

Ion-sensitive field effect transistor as a monovalent cation detector for ion chromatography and its application to the measurement of Na⁺ and K⁺ concentrations in serum

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ABSTRACT

An ion-sensitive field effect transistor, which is a small potentiometric ion-sensing probe, was applied as a detector in the ion chromatography of alkali-metal cations. The cation-sensing transistor was prepared by coating the gate part of the probe to form a poly(vinyl chloride) matrix membrane containing tetranactin, which enables detection of alkali-metal ions such as Na⁺, K⁺, Rb⁺, and Cs⁺. To be able to analyse amounts less than 1 μl and make full use of the small ion-sensing probe, we constructed a miniature cation-exchange separation column (support 10 μm polystyrene) with a PTFE tube (20 mm × 1.5 mm I.D. × 2.1 mm O.D.). As an application of the system, Na⁺ and K⁺ concentrations in serum were determined. The analytical results for these two cations were in good agreement with those obtained by flame photometry.

INTRODUCTION

The ion-selective electrode is an excellent probe that enables selective determinations of specific ions, but so far few of them have been developed to have outstanding selectivity for a single ion. Many ion-selective electrodes, however, have high sensitivity towards several ions. Using this feature, several investigations have been performed on the application of the ion-selective electrode as a potentiometric detector for ion chromatography [1–16]. Unlike conductivity or electrochemical detectors based on amperometry, which are commonly used for ion chromatography, the response of a potentiometric detector using an ion-selective electrode does not depend on the surface area of the electrode. Thus the electrode for the detector can be made very small.

Here we report the preparation and characteristics of a small potentiometric detector for ion chromatography to be used for the determination of alkali-metal

cations in a small sample size (less than 1 μl) using a miniature potentiometric ion-sensing probe, an ion-sensitive field effect transistor (ISFET), based on a tetranactin-containing cation-sensing poly(vinyl chloride) (PVC) matrix membrane. A miniature cation-exchange separation column suitable for small amounts of samples was also prepared to assist the most effective use of the small detection probe. As an application of this ion chromatographic system, Na^+ and K^+ concentrations in human serum were determined, and compared with those determined by flame photometry.

EXPERIMENTAL

Reagents

Alkali-metal chlorides, used for test-cation sample solutions, and nitric acid, used as the eluent for the ion chromatography, were the highest grade commercially available reagents. Deionized water used for diluting the reagents had a resistivity of more than $1.5 \cdot 10^7 \Omega \text{ cm}$. The PVC (p-9526, high molecular type) used for the ion-sensing electrode membrane was obtained from Sigma (St. Louis, MO, U.S.A.) and the dibenzyl ether used as the membrane solvent (plasticizer) was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Tetranactin, which was used as the cation-sensing material, was supplied from Chugai Chemical (Tokyo, Japan). The serum sample was Normal Control Serum obtained from Ortho Diagnostics (Raritan, NJ, U.S.A.).

Ion chromatography apparatus

The structure of the ISFET used as the alkali-metal cation detector is shown in Fig. 1A. The base pH-sensitive ISFET probe (Kuraray, Osaka, Japan) was placed in a PVC tube, and the tip of the probe including the gate part was covered with a PVC matrix ion-sensing membrane composed of 3% (w/w) tetranactin, 0.5% (w/w) tetrakis(*p*-chlorophenyl)borate, 68.5% (w/w) dibenzyl ether and 28% (w/w) PVC. These membrane materials were dissolved in ten times their weight of tetrahydrofuran (THF). This solution was coated onto the ISFET tip, including the ion-sensing gate part. The ion-sensing PVC matrix membrane was allowed to dry for 12 h at room temperature. The membrane thickness was *ca.* 50 nm. Fig. 1B shows a cross-section of the ion chromatograph using the ISFET probe, a small reference electrode, and a miniature ion-separation column. The 1 mm O.D. reference electrode (Kuraray) used an Ag/AgCl wire and a saturated KCl bridge solution. The column was a PTFE tube (2.1 mm O.D., 1.5 mm I.D.). Cation-exchange resin particles (Shodex IC Y-521 gel, 10 μm polystyrene support, Showa Denko, Tokyo, Japan) in a slurry form with deionized water were used to fill the PTFE tube at a hydrostatic pressure of 20 mmHg generated by an aspirator. Small amounts of PTFE wool were packed into both ends of the column to prevent the resins from spilling out. A PTFE membrane filter (bore size 5–10 μm) was attached at the outlet of the column. Two- and three-way PTFE

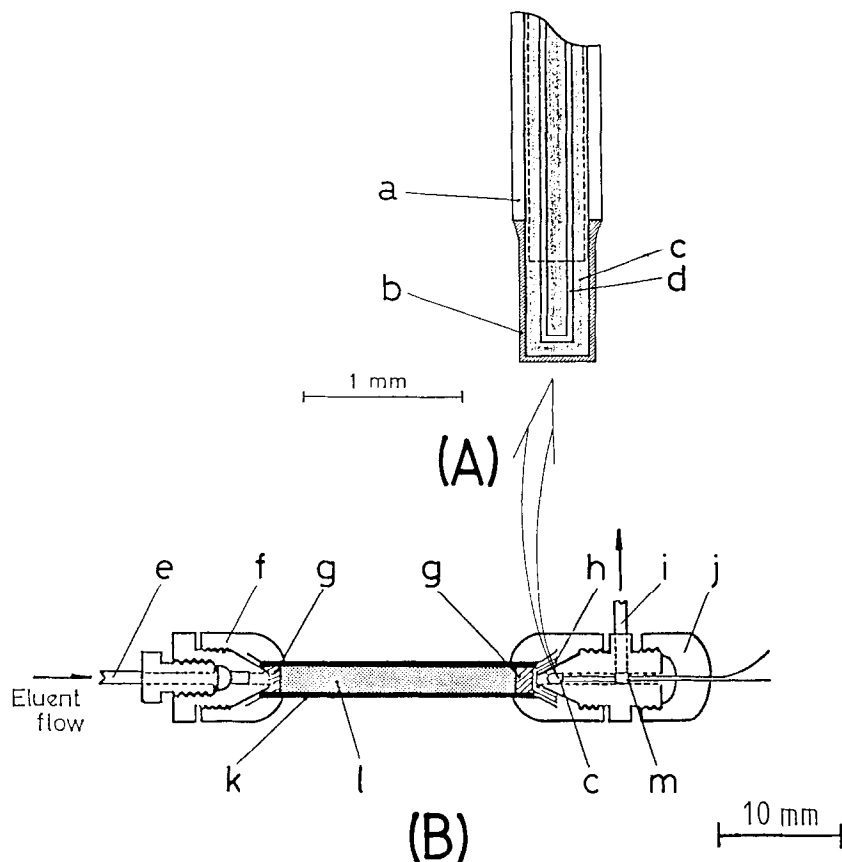


Fig. 1. Schematic representation of the ISFET detector for alkali-metal cations (A) and the ion chromatographic apparatus (B): a = PVC tube; b = PVC matrix cation-sensing membrane; c = ISFET probe; d = ion-sensing gate section; e = flow liquid inlet; f = two-way PTFE joint; g = PTFE wool; h = PTFE filter; i = flow liquid outlet; j = three-way PTFE joint; k = PTFE tube (20 mm \times 1.5 mm I.D., 2.1 mm O.D.); l = cation-exchange resin (particle diameter 10 μ m); m = reference electrode.

joints (Sanplatec, Osaka, Japan) were used at both ends of the column. The ISFET was placed in the foremost position to the flowing route, behind which a reference electrode was placed. The ISFET was positioned as close as possible to the outlet of the column. The volume of the detection section was less than 1 μ l.

The ion chromatography system consisted of a so-called 'single column system', which was composed of an eluent container (polyethylene), a liquid delivery pump (Tri-Rotor V, Jasco, Tokyo, Japan), a guard column (Y-521P, Showa Denko), a micro-injector (Model 7520, Rheodyne, Cotati, CA, U.S.A.), a laboratory made cation-exchange separation column and a detector using the ISFET. The guard column was placed between the pump and the injector, to remove small particles and polyvalent cations from in the eluent. The response of

the ISFET was detected with an ISFET monitor (Digital pH/CO₂ Monitor, Kuraray), in which an ion monitor with an anti-logarithmic converter (IM-20E, TOA Electronics, Tokyo, Japan) was connected to the signal output (mV) of the ISFET monitor.

The human serum samples were filtered through a 0.45- μ m disposable filter (Ekicrodisk 13CR, Gelman Sciences Japan, Tokyo, Japan) and diluted ten-fold with the eluent. The injected volume for the measurement of Na⁺ and K⁺ concentrations was 1 μ l. These two cations were also determined with a flame photometer (FIP-3M, Kotaki Seisakusyo, Tokyo, Japan). A conductivity detector (Shodex IC-2, Showa Denko) was used to confirm cation separation by the prepared column. Nitric acid (pH 2.5) was used as the eluent, at a flow-rate of 50–100 μ l/min.

RESULTS AND DISCUSSION

A miniature separation column was prepared in order to make the best use of the small ISFET probe. The column was made of PTFE tubing of 1.5 mm I.D., a size that is minimally affected by the so-called 'extent of the wall region' [17]. The four different lengths were used, 40, 35, 20 and 10 mm and filled with the cation-exchange resin (10 μ m diameter). Fig. 2A shows the relation between $R_{s(\text{NaK})}$ (resolution of Na⁺ and K⁺ response peaks in chromatogram) [17] and column length that was obtained with a conductivity detector by introducing a 1- μ l mix-

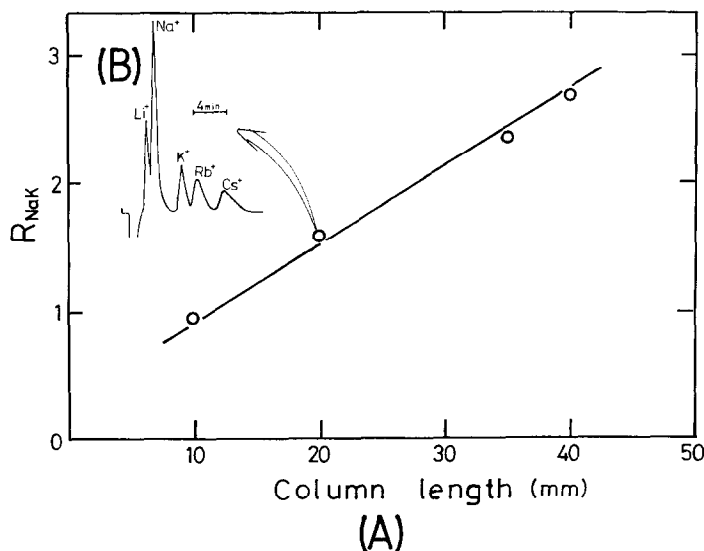


Fig. 2. Relation between $R_{s(\text{NaK})}$ (resolution of Na⁺ and K⁺ peaks in chromatogram) and column length with a bore of 1.5 mm (A), and a typical cation chromatogram obtained with a 20-mm column (B) with conductivity detection (injection volume 1 μ l).

ture of five different alkali-metal ions (Li⁺, Na⁺, K⁺, Rb⁺ and Cs⁺) in an equal concentrations ($1 \cdot 10^{-3} M$; flow-rate, 50 $\mu\text{l}/\text{min}$). The $R_{s(\text{NaK})}$ value increased with increasing column length. As shown in Fig. 2B, Na⁺ and K⁺ were completely separated with the 20-mm column ($R_{s(\text{NaK})} = 1.58$; complete separation: $R_{s(\text{NaK})} \geq 1.5$). Thus, the 20-mm column was used for all subsequent measurements. The pressure limit for the PTFE column was *ca.* 40 kg/cm². For the 20-mm column, the observed pressure was 8–9 kg/cm² with the ion chromatographic system at a flow-rate of 50 $\mu\text{l}/\text{min}$ of nitric acid (pH 2.5). Therefore, the maximum flow-rate can be up to *ca.* 250 $\mu\text{l}/\text{min}$ ($\approx 40 \text{ kg}/\text{cm}^2$).

The life of the column depends, of course, on the kinds of test sample, but it proved durable for at least two weeks if used for the measurements of alkali-metal ion standard samples for only 5–6 h per day. However, it showed poorer separation after being used for three to four weeks.

Fig. 3A shows typical chromatograms obtained with the ISFET detector based on tetraoctylammonium for the five different alkali-metal ions. No response to Li⁺ was shown by this detector, whereas the determination of Na⁺, K⁺, Rb⁺ and Cs⁺ was feasible. Fig. 4 shows typical calibration curves for the ions Na⁺, K⁺, and Rb⁺ with the potentiometric detector. The detection limits of Na⁺ and K⁺ for 1- μl sample injection were $1 \cdot 10^{-4}$ and $1 \cdot 10^{-5} M$, respectively, which corresponded to 2.3 ng of Na⁺ and 0.39 ng of K⁺.

For the response of an ion-selective electrode, the relation between the loga-

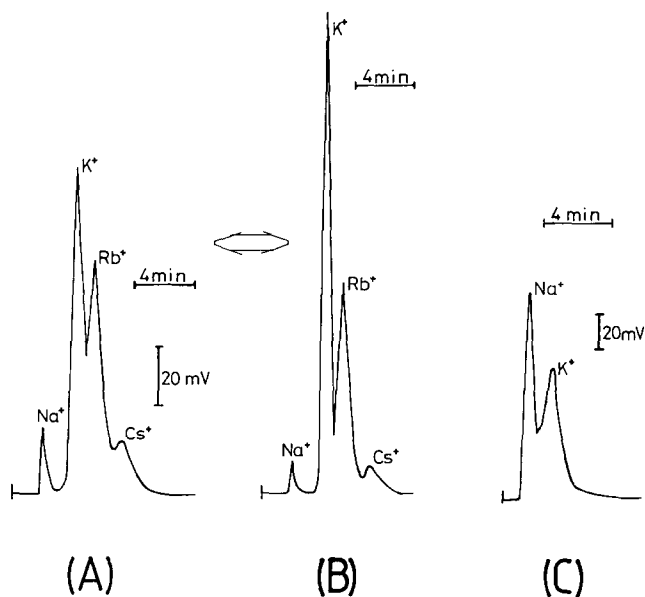


Fig. 3. (a) Typical alkali-metal cation chromatogram (normal response) and (B), its anti-logarithmic conversion drawing, obtained with a $1 \cdot 10^{-3} M$ (1- μl) injection for each alkali-metal cation (Li⁺, Na⁺, K⁺, Rb⁺ and Cs⁺). (C) Typical cation chromatogram (normal response) for a human serum sample.

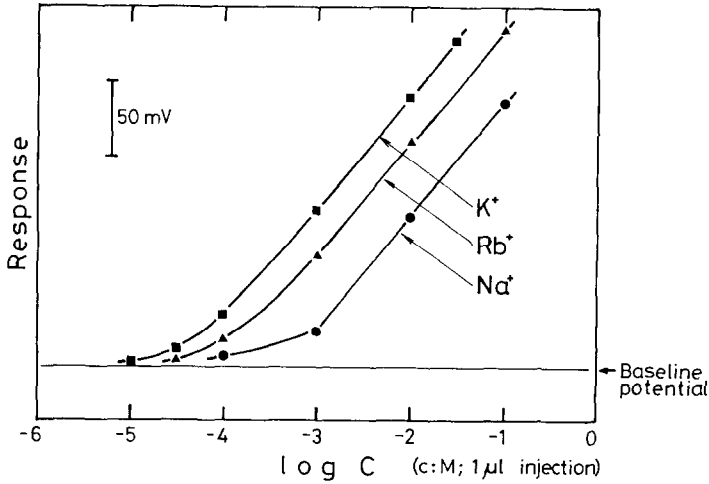


Fig. 4. Typical calibration curves for alkali-metal cations (Na^+ , K^+ and Rb^+) obtained with the ISFET detector.

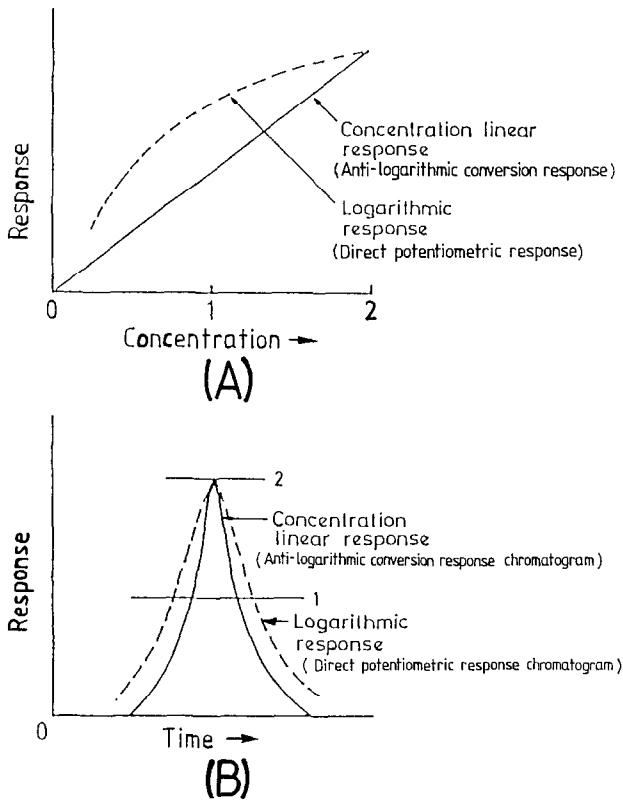


Fig. 5. Comparison of the response profiles with respect to concentration (A) and chromatographic peak shape (B) of the ISFET detector. Dashed lines indicate normal response (see eqn. 1) and solid lines indicate anti-logarithmic conversion response (see eqn. 2). Arbitrary units for all axes.

rhythmic ion activity for the ion i ($\ln a_i$) and the response potential (E_i) generally becomes linear in accordance with the Nernst equation (eqn. 1):

$$E_i = E_i^0 + (RT/nF) \ln a_i \quad (1)$$

where E_i^0 is the standard electrode potential for the ion i , R is the gas constant, T is the absolute temperature, n is the charge of the ion i and F is the Faraday constant. If the response potential is subjected to anti-logarithmic conversion, the relation between the ion activity, a_i , and the response potential (E_i^*) becomes linear as in conductivity detection and in eqn. 2:

$$E_i^* = \exp(RT/nF) a_i + c \quad (2)$$

where c is a constant. The relation of eqns. 1 and 2 is schematically shown in Figs. 5A and 5B, where dashed and solid lines indicate the response potentials E_i and E_i^* , respectively. Using the relation between the ion concentration and the resulting response (Fig. 5A), the peak shape in the chromatogram can be drawn as shown in Fig. 5B. The peak obtained with an anti-logarithmic converter is sharper than that obtained without such equipment. Fig. 3B shows an ion chromatogram for alkali-metal cations with anti-logarithmic conversion of the response from the ISFET detector. Though the separation of the peak in the chromatogram of Fig. 3A seems insufficient, the separation was actually satisfactory, as shown in Fig. 3B.

As an example of an application for the described system, the content of Na⁺ and K⁺ in human serum was determined. The potentiometric detector response is higher for K⁺ and lower for Na⁺. Thus, it is opposite of the balance of Na⁺ and K⁺ in serum, which is convenient for the determination of the two cations. The test sample of the serum was filtered and diluted ten-fold. A 1- μ l sample of the diluted serum was introduced into the ion chromatograph. Fig. 3C shows a typical chromatogram of the diluted serum. The measured concentrations in the repeated ($n = 20$) serum sample injections were $1.33 \cdot 10^{-1} M$ (C.V. = 2.1%) for Na⁺ and $4.54 \cdot 10^{-3} M$ for K⁺ (C.V. = 2.4%). These values are coincident with the results from the serum samples using flame photometry: for Na⁺ = $1.35 \cdot 10^{-1} M$ (C.V. = 3.2%, $n = 3$) and for K⁺ = $4.6 \cdot 10^{-3} M$ (C.V. = 3.8%, $n = 3$). The measurement of the serum sample (diluted ten-fold) can be used for at least 60 repeated 1- μ l injections for Na⁺ and K⁺ determinations without re-calibration. However, as its ability for ion separation becomes gradually poorer, it is necessary to replace the column after *ca.* 200–300 injections. In this case, the ISFET could be used even after 300 determinations. The column and the ISFET are small enough to be considered disposable, even from an economic viewpoint.

In conclusion, the ISFET, as a small ion-sensing detector for ion chromatography, is suitable for the measurement of ions in sample and detection volumes of less than 1 μ l. Using tetranactin as an ion-sensing material, the ISFET detector can be successfully applied for the measurement of Na⁺ and K⁺ concentrations in serum. Although the concentration ratio of the two cations is large ($C_{Na}/C_K \simeq$

50), both ions can be detected in an ion chromatogram using the large difference in response sensitivities ($\text{Na}^+ \ll \text{K}^+$) of the tetranactin-based ISFET detector. This 'ion-selectivity' feature is a unique advantage of this type of detector. Thus, many ISFET detectors, purpose-designed for each measurement, could be prepared using a variety of ion-selective materials.

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