Equilibrium Analysis of Acid Dissociation Reactions of Phenolphthalein by Using Mobility Change in Capillary Zone Electrophoresis

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An equilibrium analysis method by capillary zone electrophoresis was utilized for the determination of acid dissociation constants of phenolphthalein, which is unstable in an alkaline aqueous solution. Apparent electrophoretic mobility of phenolphthalein increased with increasing pH of a migrating solution. Acid dissociation constants (pK_a) of phenolphthalein were determined as 8.84 and 9.40 by applying a non-linear least-squares analysis to the mobility change.

Phenolphthalein is one of well-known pH indicators,[1] and is used in wide area of studies and of practical analyses. In spite of the familiar feature of phenolphthalein, a fundamental chemical property, acid dissociation reactions, had long been unsolved. It was quite recent that Ozeki et al.[2] and Tamura et al.[3] determined the acid dissociation constants by spectrophotometric methods. The reason for such backward status should be attributed to the instability of anionic form of phenolphthalein in an aqueous solution. A new method is desired to be developed for analyzing the equilibrium of such unstable compounds. Acid dissociation constants of phenolphthalein would be also desired for designing precise analysis method.

Electrophoretic methods have been proved to be a powerful tool for the analysis of equilibrium reactions, and some acid dissociation constants have been determined by capillary zone electrophoresis (CZE).[4–7] In such studies, one merit by CZE was claimed that analyte concentrations can be lowered down to µM (1 M = 1 mol dm^{-3}) levels. However, another valuable point is missed; that is, CZE methods involve "separation" and unnecessary or unrelated species, such as impurities and decomposed species, can be separated from the analyte of interest, whether the species are stable or not in solutions, and are formed or not during the electrophoresis. This study aims not only applying the CZE method to the determination of acid dissociation constants of phenolphthalein as an example of unstable species, but also evaluating the validity of the equilibrium analysis of such unstable compounds using mobility change in CZE.

A Hewlett Packard ^{3D}CE capillary electrophoresis system with a diode-array detector was used. A fused silica capillary ((56 + 8.5) cm, 50 µm i.d.; Hewlett Packard) was attached to the system.

Migrating buffer solutions were prepared by using sodium tetraborate (5 mM) mixed with HCl or NaOH. Ionic strength (I) of the solutions was controlled to 0.05 M by adding appropriate amounts of NaCl. Sample solutions were prepared by diluting phenolphthalein 50%(v/v) ethanol solution (1×10^{-3} M) with water to give a 2×10^{-5} M solution, which contained Methyl Orange (2×10^{-5} M), the borate buffer, and NaCl (I = 0.05). One percent (v/v) of ethanol coexisted in the sample solution was utilized for monitoring the electroosmotic flow (EOF). The sample solution was injected from an anodic end by applying pressure (150 mbar•s). A voltage of 15 kV was then applied, and the analytes, phenolphthalein and Methyl Orange, were photometrically detected at 230 nm. Throughout the experiments, the temperature of the capillary, the reservoir of the migrating solutions, and the sample vials was kept at 25.0 ± 0.1 °C. The electric current produced during the electrophoresis was less than 30 µA under the experimental conditions. The electrophoretic mobility of the analytes was calculated in an ordinary manner.

Electropherograms for phenolphthalein and Methyl Orange are shown in Figure 1. Migration time for phenolphthalein increased with increasing pH of the migrating solution, which suggested that phenolphthalein became more anionic. However, signal shapes for phenolphthalein became much broad at pH range above 11.5 as is shown in Figure 1 d), which can be attributed to the formation of $PP(OH)^{3-}$ as is reported.[2,3] The broadening of the signal suggests that a considerable amount of phenolphthalein species once equilibrized changes to $PP(OH)^{3-}$ during the migration, continuously.

Changes in the apparent electrophoretic mobility of phenolphthalein and Methyl Orange, $-\mu_{ep}'$, were plotted against the pH (Figure 2). The $-\mu_{ep}'$ values of phenolphthalein increased with increasing pH of the migrating solution from around zero, while those of Methyl Orange, monovalent anion over the pH range, was almost identical. Such results suggest that phenolphthalein species in the aqueous solution changes from a neutral species to an anionic one. Almost identical mobility of Methyl Orange indicates that the mobility was successfully measured and that external causes affecting electrophoretic

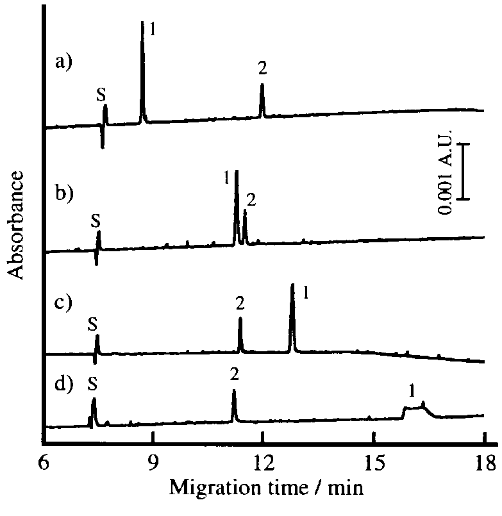

Figure 1. Electropherograms for Phenolphthalein and Methyl Orange at different pH conditions.
CZE conditions and the solutions are written in the text, except for pH conditions. pH conditions: a) 8.64; b) 9.42; c) 9.81; d) 11.60. Signals: 1, Phenolphthalein; 2, Methyl Orange; S, ethanol (EOF marker).

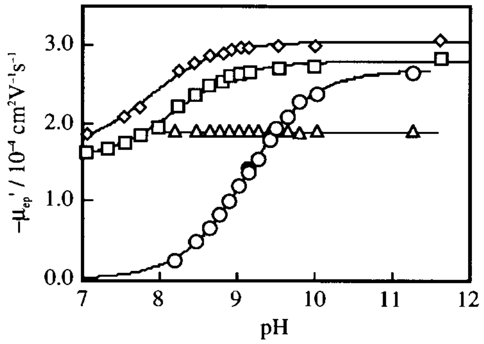

Figure 2. Changes in electrophoretic mobility of the reagents by the change in pH of migrating solution.
CZE conditions and the solutions are written in the text. ○, ●: Phenolphthalein; △: Methyl Orange; ◇: Phenol Red; □: Cresol Red. Open circles: measured using freshly prepared solution. Filled circle: measured after 24 h standing in the borate buffer solution. Solid curves show the simulation of the mobility change obtained by using Eq. (3) and the optimized values.

mobility, such as the change in viscosity of the migrating solutions, can be well excluded. Filled circle in Figure 2 shows the apparent electrophoretic mobility of phenolphthalein measured after 24 h standing in the borate buffer solution. The plot fits to the results with freshly prepared solution (open circles), which also suggests that the experiments in this study are valid.

Two steps of acid dissociation reactions and the equilibria are written in eqs (1) and (2), respectively, where the activity of oxonium ion, a_{H^+}, obtained by the pH measurement was used in equilibrium constants, instead of its concentration.

$$H_2PP \overset{K_{a1}}{\rightleftarrows} H^+ + HPP^- \quad ; \quad HPP^- \overset{K_{a2}}{\rightleftarrows} H^+ + PP^{2-} \qquad (1)$$

$$K_{a1} = \frac{a_{H^+}[HPP^-]}{[H_2PP]} \quad ; \quad K_{a2} = \frac{a_{H^+}[PP^{2-}]}{[HPP^-]} \qquad (2)$$

The apparent electrophoretic mobility of phenolphthalein can be written as in eq (3) by using the mass balance for phenolphthalein and eq (2).

$$-\mu_{ep}' = \frac{(a_{H^+}{}^2/K_{a1}K_{a2})(-\mu_{ep,H2PP}) + (a_{H^+}/K_{a2})(-\mu_{ep,HPP}) + (-\mu_{ep,PP})}{(a_{H^+}{}^2/K_{a1}K_{a2}) + (a_{H^+}/K_{a2}) + 1} \qquad (3)$$

where $-\mu_{ep,H2PP}$, $-\mu_{ep,HPP}$, and $-\mu_{ep,PP}$ are the electrophoretic mobility of H_2PP, HPP^-, and PP^{2-}, respectively. In eq (3), the value of $-\mu_{ep,H_2PP}$ is considered to be zero from the view point of its charge, and the value of $-\mu_{ep,HPP}$ seems to be almost one half of that of $-\mu_{ep,PP}$ on the basis of the principle of electrophoresis.[8] The postulation for $-\mu_{ep,HPP}$ was also ascertained to be valid through the following experimental results. Electrophoretic mobility of Phenol Red and Cresol Red was also measured over the pH range from acidic to alkaline conditions, and the results are shown in Figure 2 and Table 1. Electrophoretic mobility of the divalent sulfophthaleins was about twice as large as that of monovalent forms. A non-linear least-squares analysis was applied to the determination of K_{a1} and K_{a2}, where a series of pairs of a_{H^+} and $-\mu_{ep}$' were put into eq (3), and the values of K_{a1}, K_{a2}, and $-\mu_{ep,PP}$ were optimized. Measured values of $-\mu_{ep}$' at pH range above 11.5 were not used, because the contribution of $PP(OH)^{3-}$ is considerably large. Obtained values are summarized in Table 1. Acid dissociation constants of Phenol Red and Cresol Red are also examined as examples of stable compound to validate the proposed method; the results are also summarized in Table 1. Since pK_a values for Phenol Red and Cresol Red are in good agreements with the reported values, the validity of the method is ascertained. Optimized electrophoretic mobility of divalent phenolphthalein, $-\mu_{ep,PP}$: 2.70 × 10^{-4} cm^2 V^{-1} s^{-1}, is very close to those of divalent Phenol Red and Cresol Red, which also suggests that the present analysis method is valid. Strictly, the mobility of the monovalent form is not one half of that of divalent one, and therefore, the non-linear least-squares analysis was also carried out by varying the values of $-\mu_{ep,HPP}$ in the ranges from one third to two thirds of $-\mu_{ep,PP}$ value. Obtained pK_a values are in the range from 8.67 to 8.97 for pK_{a1} and the one from 9.28 to 9.54 for pK_{a2}; the variation of the constants are narrow in spite of the wide range of assumed $-\mu_{ep,HPP}$ values.

Table 1. Electrophoretic mobility and acid dissociation constants obtained in this study

Value or constant	Dyes Phenolphthalein	 Phenol Red	 Cresol Red
$-\mu_{ep,HPP}$	–	1.68±0.18[a]	1.54±0.12[a]
$-\mu_{ep,PP}$	2.70±0.10[a]	3.06±0.10[a]	2.81±0.10[a]
pK_{a1}	8.84±0.09[b] (10.1)[c] (9.05)[d]	–	–
pK_{a2}	9.40±0.09[b] (9.3)[c] (9.50)[d]	7.88±0.20[b] (7.9)[e]	8.20±0.15[b] (8.3)[e]

[a]Error: 3σ; dimension: 10^{-4} cm^2 V^{-1} s^{-1}. [b]I = 0.05 M. Error: 3σ. [c]Reported values cited from Reference 2 obtained by spectrophotometric method. [d]Reported values cited from Reference 3 obtained by spectrophotometric method. [e]Cited values from Reference 1.

The pK_a values obtained in this study are compared with the reported values to evaluate the validity of the proposed CZE method. Photometric methods provided the pK_a values,[2,3] however, serious differences exist in the reported values as is summarized in Table 1. Our results are very close to those determined by Tamura et al.,[3] and their results should be more reliable. While spectrophotometric method requires precise control of standing time[3] and the obtained values change as a function of time, the proposed method is not time-dependent. Therefore, the values obtained in this study would be most reliable among the values reported. Acid dissociation reactions of phenolphthalein, an example of unstable compound, were properly analyzed by the CZE method.

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References

1 F. J. Green, in "The Sigma-Aldrich Handbook of Stains, Dyes and Indicators," Aldrich Chemical Company, Milwaukee (1990).
2 T. Ozeki, H. Morikawa, and H. Kihara, *Bunseki Kagaku*, **42**, 887 (1993).
3 Z. Tamura, S. Abe, K. Ito, and M. Maeda, *Anal. Sci.*, **12**, 927 (1996).
4 J. L. Beckers, F. M. Everaerts, and M. T. Ackermans, *J. Chromatogr.*, **537**, 407 (1991).
5 J. A. Cleveland, M. H. Benko, S. J. Gluck, and Y. M. Walbroehl, *J. Chromatogr. A*, **652**, 301 (1993).
6 S. J. Gluck and J. A. Cleveland, *J. Chromatogr. A*, **680**, 43 (1994).
7 S. J. Gluck and J. A. Cleveland, *J. Chromatogr. A*, **680**, 49 (1994).
8 Y. Kiso, in "Topics surrounding Zone Electrophoresis – Ionic Processes through Matrices," Nankodo, Tokyo (1972), Chap. 4.