

Ionophore-Based Lithium Ion Film Optode Realizing Multiple Color Variations Utilizing Digital Color Analysis

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Digital color analysis (DCA), utilizing colors themselves or digital information of colors, can not only be applied to various quantitative analysis using chromaticity coordinates but can also be used to develop suitable sensors for visual colorimetry based on the characteristics of human visual perception by virtual simulations based on digital color information. To achieve a clear visual color variation for lithium ion determination, we designed and prepared a color-changeable film sensor (film optode) by the use of two kinds of lipophilic dyes, KD-C4 and KD-M11, whose colors and pK_a values are different. This film sensor is a plasticized PVC membrane containing the mixture of two kinds of dyes with the lithium ionophore TTD14C4 and the lipophilic anionic additive tetrakis-[3,5-bis(trisfluoromethyl)phenyl]borate sodium salt dihydrate. The simulation of the color variation using the mixed dyes was evaluated by plotting the values on a uniform chromaticity scale diagram in a^*b^* coordinates, after converting the tristimulus values of each dye into its $L^*a^*b^*$ values. When the lithium ion concentration was actually determined by the PVC film optode containing the mixed dyes whose molecular ratio of KD-C4/KD-M11 was 3:1, the hues of red \rightarrow orange \rightarrow yellow \rightarrow green \rightarrow blue could be realized in the range of 10^{-6} –1 M. This observed color variation was similar to the result of the virtual simulation based on DCA.

The usual spectrophotometry for quantitative analysis has generally been performed by the use of spectral and absorbance information. We have proposed digital color analysis (DCA) as a new optode instead of conventional spectrophotometry.^{1,2} This analytical method not only makes use of the “colors” themselves but also utilizes numerical values converted from color information. DCA treats “spectral information” as “digital information”;

it makes it possible to quantify while the characteristics of human visual perception are considered. As an example of DCA, we previously developed a lithium ion sensor that was suitable for visual determination by utilizing a screening dye for a film-type ion-selective optode.¹ This film optode was made from a plasticized PVC membrane as a matrix that contained a lithium ionophore, a lipophilic cationic dye, a screening dye, and an anionic additive. It was designed to show the gray point at 10^{-3} M lithium ion concentration in order to monitor Li^+ in saliva of patients with manic-depressive psychosis and hyperthyroidism. Preparing the membrane, we optimized the amount of the screening dye by calculations using the complementary chromaticity coordinates Q_iQ_j . As human eyes are quite perceptive for a slight color difference around the colorless gray point, it is easy to judge by this sensor whether the Li^+ concentration in biological samples indicates medical treatment or not.

In this study, as another novel example of DCA, we tried to develop a lithium ion sensor realizing a multicolor variation using the uniform chromaticity scale diagrams $L^*a^*b^*$ color space and a^*b^* coordinates proposed by Commission Internationale de l'Éclairage (CIE) in 1976, as shown in Figure 1. The previously reported optode described above utilizing the “screening dye” showed a sharp and effective visual color variation in a narrow concentration range by setting up the color transition through the gray point.^{1,3,4} In this case, the hues perceived by human eyes were orange and purple, and the largest achievement of that optode was making use of the high sensitivity of human visual perception for these hues to appear or disappear across the colorless gray point. On the other hand, when we focus on “hues” in visual colorimetry, it is assumed that the more hues appear during transitions, the easier the visual perception becomes. It is supposed that accurate quantification can be realized by large hue variations. However, so far ion-sensing films with multiple color variations have not been developed for selective ion determination despite of the usefulness as a chemical ion sensor similar to a multiple pH indicator-based sensor. As shown in Figure 2a, when a mixture of two kinds of pH indicators 1 and 2 with different pK_a values is used in an optode, the color of indicator 1 (lower pK_a)

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(1) Hirayama, E.; Sugiyama, T.; Hisamoto, H.; Suzuki, K. *Anal. Chem.* **2000**, 72, 465–474.

(2) Reilly, C. N.; Flaschka, H. A.; Laurent, S.; Laurent, B. *Anal. Chem.* **1960**, 32, 1218–1232.

(3) Prasad, K. M. M. K.; Rahhem, S. *Analysis* **1992**, 20, 401–406.

(4) Prasad, K. M. M. K.; Rahhem, S.; Vijayalekshmi, P.; Sastri, C. K. *Talanta* **1996**, 43, 1187–1206.

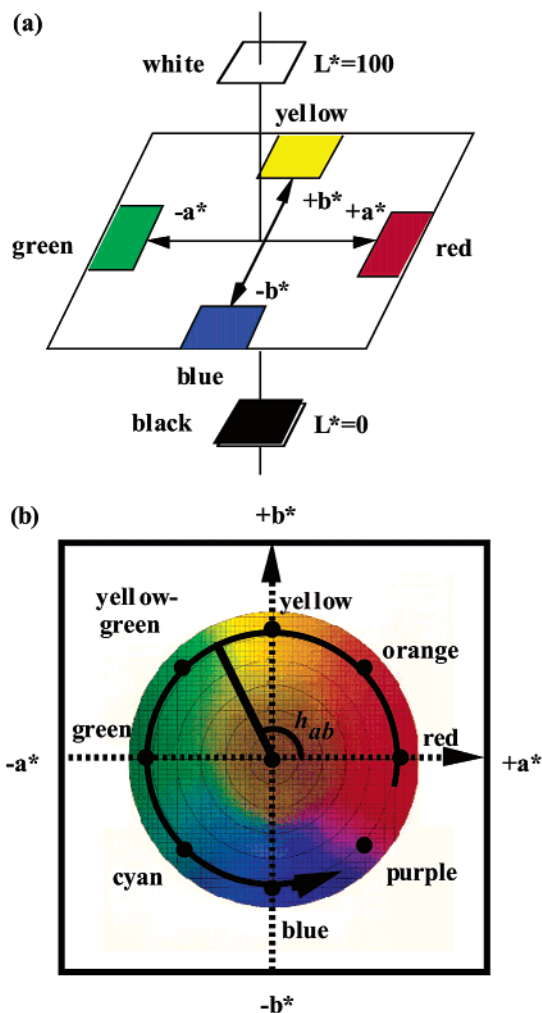


Figure 1. (a) $L^*a^*b^*$ color space proposed by CIE in 1976 and (b) color circle and hue angle shown on an a^*b^* chromaticity diagram.

will change at lower ion concentration, whereas indicator 2 (higher pK_a) will change at higher ion concentration. Hence, a large variety of hues will appear over the whole concentration range by the combination of the indicators that show suitable color variation. In addition, the colors of the indicators used in combination will change over a wider dynamic concentration range than an individual indicator used alone. Universal indicators for pH including Methyl Red, Bromothymol Blue, and phenolphthalein are given as representative examples for the application of this theory. The combinations of indicators are important for obtaining suitable effects of mixing. As a first condition, the pK_a values of the two indicators need to be appropriately apart. If two indicators whose pK_a values are too close are used, their colors will change at the same time. It will improve neither the dynamic range nor the quality and the quantity of the color variation. When the pK_a values are too far apart, the colors of both indicators will show no changes at the intermediate concentration range. The direction of the color variation is important as a second condition. If the direction of the color variation of two indicators is continuous on the a^*b^* chromaticity diagram, there will be no offset between both of the color variations and many hues will appear as shown in Figure 2b. On the other hand, if the initial colors are close or the direction of the color variations are opposite to each other across the gray point as shown in Figure 2c, a positive effect from

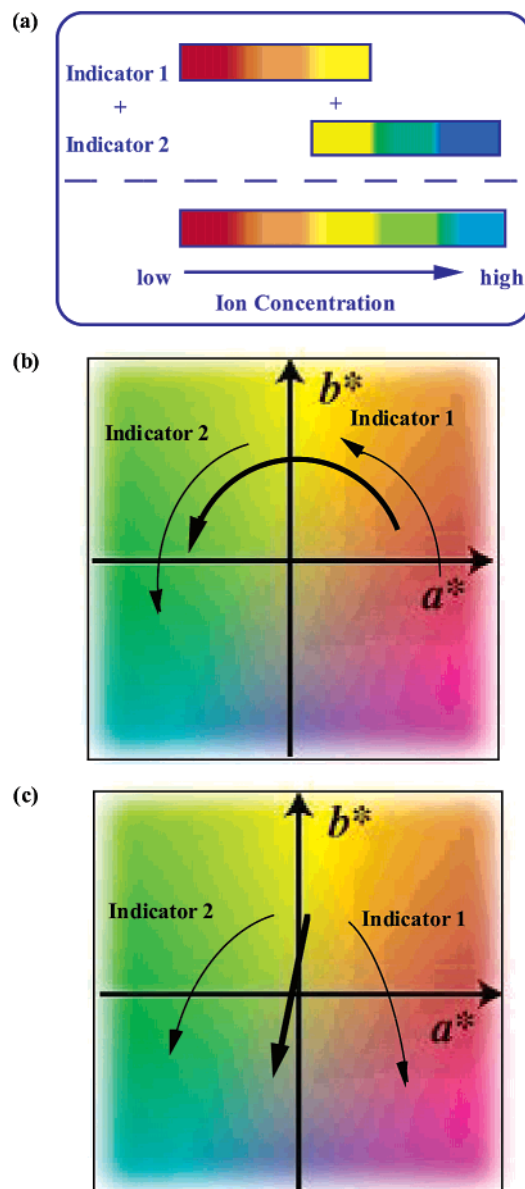


Figure 2. (a) Color variation of an ion optode based on mixing pH indicators of different pK_a values and different color variations. The color transition of mixed indicators is illustrated on an a^*b^* chromaticity diagram showing a suitable (b) and an ineffective combination of indicators (c) for developing an optode based on "hue angle" variations.

mixing will not be expected. In this study, two kinds of lipophilic cationic dyes, KD-C4 and KD-M11⁵ whose pK_a values are different, were used as indicators contained in an optode membrane. KD-C4 shows color changes red \rightarrow orange \rightarrow yellow, and KD-M11 changes yellow \rightarrow green \rightarrow blue with increasing pH. Therefore, a suitable color variation such as red \rightarrow orange \rightarrow yellow \rightarrow green \rightarrow blue would be expected by mixing the indicators. We tried to prepare a plasticized PVC membrane that contained two kinds of lipophilic cationic dyes and a lithium ionophore as a new optode for multicolor visual colorimetry over a large concentration range of lithium ions.

(5) Hisamoto, H.; Tohma, H.; Yamada, T.; Yamauchi, K.; Siswanta, D.; Suzuki, K. *Anal. Chim. Acta* **1998**, *373*, 271–289.

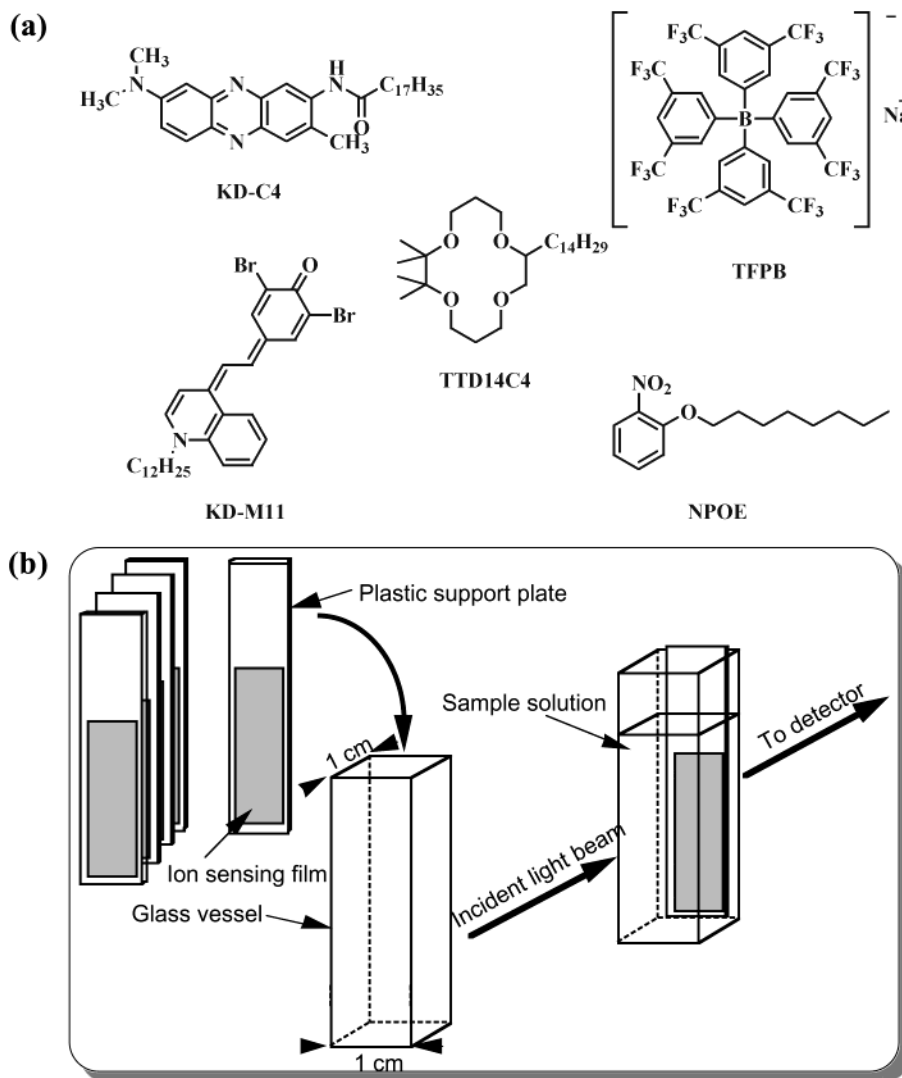


Figure 3. (a) Chemical structures of the compounds incorporated into the PVC membrane film optode and (b) a schematic view of the sensing film and the experimental setup.

EXPERIMENTAL SECTION

Reagents. The lithium ionophore (TTD14C4), tetrakis[3,5-bis-(trifluoromethyl)phenyl]borate sodium salt dihydrate (TFPB) and 2-nitrophenyl octyl ether (NPOE) were purchased from Dojindo Laboratory (Kumamoto, Japan), where TTD14C4 was developed by our research group.⁶ Poly(vinyl chloride) (PVC, high molecular weight) was purchased from Sigma (St. Louis, MO). The lipophilic cationic dye (KD-M11) was synthesized according to our previous report.⁵ The chemical structures of TTD14C4, KD-M11, and TFPB are shown in Figure 3a.

Synthesis of KD-C4 [Octadecanoic Acid (8-(Dimethylamino)-3-methylphenazin-2-yl)amide]. Neutral Red (1.6 g) was dissolved in distilled water, and 0.1 M aqueous NaOH was added. The desalted Neutral Red was extracted into chloroform. The dried free-base form of Neutral Red (0.915 g) was obtained after drying over Na_2SO_4 and evaporation. Triethylamine (1.0 g, 9.9 mmol) and the free base of Neutral Red (0.91 g, 3.6 mmol) were dissolved in tetrahydrofuran (THF, 300 mL) and the resultant

mixture was stirred for 1 h at room temperature. A 10-mL sample of THF containing stearoyl chloride (1.7 g, 5.6 mmol) was added to the reaction mixture, and the resultant mixture was stirred for 24 h at room temperature. After the reaction, the THF was evaporated and the residue obtained was dissolved in chloroform. The chloroform phase was washed with aqueous HCl two times, NaOH two times, and water one time and dried over Na_2SO_4 , and the solvent was evaporated. The residue was purified by silica gel column chromatography with *n*-hexanes–ethyl acetate (1:3) as the eluent to yield the final product, octadecanoic acid (8-(dimethylamino)-3-methylphenazin-2-yl)amide (yield: 0.359 g, 19%): ^1H NMR (CDCl_3 , 300 MHz) δ 0.88 (t, $J = 6.71$ Hz, CH_3 , 3H), 1.22–1.43 (m, CH_2 , 30H), 2.48 (t, $J = 7.69$ Hz, COCH_2 , 2H), 2.50 (s, Ar- CH_3 , 3H), 3.18 (s, NCH_3 , 6H), 7.04 (d, $J = 2.69$, Ar- H , 1H), 7.34 (s, Ar- H , 1H), 7.52 (dd, $J = 9.64$ Hz, Ar- H , 1H), 7.89 (s, Ar- H , 1H), 7.97 (d, $J = 9.52$ Hz, Ar- H , 1H). Anal. Calcd for $\text{C}_{33}\text{H}_{51}\text{N}_4\text{O}$ (520.411): C, 76.25; H, 9.89; N, 10.78. Found: C, 75.72; H, 9.42; N, 10.27.

Standard Solutions. Standard solutions of Li^+ (Li^+ test solutions) used in this study were 10^{-6} – 1 M LiCl in 0.01 M

(6) Suzuki, K.; Yamada, H.; Sato, K.; Watanabe, K.; Hisamoto, H.; Tobe, Y.; Kobihiro, K. *Anal. Chem.* **1993**, *65*, 3404–3410.

magnesium acetate/0.02 M acetic acid buffer (pH 7.0). Standard solutions of Na⁺ and K⁺ (10⁻⁵–1 M) were prepared with NaCl and KCl dissolved in the same buffer (pH 7.0). Test acid and base used were aqueous solutions of HCl (pH 2) and NaOH (pH 11).

Preparation of the PVC Film Optode Containing the Individual Dyes. The plasticized PVC membrane used for the lithium ion film optode was prepared by the standard procedure reported previously.^{7–9} The membrane compounds PVC (20 mg), NPOE (40 mg), and equimolar amounts of TTD14C4, TFPB, and the dye (4.06 μ mol, KD-M11 or KD-C4) were dissolved in THF. The cocktail solution was dropped on a transparent plastic sheet fixed on a spin coater, which was rotated for 5 s at 4000 rpm to prepare the thin film. The film was dried under vacuum and cut into 9 mm \times 45 mm segments to fit into a standard glass cell (10 mm \times 10 mm \times 50 mm) for a spectrophotometer.

Preparation of the PVC Film Optode Containing the Mixed Dyes. The membrane cocktail was prepared by dissolving KD-M11 (1.57 μ mol), KD-C4 (4.52 μ mol), TTD14C4 (6.09 μ mol), TFPB (6.09 μ mol), PVC (30 mg), and NPOE (60 mg) in THF, resulting in a total weight of 0.422 g. The thin film was prepared by the use of a spin coater as described above.

Ion Determination with the PVC Film Optode. According to the known response mechanism,¹ Na⁺ as the counterion of the anionic additive was released by immersing the freshly prepared PVC membrane in 0.1 M aqueous HCl before a sample solution was measured. The dyes were completely protonated at the same time. The PVC membrane was fixed to the wall of a glass cell for the double-beam spectrophotometer (U-2000, Hitachi Co., Ltd.) as shown in Figure 3b and subsequently immersed in the ion standard solution of each concentration (10⁻⁶–1 M Li⁺, pH 7). The absorbance spectra were recorded after the absorbance readings were constant. The membrane was then removed from the glass cell. After the water droplets were gently removed from the membrane surface, the membrane was placed on a white Teflon plate. The color of the membrane was measured by a digital color analyzer (COLORTRON, Light Source Computer Images, Inc., Larkspur, CA) in the reflection mode. The same measurements were carried out three times consecutively, and the average data were adopted. To measure the selectivity of the optode membrane, we immersed the membrane in Na⁺- or K⁺-containing aqueous solutions (pH 7) in a manner similar to that as described for Li⁺. The normalized absorbance α at the maximum absorption wavelength was calculated according

$$\alpha = \frac{a_{Co}}{a_{C}^{tot}} = \frac{A - A_0}{A_1 - A_0} \quad (1)$$

where a_{Co} stands for the activity of the protonated dye, A_0 and A_1 represent the absorbance values of the dye in the fully protonated and deprotonated form, A is the absorbance value at a certain equilibrium, and a_{C}^{tot} represents the total activity of the dye, respectively.

Optimization of the Mixing Ratio of the Dyes. To select the most suitable color variation determined by the mixing ratio

of two dyes, we performed a simulation of the color variations for various mixing ratios. According to the Lambert–Beer law, the absorbance $A(\lambda)$ of the membrane containing mixed dyes is expressed as follows

$$A(\lambda) = x_1 A_1(\lambda) + x_2 A_2(\lambda) \quad (2)$$

where x_1 and x_2 represent the amount of each dye (mole) in the membrane and $A_1(\lambda)$ and $A_2(\lambda)$ indicate the molar absorbance of each dye, determined using the membrane containing the individual dyes. The transmittance, T is given by

$$T(\lambda) = 10^{-A(\lambda)} \quad (3)$$

The tristimulus values XYZ are expressed by the following

$$X = k \int_{380}^{780} 10^{-A(\lambda)} P(\lambda) \bar{x}(\lambda) d\lambda \quad (4)$$

$$Y = k \int_{380}^{780} 10^{-A(\lambda)} P(\lambda) \bar{y}(\lambda) d\lambda \quad (5)$$

$$Z = k \int_{380}^{780} 10^{-A(\lambda)} P(\lambda) \bar{z}(\lambda) d\lambda \quad (6)$$

where $\bar{x}(\lambda)$, $\bar{y}(\lambda)$, and $\bar{z}(\lambda)$ are the color-matching functions for the standard observer, $P(\lambda)$ is the intensity of the illuminant as a function of the wavelength, and k is defined

$$k = \frac{100}{\int_{380}^{780} P(\lambda) \bar{y}(\lambda) d\lambda} \quad (7)$$

The $L^*a^*b^*$ values for mixed dyes were calculated by substituting the tristimulus values, XYZ , in eqs 4–6. This calculation was performed using the software Mathematica. The membrane colors obtained by the simulation were represented as color charts by entering the XYZ or $L^*a^*b^*$ values into the COLORTRON software.

RESULTS AND DISCUSSION

Response of the Ion Optode Film Containing an Individual Dye. We investigated the response of the optode film containing the individual dye, KD-C4 or KD-M11 toward Li⁺. The spectra are shown in Figure 4a and b. After normalizing the absorbance (α) at the maximum absorption wavelength by eq 1, the response curves were obtained by plotting the normalized absorbance against the logarithm of the ion concentration as shown in Figure 4c. The corresponding colors observed by the COLORTRON are shown in Figure 4d. As can be observed from the response curves and the color charts, the KD-C4-based optode film responded in the range between 10⁻⁶ and 10⁻² M while changing the color from red to yellow. On the other hand, the optode film containing KD-M11, whose pK_a value is higher than that of KD-C4, responded in the range between 10⁻⁴ and 1 M with color changes from yellow to blue via green. Each dye responded over ~ 4 orders of magnitude to the ion concentration. We assumed that a desirable color variation will be obtained when the pK_a values of the two indicators to be mixed satisfy one of three conditions described as follows: (1) the difference in the

(7) Hisamoto, H.; Miyashita, N.; Watanabe, K.; Nakagawa, E.; Suzuki, K. *Sens. Actuators, B* **1995**, *29*, 378–385.

(8) Suzuki, K.; Tohda, K.; Tanada, Y.; Ohzora, H.; Nishihama, S.; Inoue, H.; Shirai, T. *Anal. Chem.* **1989**, *61*, 382–384.

(9) Suzuki, K. *J. Synth. Org. Chem. Jpn.* **1998**, *59*, 291–302.

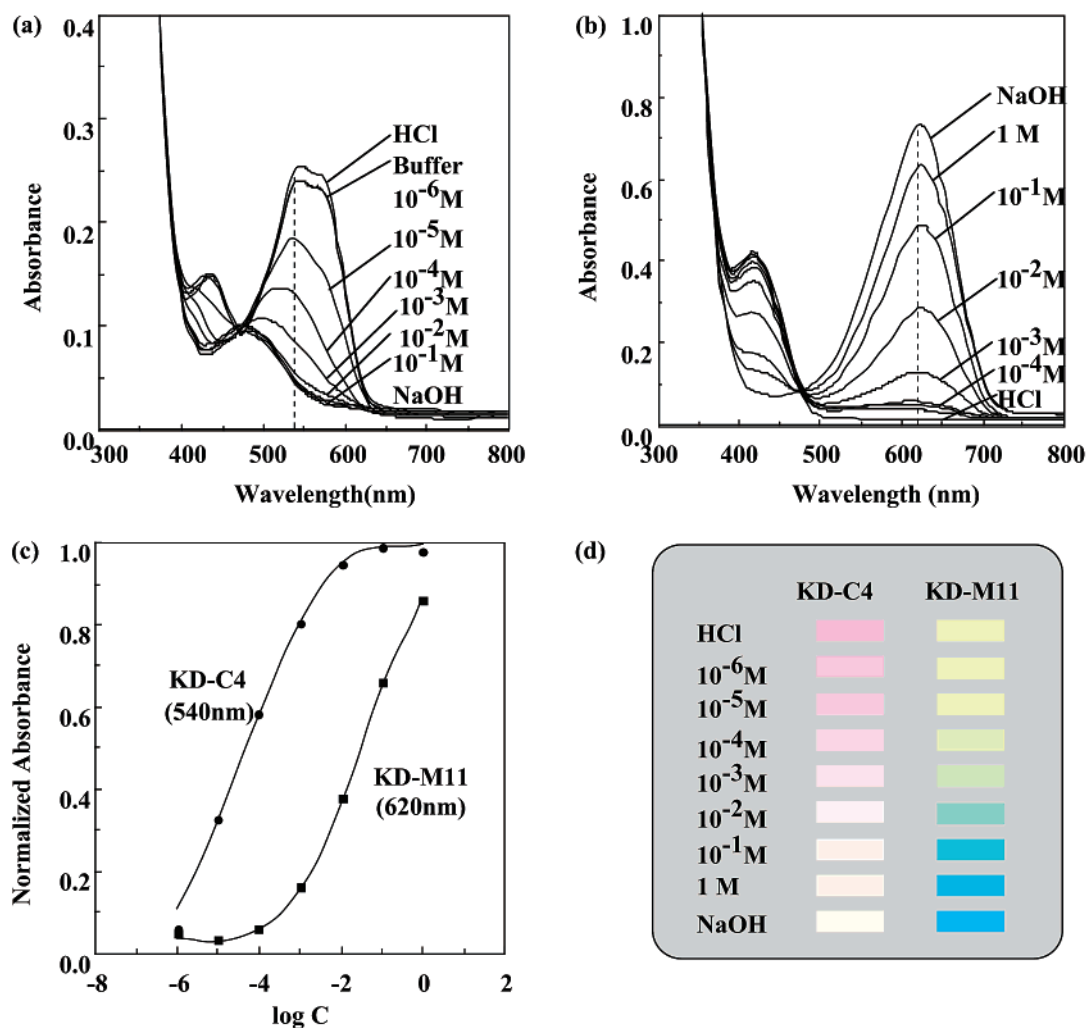


Figure 4. Absorption spectra obtained with the PVC membrane optodes based on TTD14C4, TFPB, NPOE, and KD-C4 (a) or KD-M11 (b) and the corresponding response curves for Li^+ (c); (d) color variations of the Li^+ optode membranes containing KD-C4 and KD-M11, respectively.

$\text{p}K_a$ between the two indicators is more than 4; (2) the difference in the $\text{p}K_a$ between the two dyes is 3; (3) the difference in the $\text{p}K_a$ between the two dyes is less than 2. The widest dynamic range is obtained when the difference of the $\text{p}K_a$ is large as in condition 1; however, a region of no color variation appears in the medium-concentration range, which is disadvantageous for concentration determination in that region. When the difference of the $\text{p}K_a$ is small as in condition 3, the maximum response range is not achieved and there is no sufficient positive effect due to mixing of two dyes. We determined that (2) is the optimum condition leading to a continuous and uniform change in color and, furthermore, to an extension of the dynamic response range. We described the color variation of indicators for pH in the introduction section. It seems likewise that the color variation of the ion optode used in this study depends on the variation of the Li^+ concentration ($\log C$). In this case, the value corresponding to the $\text{p}K_a$ is the logarithm of the lithium ion concentration at $\alpha = 0.5$.

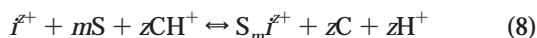
Lithium Ion Optode Based on a Lipophilic Cationic Dye.

The response mechanism of the Li^+ film optode used in this study is based on the ion extraction from a sample solution as an

aqueous phase into a PVC membrane as an organic phase.¹⁰ The PVC membrane contains a lithium ionophore as an ion recognition element, lipophilic cationic dyes as indicator reagents, and a lipophilic anionic additive. A 14-crown-4 derivative (TTD14C4) was chosen as the lithium ionophore.⁹ Crown ethers easily form complexes with metal ions, and the cavity size of a 14-membered crown particularly fits the Li^+ ionic radius. To avoid the formation of sandwich complexes with Na^+ and K^+ , whose radii are larger than the crown cavity, tetramethyl groups acting as steric hindrance subunits are introduced into TTD14C4. When the PVC membrane is immersed in the sample solution, Li^+ forms a complex with the ionophore and is extracted into the membrane. At the same time, the cationic dyes are deprotonated in order to maintain the electroneutrality in the membrane phase. Thus, at constant pH, the color variations correspond to the desired target ion concentration.⁵ In the case where a nonpolar plasticizer is used, it is well known that ion pairs are formed between the dyes and the anionic additive. However, NPOE used in this study is comparatively polar and the formation of ion pairs is additionally obstructed by steric hindrance and shielding of the electric charge

(10) Bakker, E.; Buhlmann, P.; Pretsch, E. *Chem. Rev.* **1997**, *97*, 3083–3132.

of the anionic additive. Therefore, in this study, we adopted a simple theory that does not consider the formation of ion pairs.⁹ The equilibrium of the ion exchange reaction and the ion exchange constant (K_{exch}^i) can be described by the following eqs 8 and 9 where i represents the cation with the charge z to be



$$K_{\text{exch}}^i = \frac{(a_{H^+})^z}{a_{i^{z+}}} \left(\frac{[C]}{[CH^+]} \right)^z \frac{[S_m i^{z+}]}{[S]^m} = \frac{k_{i^{z+}}}{(k_{H^+})^z} \left(\frac{\gamma_{CH^+}}{\gamma_C} \right)^z \frac{(\gamma_S)^m}{\gamma_{S_m i^{z+}}} \beta_{S_m i^{z+}} (K_a)^z \quad (9)$$

extracted (the analyte Li^+ in the present case). The abbreviations S, CH^+ , Si, and C in eqs 8 and 9 stand for the neutral ionophore, the protonated dye, the ion–ionophore complex, and the deprotonated dye, respectively. The terms a , k , γ , β , and K_a in eq 9 stand for the activity in the aqueous phase, the partition coefficient between the aqueous and the organic phase, the activity coefficient of each chemical species in the organic phase, the formation constant of the ion–ionophore complex, and the acidity constant of the dye, respectively. As the lithium ion is univalent and it is assumed to form a 1:1 complex with TTD14C4, the ion exchange constant (K_{exch}) can be expressed by eq 10. The ionic strength

$$K_{\text{exch}}^i = \frac{k_{i^{z+}}}{k_{H^+}} \frac{\gamma_{CH^+}}{\gamma_C} \frac{\gamma_S}{\gamma_{S_m i^{z+}}} \beta_{S_m i^{z+}} K_a \quad (10)$$

within the optode membrane is assumed to be constant due to the presence of the anionic additives, and therefore, the activity coefficients (γ) in eq 10 can be regarded as constants. In addition, as the complex formation constant (β) and the partition coefficient (k) are fixed, the difference in $\log K_{\text{exch}}$ will be 3 if the $\text{p}K_a$ difference between the two dyes is 3. In that case, it is supposed that the optode shows a suitable color variation due to a shift of 3 orders of magnitude in the dynamic response range for Li^+ . The $\text{p}K_a$ values of KD-C4 and KD-M11 in $\text{EtOH:H}_2\text{O} = 1:1$ are 2.7 and 5.7, respectively. Determined from the experimental results obtained with separate membranes for each dye, the $\log K_{\text{exch}}$ of KD-C4 and KD-M11 were -2.7 and -5.4 , respectively. The experimentally evaluated difference of $\log K_{\text{exch}}$ between KD-C4 and KD-M11 was 2.7. Consequently, it seems that these dyes are a suitable combination.

Examination of the Mixing Ratios of the Dyes by Simulation. We performed a simulation of the color variation for various mixing ratios of the dyes using the 1976 CIE $L^*a^*b^*$ color space and a^*b^* coordinates as described in the Experimental Section. Figure 1a shows the concept of the $L^*a^*b^*$ color space, which was proposed by the Commission Internationale de l'Éclairage as a visually uniform color expression system. The a^* , b^* , and L^* axis show red–green, yellow–blue, and brightness, respectively. The $L^*a^*b^*$ values are expressed as follows

$$a^* = 500 \{ (X/X_n)^{1/3} - (Y/Y_n)^{1/3} \} \quad (11)$$

$$b^* = 200 \{ (Y/Y_n)^{1/3} - (Z/Z_n)^{1/3} \} \quad (12)$$

$$L^* = 116(Y/Y_n)^{1/3} - 16 \quad (13)$$

where X_n , Y_n , and Z_n are the tristimulus values of a completely diffuse and reflective surface (standard white plate of magnesium oxide), $X_n = 96.422$, $Y_n = 100.00$, and $Z_n = 82.521$. The color difference, ΔE is expressed as follows

$$\Delta E_{ab}^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (14)$$

and corresponds to the color variation perceived by human eyes. Hence, the $L^*a^*b^*$ color space is a visually uniform color space and is quite useful for designing devices suitable for visual colorimetry. The angle originating from the positive direction of the a^* axis in Figure 1b expresses the hues themselves and is defined as the hue angle, h_{ab} , expressed by eq 15.¹¹ When we

$$h_{ab} = \tan^{-1}(b^*/a^*) \quad (15)$$

evaluate color variation qualities of sensors, it is important to evaluate both a color difference (ΔE) and a hue angle variation (Δh_{ab}). As an example of an investigation using h_{ab} , Tlaczala and Bartecki^{12,13} evaluated the solvatochromism for metal ion complexes using h_{ab} . They studied the relationship between the acceptor number (E_T values) of solvents and the h_{ab} values. In general, solvatochromism refers to variations of absorbance spectra influenced by solvents. The maximum absorption wavelength (λ_{max}) shifts by changing the electronic energy levels under the influence of solvent polarities. Hues, chromaticity, and brightness as parameters expressing colors are related to the λ_{max} values, the sharpness of peaks, and the absorbance values of spectra. It is concluded that h_{ab} values are useful for evaluating color variations of solvatochromic dyes induced by solvent polarity variations.

The $L^*a^*b^*$ values were calculated at each concentration of Li^+ in the range 10^{-6} – 1 M when the mixing ratio of KD-C4/KD-M11 was 9/2, 3/1, 2/1, and 3/2 (mole/mole), respectively. The appearance of the color variations was expressed by plotting the values on the a^*b^* coordinates as shown in Figure 5a. The color differences (ΔE) calculated from the beginning (10^{-6} M) to the end (1 M) of Li^+ concentration variations were 43, 40, 51, and 55 when the mixing ratio was 9/2, 3/1, 2/1, and 3/2 (mole/mole), respectively. Figure 5b shows the membrane colors obtained by the simulation and the hue angle variations (Δh_{ab}) that were calculated for changing the Li^+ concentrations between 10^{-6} and 1 M. As a result, it was shown that ΔE was largest for a mixing ratio of KD-C4/KD-M11 of 3/2 and 2/1, whereas in the case of the hue angle changes Δh_{ab} , the variation was largest when the mixing ratio was 3/1 and 9/2, respectively. It is not necessarily true that the number of hues increases with larger ΔE values because the latter just quantifies the color difference perceived by the human eye. The degree of Δh_{ab} is more important than ΔE in order to obtain a larger variety of hues allowing one to

(11) Japanese Industrial Standard, color, Z8729, 1996, Japanese Standard Association.

(12) Tlaczala, T. *Pol. J. Chem.* **1997**, 71, 823–830.

(13) Tlaczala, T.; Bartecki, A. *Monatsh. Chem.* **1997**, 128, 225–234.

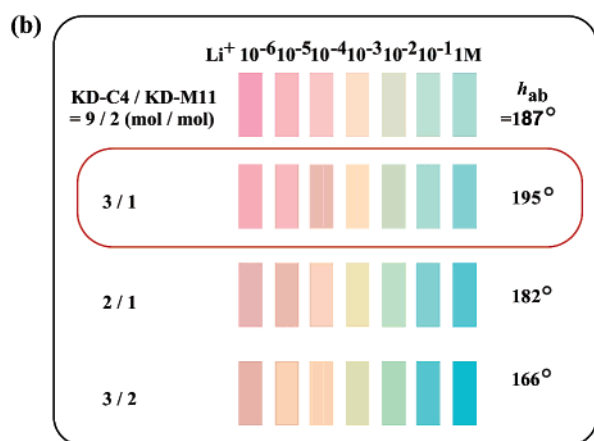
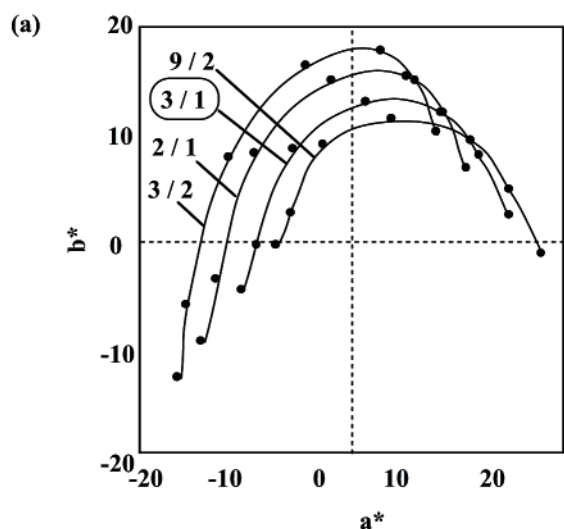


Figure 5. (a) a^*b^* values for the simulated color variation of the optode membranes incorporating the mixed dyes (KD-C4/KD-M11) in different mixing ratios and (b) the corresponding simulated color variations.

distinguish between smaller concentration changes, and therefore, the mixing ratio of 3/1 for the dyes was chosen by simulation. Additionally, the individual absorbance values of KD-C4 and KD-M11 at λ_{\max} were approximately equal when the mixing ratio of KD-C4/KD-M11 was 3/1.

Response of the Optode Containing the Mixed Dyes. On the basis of the simulation result, we investigated the actual response of the optode film containing the mixture of dyes, in which the molecular ratio of KD-C4/KD-M11 was 3/1. The colors observed with the COLORTRON are shown in Figure 6a. The hue angle variation of the mixed dyes was larger than that of an individual dye and the color variation was clear and visually distinguishable. The color differences (ΔE) for individual KD-C4, individual KD-M11, and mixed KD-C4/KD-M11 were 30, 44, and 54, respectively. The hue angle changes (Δh_{ab}) for individual KD-C4, individual KD-M11, and mixed KD-C4/KD-M11 were 98, 112, and 187°, respectively. The dynamic concentration response range for individual KD-C4, individual KD-M11, and mixed KD-C4/KD-M11 was 10^{-6} – 10^{-2} , 10^{-4} –1, and 10^{-6} –1 M, respectively. The values observed for the mixed dyes were larger in all cases than for an individual dye; in particular, the hue angle was improved. Comparing the simulation results with the experimental data, the predicted tendencies agreed approximately. However,

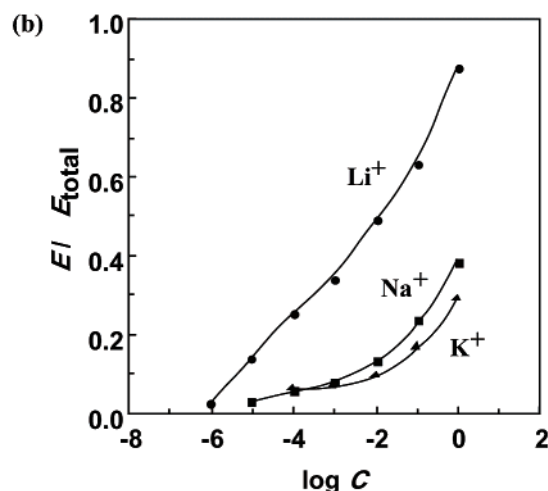
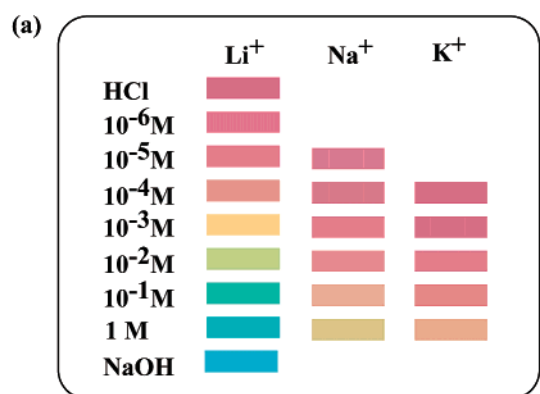


Figure 6. Selectivity of the optode based on the mixed dyes for Li⁺ over Na⁺ and K⁺: (a) color changes for the optode membrane based on the mixed dyes (KD-C4 + KD-M11), TTD14C4, TFPB, PVC, and NPOE measured for Li⁺, Na⁺, and K⁺ sample solutions using the COLORTRON; (b) correlation between the ion concentration ($\log C$) and $\Delta E/\Delta E_{\text{Total}}$.

the a^*b^* coordinates and the Δh_{ab} values obtained by simulation were slightly different from those of the experimental results. Concerning this point, it has to be noted that the a^*b^* values were large on the whole because thicker membranes were prepared for the mixed dyes than for the individual dyes. Thicker membranes were applied to guarantee sufficient color perception since the amount of each dye is kept small. As we evaluated the experimental results, the direction of the color variation of the two dyes on the a^*b^* coordinates was found to be continuous and satisfying the condition as shown in Figure 2b. The color variation of the two dyes had no offset, and multicolor variation was realized over a wide dynamic range of the ion concentration. Therefore, we were able to develop an advantageous optode device for visual colorimetry by mixing dyes after selecting the optimal combination by making use of characteristics such as color variations and pK_a differences of indicators.

Evaluation of Selectivity. We investigated the selectivity of the optode film containing the mixed dyes. To determine the selectivity of a conventional optode, the selectivity coefficient (K_j^{opt})⁸ can be graphically estimated by plotting the normalized absorbance (α) against the $\log C$ values of each ion and measuring the horizontal distance between the calibration curves. Because the optode membrane in this study contains two kinds of dyes, K_j^{opt} cannot be calculated in this manner. Hence, we evaluated

the selectivity of the optode film for Li^+ over other ions using $\Delta E/\Delta E_{\text{total}}$ values. ΔE was calculated from the beginning of the color variation on the a^*b^* coordinates (10^{-6} M Li^+ in this study) to the point of each respective ion concentration. ΔE_{total} expresses the maximal possible color difference of the optode film measured from the completely protonated (HCl, pH 2) to the fully deprotonated state (NaOH, pH 11). Because the calibration curves of the color variation form arcs in the a^*b^* coordinate system, a simple calculation that does not consider the pathways of the color variations must be expressed by the shortest distance. ΔE and ΔE_{total} were calculated by accumulating each color difference ($L^*a^*b^*$ values) from the beginning to the end of the variation. Figure 6a shows the color variations measured by the COLOR-TRON for Li^+ , Na^+ , and K^+ . Figure 6b shows the correlation between the ion concentrations $\log C$ and $\Delta E/\Delta E_{\text{total}}$. When the values corresponding to the conventional selectivity coefficient were calculated from Figure 6b in the usual manner, the selectivity for Li^+ over Na^+ and K^+ was about 1000 and 5000 times, respectively. These selectivity values correspond quite well to $\log K_{\text{Li}^+,\text{Na}^+}^{\text{pot}} = -3.1$ (~ 1300 times) and $\log K_{\text{Li}^+,\text{K}^+}^{\text{pot}} = -3.7$ (~ 5000 times), found for ion-selective electrodes using the same ionophore.

CONCLUSION

In this study, a lithium ion multicolor film optode suitable for visual colorimetry based on DCA was designed and prepared. By applying mixed indicators for the optode film after selecting the best combination based on characteristics such as color variations and $\text{p}K_{\text{a}}$ of the indicators, this film showed many hues that varied over the desired ion concentration range. This color variation allows accurate visual colorimetry. In the case of using the color-changeable dyes KD-C4 and KD-M11 individually for the lithium ion optode, the dynamic range of the optode film was 10^{-6} – 10^{-2} and 10^{-4} – 1 M, respectively. However, visual colorimetry became feasible over the range of 10^{-6} – 1 M using the mixed dyes. This simple and easily handled membrane optode based on DCA is suitable for easy measurements such as visual colorimetry, which is expected to be applicable for field assays of environmental samples and clinical examinations in private households. We demonstrated a lithium ion film optode in the present study. However, other optodes for other ions can be prepared in the same manner by choosing a suitable ionophore from the wide selection that we have developed.⁹

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