pH Indicator with Dual ^{19}F NMR and VIS Responses

Kunihiro Hamada,*[a] Takeshi Hirano,[a] Masaru Mitsuishi,[a] Manabu Ohira[b] and Koyuki Mesuda[b]

[a] *Faculty of Textile Science and Technology, Shinshu University, 3-15-1 Tokida, Ueda-shi, Nagano 386, Japan*
[b] *Central Research Laboratory, Showa Denko K. K., 2-24-25 Tamagawa, Ohta-ku, Tokyo 146, Japan*

A pH indicator, sodium 1-(4-*N*,*N*-dimethylaminophenylazo)-2-trifluoromethyl-4-benzenesulfonate, has been prepared, which makes it possible to measure using two kinds of spectroscopy, *i.e.* ^{19}F NMR and VIS spectroscopy.

pH Values in microenvironments such as micelles, lipid bilayers and cells are one of the most important factors to establish their properties. Useful pH range is *ca.* 7 in cells, but a wider range is useful for bipolymers such as BSA. Many researchers have attempted to exploit indicators sensitive to pH change. Azo indicators are the most popular[1] and fluorescent indicators are often used.[2] Recently a new class of ^{19}F pH indicators was reported,[3] whose chemical shifts are a function of pH. Most of these pH indicators give information from only one kind of spectroscopy, although their benzene rings absorb in the UV region.

In the present study, we designed the preparation of a pH indicator which is measurable by means of two kinds of spectroscopy, *i.e.* ^{19}F NMR and VIS spectroscopy. Taking account of the frequent use of methyl orange as a pH indicator,[1] a trifluoromethyl group was introduced into the benzene ring of methyl orange: sodium 1-(4-*N*,*N*-dimethylaminophenylazo)-2-trifluoromethyl-4-benzenesulfonate **1** was synthesized.

The buffer solutions with various pH values and a constant dye concentration were prepared; the buffer compositions were as follows: pH = 1.03, KCl (0.05 mol dm^{-3}) + HCl (0.1 mol dm^{-3}); pH = 1.59, KCl (0.05 mol dm^{-3}) + HCl (0.026 mol dm^{-3}); pH = 1.89, KCl (0.05 mol dm^{-3}) + HCl (0.013 mol dm^{-3}); pH = 2.16, KCl (0.05 mol dm^{-3}) + HCl (0.0067 mol dm^{-3}); pH = 2.34, potassium dihydrogen citrate $KH_2C_6H_5O_7$ (0.05 mol dm^{-3}) + HCl (0.0434 mol dm^{-3}); pH = 2.69, $KH_2C_6H_5O_7$ (0.05 mol dm^{-3}) + HCl (0.0335 mol dm^{-3}); pH = 3.00, $KH_2C_6H_5O_7$ (0.05 mol dm^{-3}) + HCl (0.0236 mol dm^{-3}); pH = 5.06, $KH_2C_6H_5O_7$ (0.05 mol dm^{-3}) + NaOH (0.0467 mol dm^{-3}); pH = 7.01, KH_2PO_4 (0.05 mol dm^{-3}) + NaOH (0.028 mol dm^{-3}). The VIS spectra of the buffer solutions (dye concentration = 3.00×10^{-5} mol dm^{-3}) were measured using a Shimadzu UV-3100 spectrophotometer at 298 K. A Bruker AM-X400 NMR spectrometer (376 MHz for ^{19}F nucleus) was used to record the ^{19}F NMR spectra of the solutions (dye concentration = 8.89×10^{-5} mol dm^{-3}, containing 11.1% D_2O) at 323 K. Chemical shifts, δ, were calculated on the basis of external reference (CF_3CO_2H, δ −76.5).

High accuracy of NMR spectra requires rather high dye concentrations. However, such high concentrations lead to dye aggregation as described for the fluorinated derivatives of sodium 1-phenylazo-2-hydroxy-6-naphthalene-sulfonate in our previous papers.[4–8] In the aqueous solutions of these dyes with 1×10^{-4} mol dm^{-3}, about 25% of the dyes formed dimers, so that in the high concentration region their ^{19}F NMR spectra cannot be analysed without considering the dye aggregation. On the other hand, the VIS spectra of the dye **1** in aqueous solutions were independent of dye concentration from 1×10^{-5} to 1×10^{-3} mol dm^{-3} in water and from 1×10^{-5} to 1×10^{-4} mol dm^{-3} in any buffer solutions (solubility is $<2 \times 10^{-5}$ mol dm^{-3} in buffers with low pH), indicating that the dye does not form any aggregates in the concentration range examined. From this, it is concluded that the spectra of the dye monomer can be recorded even in the high concentration region.

Considering the accuracy of the NMR measurements and the low solubility in the buffer, the concentration of 8.89×10^{-5} mol dm^{-3} was selected. Furthermore, to avoid the broadening of the NMR spectra,[3] the temperature of 323 K was preferred. All the NMR spectra observed over the pH range from 1 to 7 consisted of one singlet. The chemical shifts of the fluorine atoms as a function of the pH values are shown in Fig. 1. The signals showed progressive shifts to higher magnetic field with increasing pH, reflecting the change in the electron structure of the dye **1**. The VIS spectra of the dye buffer solutions with isosbestic points (275 and 360 nm) are shown in Fig. 2. The apparent extinction coefficients, ε, at the maximum absorption wavelength of 470 nm increased with an increase in the pH values (Fig. 1). Thus, the pH values significantly affect both the δ and ε values: the pH change can be detected using two kinds of spectroscopy.

The pH dependence of δ and ε is believed to be due to the

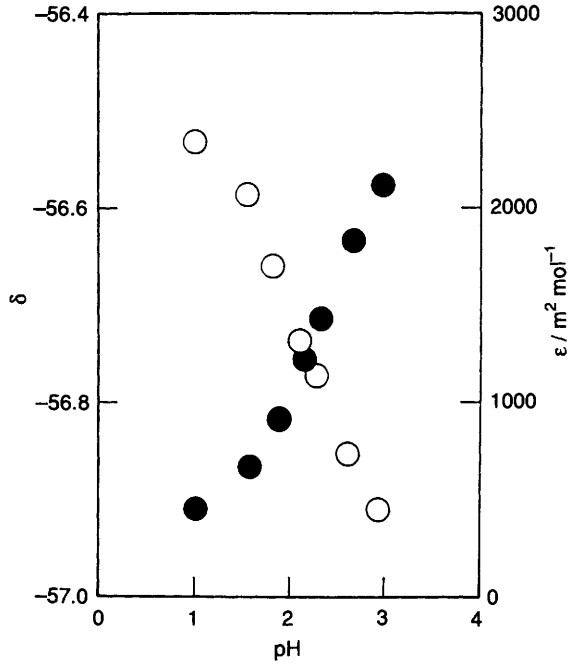


Fig. 1 Dependence of chemical shifts at 323 K (○) and the extinction coefficients at 298 K and 470 nm (●) on pH values

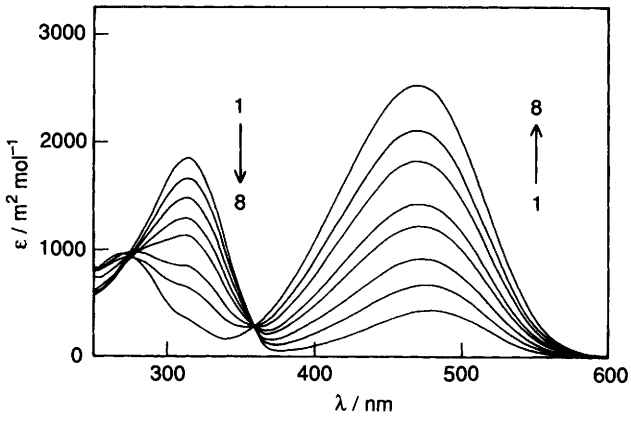


Fig. 2. VIS absorption spectra of the dye buffer solutions: 1, pH = 1.03; 2, pH = 1.59; 3, pH = 1.89; 4, pH = 2.16; 5, pH = 2.34; 6, pH = 2.69; 7, pH = 3.00; 8, pH = 5.06 or 7.01

acid-base equilibrium in eqn. (1), where DH^+ represents the

$$DH^+ \rightleftarrows D + H^+ \quad (1)$$

conjugate acid form of the azo dye **1**, D the conjugate base form and H^+ the hydrogen ion. From the above equilibrium, eqns. (2) and (3) can be derived;[9]

$$\delta = -\frac{(\delta - \delta_D)10^{pH}}{10^{pK_a}} + \delta_{DH} \quad (2)$$

$$\varepsilon = -\frac{(\varepsilon - \varepsilon_D)10^{pH}}{10^{pK_a}} + \varepsilon_{DH} \quad (3)$$

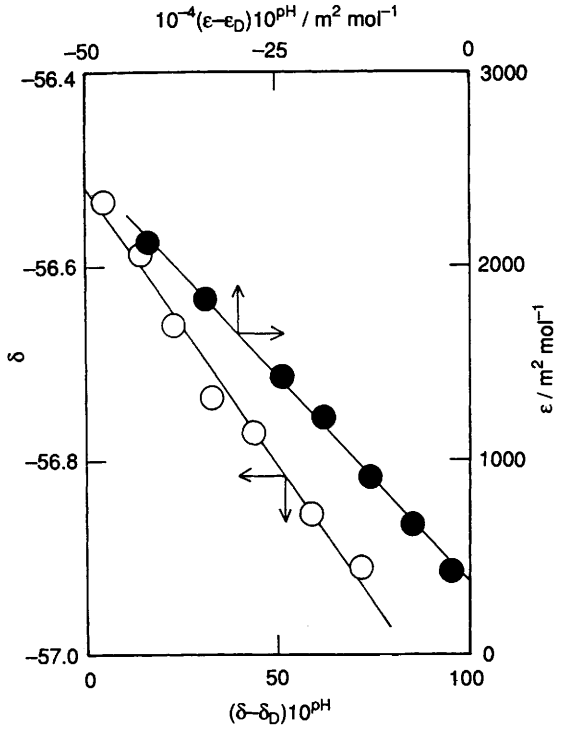
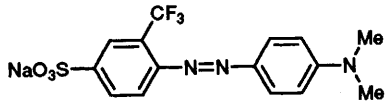


Fig. 3 Plots of δ against $(\delta - \delta_D)10^{pH}$ (○) and ε against $(\varepsilon - \varepsilon_D)10^{pH}$ (●)



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where $pK_a = -\log K_a$ is the acid dissociation constant. δ, δ_D and δ_{DH} are the observed chemical shift, the chemical shifts of the conjugate base and acid form, respectively. ε, ε_D and ε_{DH} are the corresponding extinction coefficients to the above chemical shifts. The plots of δ against $(\delta - \delta_D)10^{pH}$ and ε against $(\varepsilon - \varepsilon_D)10^{pH}$ gave straight lines (Fig. 3), where the δ and ε values measured at pH = 7 were used as δ_D and ε_D, respectively because δ and ε were constant for the buffer solutions with pH more than 5. The pK_a values determined from the slopes of the lines were 2.25 ± 0.05 and 2.37 ± 0.02, respectively for ^{19}F NMR and VIS spectroscopy, suggesting that both the values are consistent taking into consideration the difference in measured temperature. The above results demonstrate that the fluorinated azo dye **1** can be used as a pH indicator with both ^{19}F NMR and VIS spectroscopy although its low solubility in the buffer is a disadvantage.

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