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Preparation of an Ion-selective Electrode Sensitive to Flufenamic Acid Anion and Its Application to the Study of Protein Binding of Flufenamate in Aqueous Solution

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A polyvinyl chloride (PVC) matrix membrane electrode sensitive to flufenamic acid anion was prepared. The membrane contained trioctylmethylammonium flufenamate as an ion exchanger. The electrode showed the Nernstian response for the flufenamate anion in the concentration range of 3×10^{-5} to 7×10^{-4} M (25°C). The response was independent of pH in the range of pH 6.2 to 9. The selectivity coefficients for chloride and nitrate ions were less than 10^{-3}, but the electrode showed almost the same sensitivity to mefenamate anion as to flufenamate. Addition of bovine serum albumin caused a marked change in the response curve, indicating that an interaction occurred between flufenamate and the protein.

Keywords——ion-selective electrode; PVC membrane electrode; trioctylmethylammonium chloride; flufenamic acid; protein binding

Ion-selective electrodes continue to be a most interesting field of study, and electrodes responsive to ions of many medicinal drug species, such as some vitamins,[2] alkaloids,[3] and antihistamines,[4] have been reported. Some have been efficiently applied to assays of drugs in tablets,[5,6] or biological fluids.[7] Most of them were of liquid membrane type, but recently polyvinyl chloride (PVC) matrix membrane electrodes, including some of coated wire type,[5,7] have also been developed and applied to medicinal drug assays. The PVC matrix membrane electrodes possess response properties similar to those of liquid membrane type electrodes and longer life times, and are much easier to prepare. Although direct potentiometry using ion-selective electrodes cannot be considered to be a very precise or sensitive method in comparison with other analytical techniques such as spectrophotometry, it has some advantages; it permits real-time measurement simply by dipping the electrode into sample solution over a wide range of concentration, and in principle makes possible an *in situ* study of ionic association or the binding of ions to polymer in aqueous solution, *e.g.* the study of the binding of an anionic surfactant to serum albumin.[8]

This report describes the preparation of a PVC matrix membrane electrode sensitive to the anion of flufenamic acid (hereafter referred to as FA), an anti-inflammatory drug, and its application to the study of the binding of FA to bovine serum albumin (BSA).

Experimental

Materials——Commercial FA and mefenamic acid (J.P.X) were purified by recrystallization from methanol; mp FA 134°C, mefenamic acid, 225°C. Trioctylmethylammonium chloride (Capriquat®) was obtained from Dojin Kagaku Lab. Ltd., and BSA was from Sigma (Fraction V powder). All other chemicals were of guaranteed grade and were used without further purification.

Preparation of the FA-sensitive Membrane——FA (0.28 g) and Capriquat (0.3 g) were dissolved in 100 ml of 1,2-dichloroethane, and chloride ions were removed by shaking with an equal volume of deionized water repeatedly. After each shaking, the aqueous phase was tested for chloride with acidic $AgNO_3$. The dichloroethane solution, 10% PVC–tetrahydrofuran solution and dioctylphthalate were mixed in a weight ratio of 1: 4: 2, and the mixture was poured on a glass plate. Evaporation of the solvent at room temperature left a PVC membrane of about 1 mm thickness.

Construction of the Electrode——A 12 mm diameter membrane was affixed to one end of a PVC tube (12 × 140 mm) using PVC–tetrahydrofuran solution. Inner reference solution containing 10^{-4} M FA and

2×10^{-3} M NaCl (pH 7.3), and an Ag–AgCl inner electrode were used.

EMF Measurement——A Pyrex vessel of 200 ml, with a jacket through which water of constant temperature (±0.1°C) was circulated, was used to hold the sample solution. The potential *vs.* SCE was measured with an Orion model 901 ion analyzer. A pH electrode and a magnetic stirrer were also used. Buffer solutions were not used and the pH of the solution was adjusted by addition of HCl or NaOH solution.

Results and Discussion

Response Potential of the FA-sensitive Electrode

Response potential, E_i, of an electrode for an ion i can be described as follows;

$$E_i=E_o+(RT/zF)\ln a_i \qquad \text{Eq. 1}$$

where a_i is the activity, z the charge of the ion, and F the Faraday equivalent. In the case that the temperature is 25°C, $z=-1$, and a_i can be replaced by the concentration C_i, the Nernst equation (Eq. 1) can be expressed as follows;

$$E_i=\text{constant}-59.2\log C_i\,(\text{mV}) \qquad \text{Eq. 2}$$

In Fig. 1, a typical response curve of the present electrode is shown. The potential slope, 59.4 mV per concentration decade, is nearly Nernstian throughout the FA concentration range of 3×10^{-5} to 7×10^{-4} M. At higher concentrations, the potential could not be measured, because of the low solubility of FA in the neutral pH range. The potential reached a steady value within a few minutes.

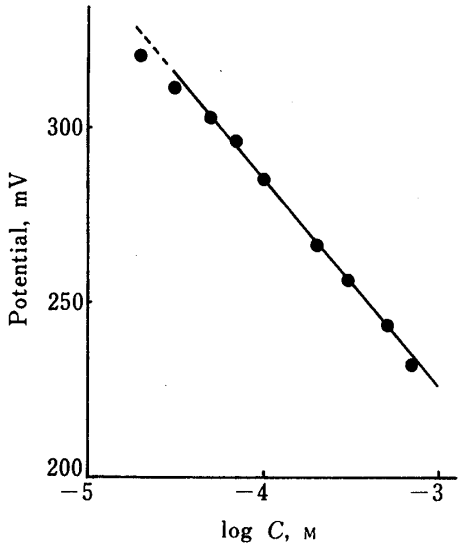

Fig. 1. Potentiometric Response to Flufenamic Acid Anion at 25°C

C: concentration of FA. pH 7.1—7.8.

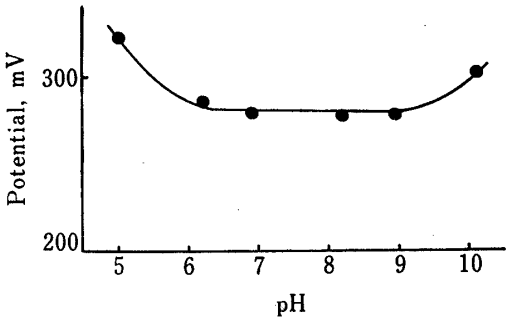

Fig. 2. Effect of pH on the Electrode Potential

Concentration of FA: 10^{-4} M. Temperature: 25°C.

Table I. Selectivity Coefficients, $K^{a)}$

Ion, j	K
Cl^-	$<10^{-3}$
NO_3^-	$<10^{-3}$
Salicylic acid	10^{-3}
Mefenamic acid	1

a) K is that in the Nicolsky equation, $E_i=\text{constant}-59.2\log(C_i+KC_j)$.

Effects of pH and Other Kinds of Anions

The effect of pH on the response potential is shown in Fig. 2. In the range of pH 6.2 to 9, the potential was not affected by pH. Selectivity coefficients for several kinds of anions obtained by the mixed solution method are summarized in Table I. It can be seen that the electrode has high selectivities for the FA ion over chloride, nitrate, and salicylate, but has almost the same sensitivity as for FA ion to the anion of mefenamic acid, a similar anti-inflammatory drug.

The Interaction between FA and BSA

The changes of the response potential for the FA ion in solutions containing 0.8×10^{-5} M and 2×10^{-5} M BSA were measured. In the case of the higher BSA concentration, the response was not stable. Measurements at various FA concentrations over the range of 7×10^{-5} to 7×10^{-4} M in 0.1 M NaCl were made at 37°C.

It can be seen in Fig. 3 that the response curves for the FA anion in the solutions containing BSA deviate markedly from that in the absence of the protein, and it appears that there is an interaction between FA and BSA.

Although calculation of the binding parameters was not possible, since the concentration data obtained from the potential measurement are not sufficiently accurate for quantitative analysis, the binding tendencies were clearly apparent.

It is concluded that electrodes sensitive to medicinal drug ions can be useful for studies of ionic interactions of drugs in the aqueous phase.

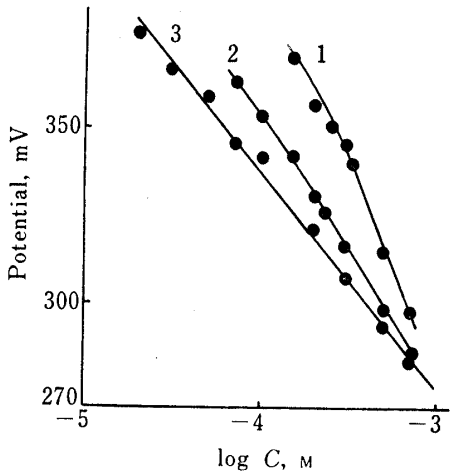

Fig. 3. Effect of Bovine Serum Albumin on the Potentiometric Response to Flufenamic Acid Anion

1: BSA 2×10^{-5} M, 2: BSA 0.8×10^{-5} M, and 3: no BSA in 0.1 M NaCl. pH 7.1—7.8. Temperature: 37°C.

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References and Notes

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