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Capillary electrophoretic and spectrophotometric investigations of the complexation of Methylene Blue with 2-naphthol-6-sulfonate and 1,2-naphthoquinone-4-sulfonate in solution

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Abstract

In capillary electrophoresis of Methylene Blue (MB), a signal was observed below about pH 5.4. Upon the addition of 2-naphthol-6-sulfonate (NS) or 1,2-naphthoquinone-4-sulfonate (NQS) to MB solution buffered at pH 2.7, the migration time of MB was prolonged. The delay of the migration time has been attributed to the formation of an organic cation–organic anion complex between MB and NS (or NQS). From variations of the migration time, the equilibrium constants (K) for the formation of 1:1 complexes with NS and NQS have been found to be 430 ± 10 and 480 ± 70 mol^{-1} dm^3, respectively. In the presence of NS or NQS, the absorption spectrum of MB was shifted to longer wavelengths, supporting the suggestion of formation of a complex between MB with NS or NQS. From absorbance changes, the K values for NS and NQS were found to be 350 ± 20 and 730 ± 260 mol^{-1} dm^3, respectively.

Keywords: Capillary electrophoresis; Absorption spectra; Methylene Blue; Organic cation–organic anion complexes; Equilibrium constants

1. Introduction

Capillary electrophoresis is a very useful, analytical technique for separating various kinds of isomers, such as structural, positional, and enantiomeric isomers [1–11]. Although capillary electrophoretic separation of fluorescein dyes has been examined, few organic dyes have been investigated by capillary electrophoreses [12]. In spite of many capillary electrophoretic studies concerning the separation of analogous substances etc., there are few capillary electrophoretic studies concerning the properties of substances.

The interactions between an organic cation and an organic anion have mainly been studied through electronic absorption and fluorescence spectroscopy [13–17]. We have examined the formation of organic cation–organic anion complexes of Methylene Blue (MB) with 1- and 2-naphthalene-

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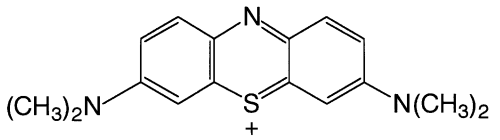
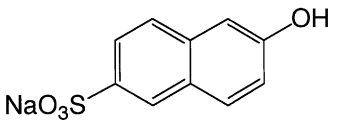
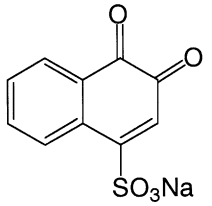


Methylene Blue (MB)



Sodium 2-Naphthol-6-sulfonate (NS)



Sodium 1,2-Naphthoquinone-4-sulfonate (NQS)

sulfonates and 2-anthracenesulfonate [17]. MB also forms a complex with anionic dyes such as Acid Orange 7, α-Naphthol Orange, and tetrakis(4-sulfonatophenyl)porphyrin [18–20]. Furthermore, we have examined the interactions of γ-cyclodextrin with several organic cation–organic anion complexes, such as complexes of MB–Acid Orange 7, MB–α-Naphthol Orange, MB–tetrakis(4-sulfonatophenyl)porphyrin and thionine–2-napthalenesulfonate [18–21].

Using capillary electrophoresis, Takayanagi et al. have recently investigated ion association between primary or quaternary alkylammonium ions with aromatic anions [22–24]. They have further examined the ion association between aromatic cations and aromatic anions [25]. In the case of ion association between aromatic cations and aromatic anions, however, the interactions between their aromatic rings seem to occur besides the electrostatic interactions. Thus, we examined the formation of organic cation–organic anion complexes by means of capillary electrophoresis as well as electronic absorption spectroscopy. For this purpose, we selected MB as an organic cation, and 2-naphthol-6-sulfonate and 1,2-naphthoquinone-4-sulfonate as organic anions.

2. Experimental

2.1. Reagents

Methylene Blue (MB), which was obtained from Wako Pure Chemical Ind., was used as received. Sodium 2-naphthol-6-sulfonate (NS) and sodium 1,2-naphthoquinone-4-sulfonate (NQS), which were purchased from Tokyo Kasei Kogyo, were recrystallized from a water–ethanol mixture.

After the recrystallization of NQS, the color of its crystals became slightly light, although the purity of NQS could not be examined quantitatively. Buffers of pH 2.7 comprised HCl (1.9×10^{-2} mol dm^{-3}) and CH_3COONa (2.0×10^{-2} mol dm^{-3}).

2.2. Apparatus

Capillary electrophoresis was performed using a Hitachi U-2000 spectrophotometer and a Matsusada Precision Devices HCZE-30 PN apparatus as a detector and a high-voltage power supply, respectively. The detection wavelength used was 660 nm. The applied voltage was maintained at 15.0 kV throughout this study. A GL Science uncoated fused-silica capillary (70.0 cm×0.05 mm I.D.) was employed for capillary electrophoresis; the effective length of the capillary was 30.0 cm (from an injection end to a detection window). Samples were hydrodynamically injected into the capillary. Absorption spectra were run on a Shimadzu UV-260 spectrophotometer. All measurements were made at 25 °C.

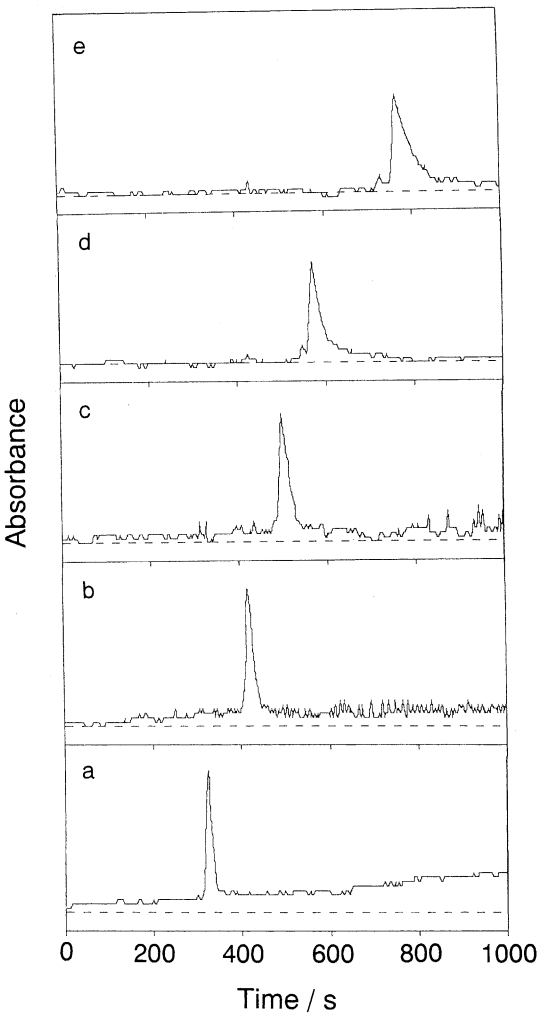


Fig. 1. Electropherograms of MB (5.0×10^{-5} mol dm^{-3}) in running buffer (pH 2.7) containing various concentrations of NS. Concentration of NS: (a) 0, (b) 1.0×10^{-3}, (c) 2.0×10^{-3}, (d) 3.0×10^{-3}, and (e) 5.0×10^{-3} mol dm^{-3}. The ordinate scales of the electropherograms (a)–(e) are the same.

3. Results and discussion

3.1. Capillary electrophoresis of MB in the absence and presence of NS and NQS

In the capillary electrophoresis of MB, we could not detect a signal for neutral or alkaline solutions of MB, probably because MB had been adsorbed on the capillary wall. Although, at pH 5.4, a signal was observed, it was broad in width, suggesting slight adsorption of MB on the capillary wall (not shown). Below about pH 4, however, the signal had a shape typically observed in capillary electrophoresis, suggesting no adsorption of MB. Fig. 1 shows the electropherograms of MB (5.0×10^{-5} mol dm^{-3}) in pH 2.7 buffers containing various amounts of NS. In the absence of NS (Fig. 1a), the signal due to MB appeared at 330 s. When NS was added to the MB solution, the migration time of MB was increased. Because MB carries a positive charge, this finding indicates that the positive charge on an MB molecule was neutralised by a negative charge and/or that the

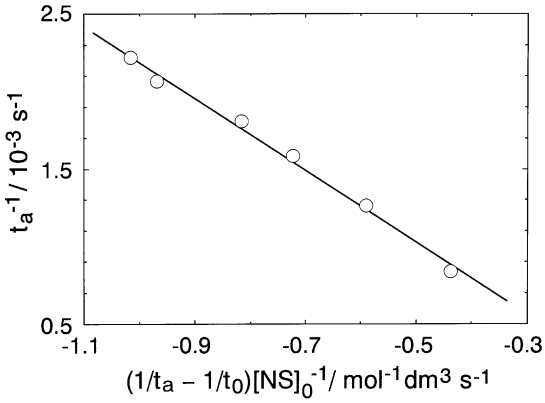


Fig. 2. Plot of $1/t_a$ against $(1/t_a-1/t_0)/[NS]_0$ for MB (5.0×10^{-5} mol dm^{-3}) in pH 2.7 buffer containing NS.

effective mass (volume) of an MB molecule had been increased. Consequently, the delayed migration time implies the formation of an organic cation–organic anion complex between MB and NS.

$$\mathrm{MB} + \mathrm{NS} \overset{K}{\rightleftharpoons} \mathrm{MB{\cdot}NS} \quad (1)$$

Here, K is the equilibrium constant for the formation of the complex (MB·NS) between MB and NS. When the MB–NS complex has a 1:1 stoichiometry, a K value can be estimated on the basis of the equation [10].

$$1/t_a = 1/t_1 - (1/t_a - 1/t_0)/\left(K[\mathrm{NS}]_0\right) \quad (2)$$

Here, t_a, t_1, t_0, and $[\mathrm{NS}]_0$ are the observed migration time of MB, the migration time of the MB–NS complex, the migration time of free MB, and the initial concentration of NS, respectively. Eq. (2) is essentially equivalent to the equation for electrophoretic mobility [10,11]. Fig. 2 shows a plot obtained for MB solution containing NS according to Eq. (2). A good fit of the data to Eq. (2) suggests that the stoichiometry of the MB–NS complex is 1:1. From the plot, K was found to be 430 ± 10 mol^{-1} dm^3. This K value was nearly the same as that (400 mol^{-1} dm^3) obtained for the formation of the complex between MB and 2-naphthalenesulfonate, which was evaluated from absorbance change [17]. Consequently, a hydroxy group of NS exerts little effect on complexation with MB. Upon the addition of NS, an absorption spectral change of MB was observed. This finding indicates the formation of a complex rather than simple ion-association, although electrostatic attraction between MB and NS plays a critical role in the formation of the complex.

We further examined the organic cation–organic anion interactions between MB and NQS. As the concentration of NQS increased, the electrophoretic signal of MB was delayed, indicating the formation of a complex between MB and NQS (not shown). As in the case of NS, analysis of the migration time afforded a K value of 480 ± 70 mol^{-1} dm^3 for the MB–NQS complex. This K value for NQS was slightly greater than that for NS, probably due to the existence of the quinone structure in NQS, which extends the conjugated system of the π-electrons to the outside of the naphthalene ring. In capillary electrophoresis of MB, the tailing of the signal by the addition of NQS was more prominent than that of NS. Although the reason for the conspicuous tailing for NQS is not clear at present, there may be the possibility that the MB–NQS complex has two conformations with respect to the relative orientation of MB and NQS.

The K values of MB for 1-naphthalenesulfonate, 2-naphthalenesulfonate, and 1-naphthaleneacetate have been reported to be 180, 400, and 240 mol^{-1} dm^3, respectively [17]. These K values are about half those for NS and NQS, except for the K value for 2-naphthalenesulfonate, which is close to those for NS and NQS.

3.2. Absorption spectral change of MB in solution in the presence of NS and NQS

Fig. 3 illustrates the absorption spectra of MB (5.0×10^{-6} mol dm^{-3}) in pH 2.7 buffer containing various concentrations of NS. In the measurement of the absorption spectra, the MB concentrations were one-tenth more dilute than those used in capillary electrophoresis. At a concentration of 5.0×10^{-6} mol dm^{-3}, MB mainly exists as a monomer. Upon the addition of NS, the absorption maximum of MB was shifted to longer

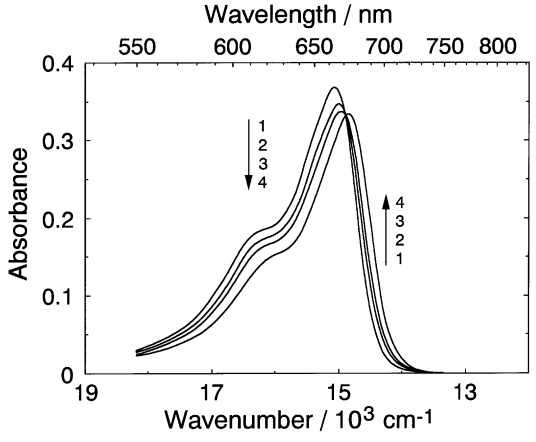


Fig. 3. Absorption spectra of MB (5.0×10^{-6} mol dm^{-3}) in pH 2.7 buffers containing various concentrations of NS. Concentration of NS: (1) 0, (2) 1.0×10^{-3}, (3) 2.0×10^{-3}, and (4) 1.0×10^{-2} mol dm^{-3}.

wavelengths, accompanied by a reduction at the λ_{max}; at the same time, an isosbestic point appeared at 671 nm. As stated previously, simple ion-association between a positively charged and a negatively charged species would not induce such a spectral change. Consequently, these spectral changes can be attributed to the formation of a complex between MB and NS, which is consistent with the change observed in the capillary electropherograms. In this complex formation, it is most likely that the π–π interaction occurs between the aromatic rings of MB and NS.

When a 1:1 complex is formed, the Benesi–Hildebrand equation can be used for the evaluation of K [26,27];

$$1/(A - A_0) = 1/a + 1/(aK[\mathrm{NS}]_0) \quad (3)$$

where A, A_0, and a are the absorbances in the presence and absence of NS and a constant, respectively. Fig. 4 shows a Benesi–Hildebrand plot for MB in pH 2.7 buffer containing NS. This plot exhibits a straight line, indicating the formation of an MB–NS complex with 1:1 stoichiometry. From the plot, the K value for the formation of the complex was found to be 350 ± 20 mol^{-1} dm^3, which was almost the same as that (430 ± 10 mol^{-1} dm^3) evaluated from capillary electrophoresis.

In addition to the MB–NS system, we examined the interactions between MB and NQS by means of absorption spectroscopy. With an increase in the concentration of NQS, the absorption maximum of MB shifted to longer wavelengths, this being accompanied by isosbestic points at 569 and 679 nm (not shown). This finding supports complex formation rather than ion-association between MB and NQS. As for NS, K value was evaluated as 730 ± 260 mol^{-1} dm^3 from a plot derived from Eq. (3). This K value was comparable to that evaluated from capillary electrophoresis. Because the error of the K value evaluated from spectrophotometry was greater than that obtained from capillary electrophoresis, however, the latter K value seems to be more reliable.

As previously stated, the MB concentrations used for measurement of the absorption spectra were one tenth of those used in capillary electrophoresis. In capillary electrophoresis, therefore, it is likely that MB partly exists in a dimeric form. Nonetheless, the K values of NS were almost the same for capillary electrophoresis and spectrophotometry, although the K values of NQS were comparable for the both methods. Since dimerization of MB leads to increases in both the effective charge and effective molecular mass (volume) of MB, the increases in the two factors may be cancelled with respect to electrophoretic mobility; the migration times were nearly the same for both the monomer and dimer of MB. Consequently, dimerization of MB seems to exert little or no effect on migration time.

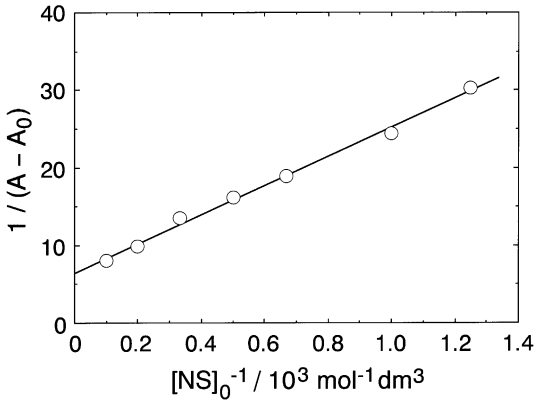


Fig. 4. Benesi–Hildebrand plot for MB (5.0×10^{-6} mol dm^{-3}) in pH 2.7 buffers containing NS. $\lambda_{obs} = 685$ nm.

4. Conclusions

In capillary electrophoresis of MB, its signal could be detected below about pH 5.4, and was not observed above about pH 5.4, probably because MB had been adsorbed on the capillary wall. In this study, we have examined the interactions of MB and NS (or NQS) in pH 2.7 buffer using capillary electrophoresis. In the presence of NS (or NQS), the migration time of MB was prolonged due to the formation of an organic cation–organic anion complex rather than simple ion-association. From the variation of the migration time, the K values for the formation of 1:1 complexes of MB with NS and NQS were found to be 430 ± 10 and 480 ± 70 mol^{-1} dm^3, respectively. Analysis using absorption spectra of MB gave K values for NS

and NQS of 350 ± 20 and 730 ± 260 mol^{-1} dm^3, respectively. In this study, it has been demonstrated that the K values obtained for complex formation of MB determined from the capillary electrophoresis are nearly the same as or comparable to those from absorbance changes.

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