

Studies of Polymer Matrix Effect for Coextraction Type Anion-Selective Optode and Determination of Thiocyanate in Human Saliva

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The polymer matrix effect on the selectivity, response rate and reproducibility for coextraction type anion-selective optode membranes were investigated with DOA-plasticized PVC, PVC/hydroxylated PVC, PU/hydroxylated PVC and DOS-plasticized CTA matrices. Optode membranes were prepared with TDMACl and ETH2412 dissolved in one of the four solvent polymeric matrices. The PU/hydroxylated PVC and PVC-based membranes have almost the same selectivity coefficients, while the CTA-based membrane is more selective toward lipophilic anions. The membrane with PU/hydroxylated PVC adhered strongly to a glass surface, and showed highly reproducible and relatively rapid response. Very poor adhesion of PVC/hydroxylated PVC and CTA-based membranes limited the usability of those membranes as sensor components. Based on these results, and considering the biocompatibility for clinical samples, the optode made with PU/hydroxylated PVC was applied to determine the thiocyanate ion in human saliva. The results obtained with this simple device were comparable to those with rather complicated ISE methods.

Introduction

Optical methods have always played a dominant role in various fields of analytical sciences. Many opto-analytical methods exploit chromogenic molecular recognition process between analyte and indicator molecules: the presence and often quantities of specific analytes are determined by an appropriate spectroscopic technique or even by one's naked eyes for distinct color changes as in many spot tests. In recent years, with the use of some chromogenic and reversible indicator molecules immobilized in (or on) an optically transparent medium, considerable efforts have been directed towards the development of chemical sensors with an optical transducer¹⁻³. Optical chemical sensors, often called as "optodes", have been applied to measure various kinds of chemical samples, such as gases, electrolytes and neutral biomolecules.

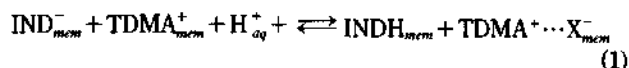
Among many chemical species, the measurement of various cations and anions in industrial, environmental and clinical samples is one of the most frequently needed information in controlling chemical process, regulating the levels of pollutants, and diagnosing many health problems. Ion-selective electrodes (ISEs), especially those prepared with electrically neutral and lipophilic ionophores as sensing components in a polymeric membrane, are being widely used for such purposes⁴. Simon *et al.* shrewdly modified those kinds of ISEs to corresponding ion-selective optodes (ISOs) by adding proton-selective chromoionophores into the ISE membranes.^{5,6} A mass transfer of the ions from the sample solution into the bulk of sensing layer induces the chromoionophores to simultaneously lose or gain protons to conserve the electroneutrality of the ISO membrane. This ion-exchange or coextraction process between ion-ionophore complex and protonated or deprotonated chromoionophore generates optical signals, which may be compared to the potentiometric signal of an ISE. Spectrophotometric responses for this type of ISO depend on the product or ratio of the ion activities

of protons and other responding ions⁵. Numerous publications have demonstrated that ISOs also exhibit the same excellent and analytically relevant selectivities for many ions as the corresponding ISEs do⁷⁻⁹.

Selectivities and response characteristics of ISOs are dictated by their active sensing components, ionophores and chromoionophores, as well as by their polymer matrices. Especially, the anion-selectivity of a polymer matrix itself is preset and rarely deviates from the well known Hofmeister series ($\text{ClO}_4^- > \text{SCN}^- > \text{I}^- > \text{NO}_3^- > \text{Br}^- > \text{Cl}^- > \text{HCO}_3^- > \text{CH}_3\text{COO}^- > \text{SO}_4^{2-} \sim \text{HPO}_4^{2-}$), which reflects the relative hydration energies (or lipophilicities) of those anions.^{8,9} The life time of an ISO is affected by the leaching out rate of the membrane components into the sample solution. The leaching out rate could be controlled, to a certain extent, either by increasing the lipophilicities of the membrane components or by employing a polymer or a blend of polymers that has stronger chemical interaction with the components.¹⁰ In addition, the mechanical properties, such as adhesion, of a polymeric layer are also very important factors in fabricating a practicable optode.¹¹ However, most studies on ISOs published to date are concerned only with the properties of active sensing components which can overcome the Hofmeister behavior of a polymer. The most popular matrix for both ISE and ISO has been poly(vinyl chloride) (PVC) because of its proven excellent electrical and optical properties. Other matrices, such as hydroxylated PVC, cellulose triacetate (CTA), polyurethane (PU) and blends of these polymers, have been seldom used for optode membranes, even though they have been occasionally used to improve certain properties of ISEs.¹¹

In order to understand the role of solvent polymeric matrices in ISOs, we fabricated coextraction type anion-selective optodes with four different polymer matrices and studied their response characteristics (selectivity, response time and reproducibility), as well as their adhesion properties to an optical material. The selection of four polymeric matrices

were based on previous ISE studies by Cha *et al.*; they were PVC, PVC/hydroxylated PVC, PU/hydroxylated PVC and CTA.^{11,12} The coextraction type optode membranes studied in this report contain a lipophilic additive (TDMACl) and a proton-selective chromoionophore (ETH2412), both in a mixture of plasticizer (DOS or DOA) and one of the polymers.^{8,9} In these optodes, coextraction is facilitated by the two charged species, $\text{IND}_{\text{mem}}^-$ and $\text{TDMA}_{\text{mem}}^+$:



where $\text{IND}_{\text{mem}}^-$ and $\text{TDMA}_{\text{mem}}^+$ represent the negatively charged chromoionophore in its non-protonated form and the positively charged lipophilic additive in the membrane phase, respectively. The positively charged sites attract anionic species in the sample electrostatically, but not selectively. It simultaneously prompts the negatively charged indicator to extract protons from the aqueous sample solution to conserve the electroneutrality of the membrane. An analytic signal, in this case the absorbance changes of the indicator molecule, is obtained from the protonated chromoionophore. In this type of optode, it is the properties of solvent polymeric matrices that determine the anion-selectivity. Thus, this type of optode would serve as a good model system for studying the characteristics of solvent polymeric matrices.

Since the optodes prepared in this experiment are highly selective toward lipophilic anions, they were applied for the measurement of thiocyanate in human saliva. It is known that highly toxic cyanide is metabolized to non-toxic thiocyanate in human body.¹³ A major source of such cyanide exposure is smoking which contains significant level of hydrogen cyanide. Since the half-life of thiocyanate in physiological fluids (about two weeks) is much longer than other smoking substances inhaled, such as carbon monoxide or nicotine, its measurement in blood, urine, or saliva would be used to assess the long-term exposure of an individual to smoking. Thiocyanate in biological samples may be measured using colorimetric complexation methods, GC/mass, ion chromatography and ion-selective electrodes. Most of the known methods, however, require extensive sample pretreatment to remove the interferences from proteins or from other anions. ISE method has been the most successful method for the measurement of thiocyanate in biological samples. Recently, Brown *et al.* reported thiocyanate-selective polymer membrane electrodes in which 5,10,15,20-tetrakis(2,4,6-triphenylphenyl porphyrinato)manganese(III) chloride served as the membrane-active component.¹⁴ To our knowledge, optodes have not been used for the measurement of thiocyanate in physiological samples. In this report, we will show that the results obtained with a very simple optode device are also comparable to those with rather complicated ISE devices.

Experimental

Reagents. Poly(vinyl chloride), cellulose triacetate, bis(2-ethylhexyl)sebacate (DOS), bis(2-ethylhexyl)adipate (DOA), 5-octadecanoyloxy-2-(4-nitrophenylazo)phenol (ETH2412) and tridodecylmethylammonium chloride (TDMACl) were obtained from Fluka (Buchs, Switzerland) while hydroxylated PVC

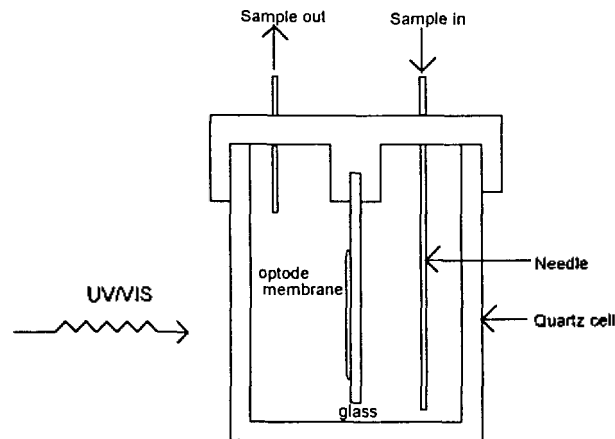


Figure 1. Flowing UV/VIS cell used in this experiment. An optode membrane is cast on a slide glass, which is then placed in a quartz cell. The sample solution flows into the cell by a peristaltic pump.

(a copolymer of vinyl chloride, vinyl acetate, and vinyl alcohol (PVC/Ac/Al), 80/5/15 wt%) was a product of Scientific Polymer Products, Inc. (Ontario, NY). Tecoflex polyurethane (SG-80A; hereafter will be denoted as PU), which was synthesized from methylene bis(cyclohexyl) diisocyanate, poly(tetramethylene ether glycol) and 1,4-butanediol chain extender, was from Thermedics, Inc. (Woburn, MA).

All other chemicals used were analytical-reagent grade. Standard solutions and buffers were prepared with deionized water.

Preparation of membranes. ETH2412 (2.3 wt%), TDMACl (5 wt%) and DOA (61.8 wt%) were mixed with 30.9 wt% of one of the following polymers: PVC, PVC(50 wt%)/hydroxylated PVC(50 wt%) and PU(80 wt%)/hydroxylated PVC(20 wt%). Each mixture was then dissolved in an appropriate solvent. For the PVC, PVC/hydroxylated PVC and PU/hydroxylated PVC membranes, the mixtures were dissolved in 0.5 ml of THF. Since the solubility of CTA was poor in THF, the CTA mixture was dissolved first in 0.2 ml of dichloromethane for 4 hours and other components were added to it with 0.4 ml of chloroform. CTA-based membrane was prepared with DOS plasticizer while the weight percents of all ingredients were the same as those of other membranes. Each cocktail was screen printed on a clean slide glass (0.7×4.0 cm) to form a thin film of 2-10 μm thickness. They were dried under a 500 ml beaker to keep dusts from the membranes. Before use, these membranes were soaked in 0.01 M Tris buffer (pH=7.4) for three hours.

Measurements of Selectivities, Response Times and Reproducibilities. The glass plates carrying the membranes were mounted in a flowing cell (Figure 1) and placed in an UV/VIS spectrometer (Shimadzu UV-240). The calibration curves for four anions were obtained by measuring the absorbance of ETH2412 at 540 nm: standard solutions containing 10^{-1} - 10^{-6} M of chloride, nitrate, thiocyanate and perchlorate in Tris buffer (pH=7.4) were flowed through the sample cell (see Figure 1) using a peristaltic pump (Ismatech 7331-00, Cole-Parmer Instrument Co.) at a flow rate of 2 ml/min. Response times and reproducibilities of optode membranes were recorded at 540 nm by alternately

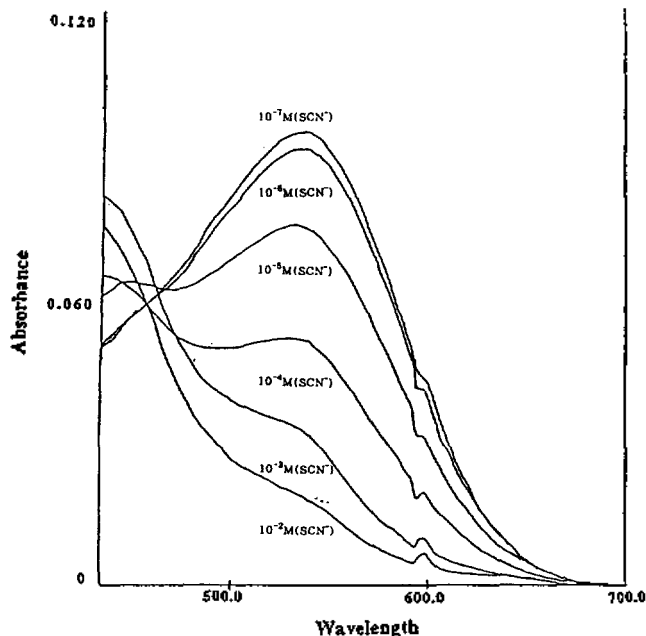


Figure 2. Spectra of an optode containing ETH2412 and TDMACl, both in DOA-plasticized PU/hydroxylated PVC matrix, for sodium thiocyanate of different concentration at pH=7.4.

changing two different thiocyanate concentrations (10^{-2} and 10^{-5} M). Since the response times are proportional to the square of membrane thickness,⁵ the membranes which have the same absorbance were carefully selected. For the determination of selectivity coefficients, the absorbance changes for the 0.1 M anion solutions at different pH values were recorded using universal buffer solutions of pH's from 2 to 12. The stock solution of universal buffer consisted of 10 mM NaH_2PO_4 , 6.6 mM citric acid and 11.4 mM $\text{Na}_2\text{B}_4\text{O}_7$ (all from Fluka). Portions of this solutions were adjusted to the desired pH value with H_2SO_4 or NaOH solution before adding the anions.

Adhesion Test. In order to test the adhesion of membranes to a glass surface, 20 spots of membranes made as described in the preparation section were cast on a slide glass. In one test, each sample spot was firmly taped with Scotch tape and removed by slow pulling up. Another test was carried out by bathing the sampled glass in a sonicator for 30 minutes. The adhesion of polymer membranes were then gauged by counting the remaining spots.

Measurements of Thiocyanate in Human Saliva. Saliva samples were obtained from both smokers and nonsmokers in the Chemistry Department at the Kwangwoon University. About 5 ml of samples were centrifuged at a speed of 4000 rpm for 10 minutes and the proteinaceous compounds were settled down. The supernatants were then taken and diluted ten times with TRIS buffer (pH=7.4), which were then flowed through the specially designed UV-cell ($1 \times 1 \times 4.5 \text{ cm}^3$) shown in Figure 1. In order to minimize the errors due to most abundant chloride ion, the standard samples containing 10^{-2} - 10^{-6} M of sodium thiocyanate were prepared with 100 mM of sodium chloride. The absorbance for the samples were read directly from the UV/VIS spectrometer and used to derive the unknown concentrations with

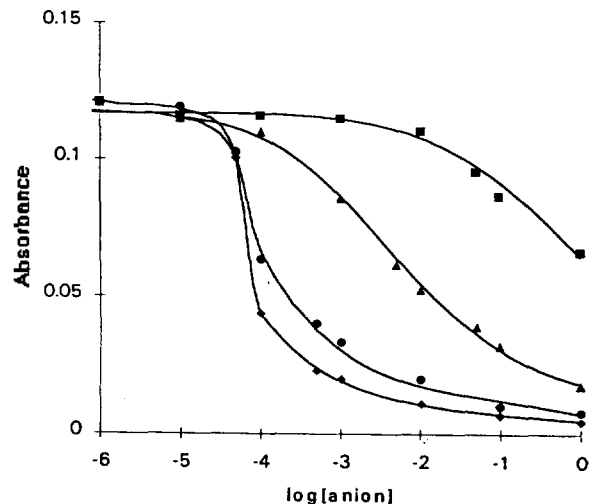


Figure 3. Calibration curves for four anions (■ chloride; ▲ nitrate; ● thiocyanate; ◆ perchlorate) at pH 7.4 obtained by measuring the absorbance at 540 nm with the DOA-plasticized PVC membrane containing ETH2412 and TDMACl.

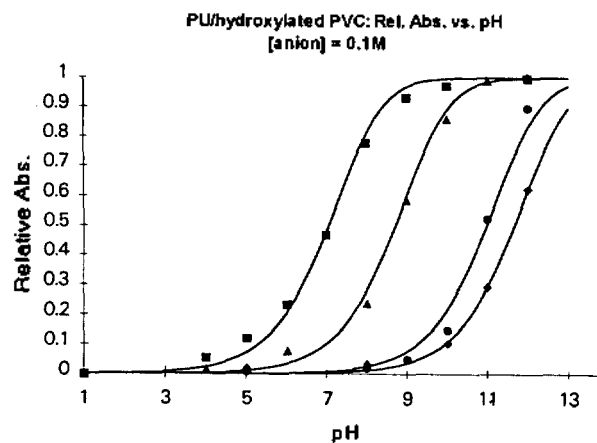


Figure 4. Relative absorbance, α , of the chromoionophore ETH2412 in DOA-plasticized PU/hydroxylated PVC at 540 nm as a function of pH for 0.1 M anion solutions. The curves are fitted to the experimental points (■ chloride; ▲ nitrate; ● thiocyanate; ◆ perchlorate) using equation (2) by adjusting the equilibrium constants.

the calibration curve obtained from the standard solutions.

Result and Discussion

Figure 2 is an example of typical absorption spectra for the system with the charged carrier ETH2412 and TDMACl embedded in a polymer membrane at varying thiocyanate concentrations; increased anion concentration induces the negatively charged ETH2412 to be protonated, which results in decreased absorbance at 540 nm. A series of similar absorption spectra was also recorded for other three membranes. For each optode membrane, calibration curves for chloride, nitrate, thiocyanate and perchlorate anions were obtained by reading the absorbance at 540 nm. Figure 3 is an example of such calibration curves obtained from the PVC-

Table 1. Selective coefficients (K_{SCN^-, Y^-}^{OPT}) for anion-selective optode membranes with different polymer matrices

Ions	Matrices			
	PVC	PU/hydroxylated PVC	PVC/hydroxylated PVC	CTA
Cl ⁻	-3.96	-3.95	-3.75	-4.31
NO ₃ ⁻	-2.11	-2.28	-2.11	-2.37
ClO ₄ ⁻	0.96	0.68	1.01	1.32

based membrane; the curves clearly reflect the order of relative hydration energies (or lipophilicities) of the four anions. Other membranes also have the same type of calibration curves as shown in Figure 3. However, their slopes and spacings between the curves vary from membrane to membrane. It indicates that the selectivities of optodes are dependent on the type of polymer matrices employed.

In order to obtain the selectivity coefficients for each membrane, a series of pH response curves was plotted at a fixed anion concentration (0.1 M). They are given in Figure 4. Considering the equilibrium constant and the law of mass action from equation (1), and introducing relative absorbance $\alpha = [IND^-]/[IND]_T = (A - A_0)/(A_1 - A_0)$, the following dependence of α on the activities of anion, a_{X^-} , and of proton, a_{H^+} , can be derived:

$$\frac{(1-\alpha)(L_T/[IND]_T - \alpha)}{\alpha^2} = K_X \cdot a_{H^+} \cdot a_{X^-} \quad (2)$$

where IND, A, L, and K represent chromoionophore, absorbance at 540 nm, lipophilic additive, and equilibrium constant for the equation (1), respectively; subscripts T, 0 and I represent total concentration, values at zero and concentrated anion concentrations (0.1 M), respectively. One may solve

equation (2) with respect to α , which may be used to fit the experimental points to obtain the equilibrium constant of a membrane for the respective anion X⁻. If other interfering anions coexist in the sample solution, equation (2) can be written analogous to the Nikolsky-Eisenman formular for ISE to include such effect⁵:

$$\frac{(1-\alpha)(L_T/[IND]_T - \alpha)}{\alpha^2} = K_X \cdot a_{H^+} \cdot (a_{X^-} + \sum_Y K_{XY}^{OPT} a_{Y^-}) \quad (3)$$

where K_{XY}^{OPT} is the selectivity coefficient of the anion Y⁻ with respect to X⁻. If the experiments are carried out for each anion, the K_{XY}^{OPT} may be expressed with the respective equilibrium constants K_X and K_Y :

$$K_{XY}^{OPT} = K_Y/K_X \quad (4)$$

These results are summarized in Table 1 and the selectivity coefficients are estimated with respect to the thiocyanate ion. Comparison of selectivity coefficients for each anion indicates that PU/hydroxylated PVC-based membrane is very similar to PVC-based membrane, while CTA-based membrane is the most lipophilic in its character.

Response times and reproducibilities for each membrane were measured by alternately changing two different thiocyanate concentrations, 10^{-5} and 10^{-2} M. Figure 5 shows the recorder traces of those experimental results; the reproducibilities of CTA-based membrane are too poor to use it as an optode, while those of other three membranes are sufficiently regular. The rising slopes of the traces, which correspond to the deprotonation process of ETH2412, determine the speed of response. The response time of an optode membrane was measured at the 95% of full response, denoted as $t_{95\%}$. Theoretically, $t_{95\%}$ is given approximately by $t_{95\%} = 1.13 d^2/D_m$ where d and D_m are the membrane thickness and the mean diffusion coefficient of the anions in the membrane

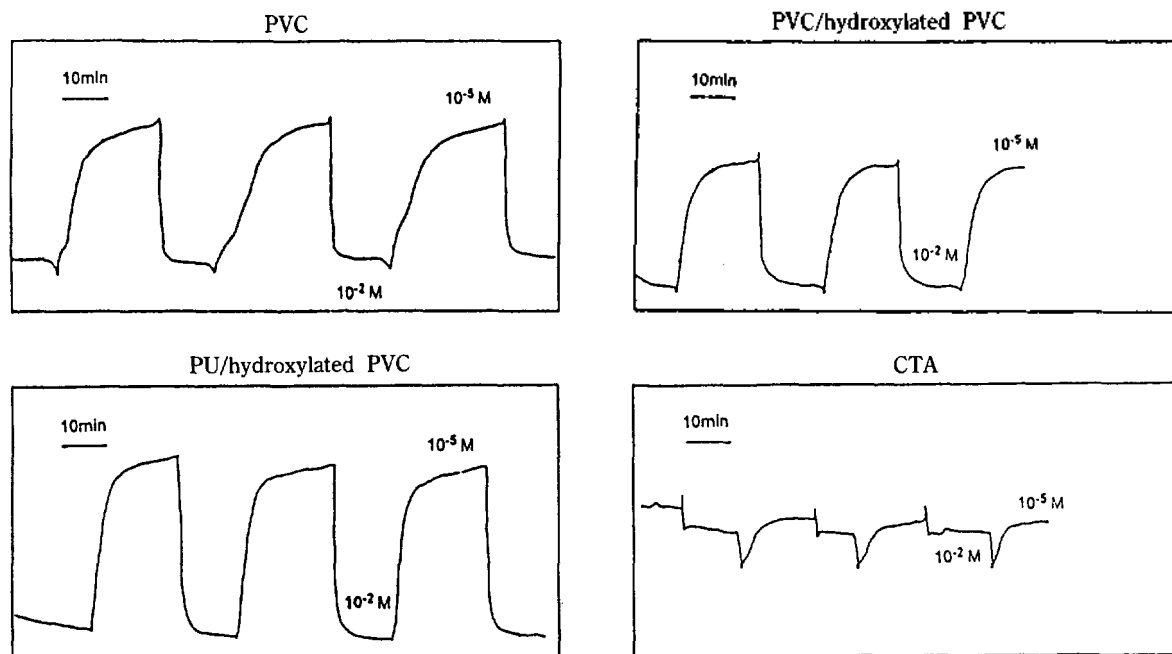
**Figure 5.** Recorder traces for four membranes obtained by alternately changing two different thiocyanate concentrations (10^{-2} and 10^{-5} M) at pH=7.4.

Table 5. The concentration of thiocyanate in human saliva determined by the anion-selective optode system

Sample No.	Smoker (mM)	Non-smoker (mM)
1	1.00	0.14
2	1.32	0.18
3	1.45	0.21
4	1.65	0.42
5	1.90	0.68

at the Kwangwoon University, and the thiocyanate levels in these samples were determined with the PU/hydroxylated PVC membrane optode. Each measurement was made in 10 minutes to ensure the complete response. The test results are calculated using the calibration curve in Figure 6 and they are summarized in Table 5. It is interesting to note that smokers have three to ten times higher thiocyanate concentrations in their saliva than non-smokers, indicating the seriousness of cyanide exposure from smoking. These values are well compared with the previously reported values measured with a thiocyanate-selective ISE.¹⁴ The thiocyanate levels determined here are neither the representative values nor of any clinical significance, since we did not pay much attention on the personal records of sample donors, such as the number of cigarettes smoked per day, their living environments and dietary habits. The purpose of this experiment was to demonstrate the workability of our simple optode system for the determination of physiological thiocyanate, and indeed the results were satisfactory.

Conclusion

In this experiment, we prepared ETH2412 and TDMACl-based coextraction type anion-selective optode. Those sensing components were dissolved in four different kinds of solvent polymeric matrices; PVC, PVC/hydroxylated PVC, PU/hydroxylated PVC and CTA. We investigated the selectivity pattern, response times and reproducibilities, as well as adhesion properties of those membranes. The ISO made with common PVC matrix exhibited the problems of slow response times for lipophilic anions. The membranes with CTA and PVC/hydroxylated PVC were too easily peeled off from a glass surface when they were in a flowing cell. On the other hand, the sensing membrane with PU/hydroxylated PVC matrix showed highly reproducible and fast responses for lipophilic anions, while its selectivity coefficients with respect to thiocyanate were comparable to those of PVC-based membrane. Furthermore, it had strong adhesion to a glass surface. It is also known that PU containing membrane provides an excellent biocompatibility for physiological fluids. On the basis of these results, we concluded that the anion-selective PU/hydroxylated PVC-based optode membrane may be used as a practicable sensor system for the

determination of some lipophilic anions in physiological fluids. Thus, we applied this optode to measure the thiocyanate ion in human saliva. The measurement of thiocyanate in physiological fluids would be a useful information in assessing an individual's exposure to cigarette smoking or other cyanide containing atmosphere. While ISEs are the most commonly used method for such purposes, we could demonstrate that comparable results could be obtained with very simple optode system.

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Table 2. Response times in minute and reproducibilities of four anion-selective optode membranes, which are standard deviation of reproducible absorbances

Concentrations	Matrices			
	PVC	PU/hydroxylated PVC	PVC/hydroxylated PVC	CTA
10^{-5} M \rightarrow 10^{-2} M	2	2	5	10
10^{-2} M \rightarrow 10^{-5} M	18	7	9	—
Reproducibilities	$\pm 6.7\%$	$\pm 2.7\%$	$\pm 6.3\%$	—

Table 3. Adhesion test of PU/hydroxylated PVC and PVC-based membranes to a glass surface

Membranes (20 spots)	% of membranes removed	
	Scotch tape test	Ultrasonic bathing (30 min)
PU/hydroxylated PVC	0	0
PVC	100	50

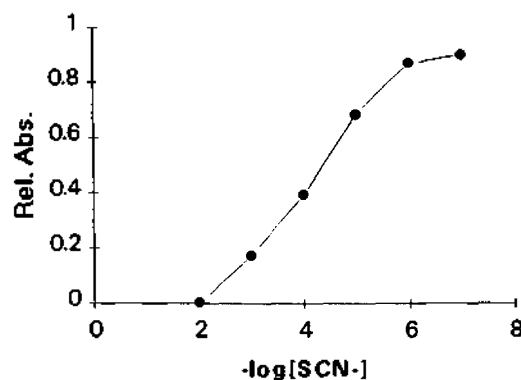
phase, respectively.⁵ Thus, the comparison of response times was made with the membranes of the same thickness. Since the amount of ETH2412 was carefully monitored for each membrane cocktails, it was assumed that the membrane thickness would be proportional to the absorbance of ETH 2412 at 540 nm. Response times for each membrane are given in Table 2. When the samples are changed from 10^{-2} M to 10^{-5} M, it appears that the response times depend on the PVC contents of each membrane: the anions in membrane phase tend to diffuse out easily with low PVC contents. On the other hand, the diffuse-in response times, i.e., when the thiocyanate ion concentrations were changed from low to high (10^{-5} \rightarrow 10^{-2} M), were not dependent on the PVC contents and much faster than the opposite case. In terms of response times and reproducibilities, the PU/hydroxylated PVC-based membrane has shown the best performance.

The adhesion of polymer membranes to an optical material is another important property to consider because it may become a major limiting factor in fabricating a practicable optode if the membranes are easily peeled off from the surface of their supporting material, in this case a glass. For example, PVC/hydroxylated PVC and CTA-based membranes would not stay on the glass surface when they are placed in a flowing cell. Thus, we tested the adhesion of PVC and PU/hydroxylated membranes to a glass surface by pulling out firmly taped Scotch tapes and sonicator bathing.¹¹ The results are summarized in Table 3. For both tests, PU/hydroxylated PVC membranes were not removed from the glass surface, indicating that they have sufficient adhesion. On the other hand, PVC-based membranes did not pass the sonicator bathing test; half of the sample spots has been removed from the glass surface after 30 minutes of sonic bathing.

Among the polymer matrices we tested, the selectivity coefficients of the membrane made with PU/hydroxylated PVC are very close to those of most popular PVC-based

Table 4. Selectivity coefficients of the optode system with ETH 2412/TDMACl pair embedded in DOA-plasticized PU/hydroxylated PVC membrane for various interfering anions in comparison with the required selectivities for blood, serum and plasma.

Anion	95% normal range (mM)	$\log K_{SCN^-, Y}^{OPT}$	
		Required	Experimental
Cl^-	95-110	-4.9	-4.0
Br^-	0.009-0.17	-2.1	
NO_3^-	0-0.1	-1.8	-2.3
I^-	0.0003-0.0005	0.5	
SCN^-	0.007-0.15	0	0
ClO_4^-	0		0.7

**Figure 6.** Calibration curve used for the determination of thiocyanate in human saliva. Relative absorbance values at 540 nm are plotted as a function of $-\log[SCN^-]$ at pH=7.4.

membrane, while exhibiting faster response times, better reproducibilities and much stronger adhesion to its glass support. Moreover, it is known that polyurethane containing membrane provides much better biocompatibility for physiological fluids than PVC-based membrane.¹¹ Thus, we expected that the anion-selective optode made with PU/hydroxylated PVC membrane may be employed as a practical sensor system for the measurement of lipophilic anions, such as thiocyanate, in biological fluids. Some representative electrolytes in human blood, serum and plasma are listed in Table 4 with the required selectivity coefficients for the determination of thiocyanate, which were calculated using the equation (3) for a maximum interference of 1% by other anions.⁹ The dilution factor was not considered in this calculation. Though we did not measure the blood samples in this experiment, those representative values could be a useful guide for the estimation of possible interferences. For example, if we assume the electrolytes in saliva are not much different from those of serum, the increase in chloride concentration would be less than 10-20 mM for the ten times diluted saliva sample. This amount would affect the result by less than 0.2% for the determination of thiocyanate, considering the selectivity coefficients ($\log K_{SCN^-, Cl}^{OPT} = -3.95$) of the PU/hydroxylated PVC membrane.

Ten human saliva samples were obtained from both smoking and non-smoking students in the Chemistry Departments