membranes presented here offer the possibility to continuously measure potassium in the clinical relevant range with low cross sensitivity to pH or sodium and without the need of additional reagents to be added to the sample. However, response times are very long and limit the applicability of such sensors to certain areas.

The authors wish to thank Dr. Marc Leiner (AVL-List GmbH, Graz) for fruitful discussions and Hannelore Brunner for perfect technical assistance. Ch. Krause thanks the AVL-List GmbH for financial support.

References

1. I. Gibb, *J. Clin. Pathol.*, **40**, 298 (1987).
2. E. Vaillo and U. E. Spichiger, *SPIE*, **2631**, pp. 127 – 135, 1996.
3. D. J. Cram, *Angew. Chem.*, **98**, 1041 (1986).
4. R. H. Ng, K. M. Sparks and B. E. Statland, *Clin. Chem.*, **38**, 1371 (1992).
5. J. M. Lehn, "*Supramolecular Chemistry Concepts and Perspectives*", Chap. 2, VCH, Weinheim, 1995.
6. Y. Takeda, *Topics Curr. Chem.*, **121**, 1 (1984).
7. M. Takagi and K. Ueno, *Topics Curr. Chem.*, **121**, 39 (1984).
8. J. P. Dix and F. Vögtle, *Chem. Ber.*, **114**, 638 (1981).

9. H. Nakamura, M. Takagi and K. Ueno, *Anal. Chem*., **52**, 1668 (1980).
10. H. Yamamoto, K. Ueda, K. R. A. Samankumara Sandanayake and S. Shinkai, *Chem. Lett*., **1995**, 497.
11. K. Toth, B. T. Thu Lan, J. Jeney, M. Horvath, I. Bitter, A. Grün, B. Agai and L. Töke, *Talanta*, **41**, 1040 (1994).
12. A. F. Sholl and O. Sutherland, *Chem. Soc. Chem. Commun*., **1992**, 1717.
13. A. M. King, Ch. P. Moore, K. R. A. Samankumara Sandanayake and I. A. Sutherland, *Chem. Soc. Chem. Commun*., **1992**, 582.
14. A. Mason and I. O. Sutherland, *Chem. Soc. Chem. Commun*., **1994**, 1131.
15. B. Valeur, J. Bourson and J. Pouget, *ACS Symp. Ser*., **538**, 25 (1993).
16. L. Fabbrizzi, M. Licchelli, P. Pallavicini, A. Perotti, A. Taglietti and D. Sacchi, *Chem. Eur. J*., **2**, 75 (1996).
17. A. Prassana de Silva and K. R. A. Samankumara Sandanayake, *Tetrahedron Lett*., **32**, 421 (1991).
18. D. Lee and J. D. R. Thomas, *Talanta*, **42**, 901 (1994).
19. S. M. S. Al-Amir, D. C. Ashworth, R. Narayanaywamy and R. E. Moss, *Talanta*, **36**, 645 (1989).
20. F. Alava-Moreno, R. Peireiro-Garcia, M. E. Diaz-Garcia and A. Sanz-Medel, *Sensors Actuators B*, **11**, 413 (1993).
21. R. P. Garcia, F. A. Moreno, M. E. Diaz Garcia, A. Sanz-Medel, R. Narayanaswamy, *Clin. Chim. Acta*, **207**, 31 (1992).
22. M. E. Diaz-Garcia, F. Alava-Moreno and A. Sanz-Medel, *Mikrochim. Acta*, **113**, 211 (1994).
23. J. F. Alder, D. C. Ashworth and R. Narayanaswamy, *Analyst* [London], **112**, 1191 (1987).
24. Yu. A. Ovchinnikova and V. T. Ivanov, "*The Cyclic Peptides. Structure, Conformation and Function*", ed. H. Neurath, R. L. Hill p. 399, Academic Press, New York, 1992.
25. K. Suzuki, H. Ohzora, K. Thoda, K. Miyazaki, K. Watanabe, H. Inoue and T. Shirai, *Anal. Chim. Acta*, **237**, 155 (1990).
26. K. Wang, K. Seiler, W. E. Morf, U. E. Spichiger, W. Simon, E. Lindner and E. Pungor, *Anal. Sci*., **6**, 715 (1990).
27. O. S. Wolfbeis, *Sensors Actuators B*, **29**, 140 (1995).
28. Y. Kawabata, K.-I. Yasunaga, T. Imasaka and N. Ishibashi, *Anal. Sci*., **7** (supplement), 1465 (1991).
29. Y. Kawabata, R. Tahara, T. Kamichika, T. Imasaka and N. Ishibashi, *Anal. Chem*., **62**, 528 (1990).
30. S. C. Charlton, R. L. Fleming and A. Zipp, *Clin. Chem*., **28**, 1857 (1982).
31. H. Sumiyoshi and K. Nakahara, *Talanta*, **24**, 763 (1977).
32. I. Oehme, B. Prokes, I. Murkovic, T. Werner, I. Klimant and O. S. Wolfbeis, *Fresenius' J. Anal. Chem*., **350**, 563 (1994).
33. D. D. Perrin and B. Dempsey, "*Buffers for pH and Metal Ion Control*", Chapman and Hall Laboratory Manuals, London, 1974.
34. J. Weber, P. Janda, L. Kavan and A. Jegorov, *J. Electroanal. Chem*., **200**, 379 (1986).
35. M. Lopez, B. Kipling and H. L. Yaeger, *Anal. Chem*., **49**, 629 (1977).

(Received November 20, 1997)
(Accepted December 19, 1997)