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Non-aqueous capillary electrophoretic separation of Brønsted acids as heteroconjugated anions

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Abstract

Capillary electrophoretic separation of phenols, carboxylic acids, and alcohols has been studied in acetonitrile. Under the condition where these compounds are not dissociated, they interact with an inorganic anion added to a running solution to form heteroconjugated anions, and migrate toward the anode. Though small anions with high charge densities (e.g. chloride) form more stable heteroconjugated anions, even perchlorate ions are involved in heteroconjugation with phenols and carboxylic acids, and allows them to behave as anionic species. The extent of heteroconjugation is dependent on the charge density in the oxygen atoms of the carboxyl group in a carboxylic acid and the hydroxyl group in a phenol or an alcohol, and thus the order of migration correlates well with various electron induction factors (e.g. Hammet σ or pK_a values). Quantitative evaluation and determination of heteroconjugated anion formation constants are also feasible.

Keywords: Heteroconjugation; Phenols; Carboxylic acids; Alcohols; Anions

1. Introduction

Capillary electrophoresis (CE) has become one of the most common separation means, and has received much attention because of its high separation performance [1–3]. Its high separation ability has allowed various practical and fundamental applications, which were impossible or very difficult by conventional methods. From a fundamental point of view, it is an advantage of CE over other separation modes that separation media are composed of only well-known homogeneous solutions; stationary phases are necessary for liquid chromatography and at least two immiscible phases are necessary for solvent extraction. In addition, interfaces are not involved, which play important roles in chromatography or solvent extraction but are often difficult to quantitatively describe. Hence, CE should be suitable for studies on fundamental solution chemistry.

Though CE separation has been mostly carried out in aqueous buffers, non-aqueous CE (not the addition of organic solvents) has proved to be effective and been applied to various analyses [4–10]. Since the separation selectivity in CE, determined by the mobility of a solute, is changed by varying medium properties, non-aqueous media have been primarily used to enhance and modify selectivity; e.g. separation of linear long-chain alkyl benzenesulfonates in methanol and methanol–acetonitrile mixtures [10], the separation of cationic drugs in various organic solvents [8], and separation of tamoxifen and its metabolites in methanol and acetonitrile [9] have been studied, which were difficult in the usual aqueous buffers or even in micellar solutions. It has

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been indicated as another practical advantage of non-aqueous CE that the lower mobility of ions in some organic solvents with high viscosity allows the use of high concentration electrolyte solutions, the use of capillaries with large diameters, and finally the application of large sample volume [6,8]. In addition, we indicated that non-aqueous electrophoresis is important to probe non-aqueous solution chemistry and that the high separation efficiency of CE allows not only separations that are difficult in aqueous media but also the evaluation of reactions not detected by other methods [4].

In the present paper, the heteroconjugated anion formation of Brønsted acids such as phenols, carboxylic acids, and alcohols with some simple anions in acetonitrile (ACN) is utilized in the CE separation of these compounds, and quantitatively evaluated. Though it has been known for a long time that heteroconjugation plays important roles in various acid–base equilibria in aprotic solvents as well as homoconjugation [11–17], very few papers have appeared dealing with the quantitative evaluation of heteroconjugation of Brønsted acids with anions in solution [13,14]. These equilibria have usually been evaluated by conductometry, potentiometry, spectrometry, etc. However, these conventional methods often required high concentrations of reactants, and sometimes failed in detecting weak interactions. CE visualizes heteroconjugation as the separation of peaks, and can be an alternative method for quantitative evaluation.

2. Experimental

The CE system was composed of a Matsusada high-voltage power supply Model HCZE-30P No. 25, a JASCO UV–visible detector Model 870-CE and a fused-silica capillary [57.8 cm (36.8 cm effective length, with a detection window located 21.0 cm from the negative end) 375 μm O.D.× 50 μm I.D.]. Currents ranged from 5–60 μA, depending on the concentration and nature of the salt used, under a 15 kV applied voltage. This CE system was set in a thermostated incubator to keep temperature constant at 25°C. Shimadzu model UV-2100PC was used for measurements of UV–visible spectra.

To obtain the appropriate migration time, the anodic solution reservoir was kept higher than the cathodic solution reservoir; the difference in the height between them was typically set to 10 cm. Thus, the hydrodynamic flow was incorporated with the electroosmotic flow. Acetone was added in all sample solutions as a flow marker. Acetone was selected because of its UV-absorption band and much weaker acceptor ability (the acceptor number of acetone is 12.5, while that of ACN is 19.3 [18]).

The running solution was prepared by dissolving an appropriate tetraethylammonium (Et_4N^+) salt in ACN, and ionic strength was not adjusted for the reason mentioned in Section 3.

Phenols and carboxylic acids of the highest purity available were used as received. ACN was distilled three times: first with P_2O_5, second with CaH_2, and finally without additives. Tetraethylammonium bromide (Et_4NBr) was synthesized from triethylamine and ethylbromide, purified by recrystallization, and used after drying over P_2O_5 under vacuum. Et_4NCl was purchased from Wako and used after drying under vacuum. Et_4NClO_4 and Et_4NBF_4 were precipitated by addition of $HClO_4$ or HBF_4 to aqueous solution of Et_4NBr, recrystallized from water, and used after drying. Et_4NNO_3 was synthesized by removing Br^- in Et_4NBr by oxidation in the presence of HNO_3, purified by recrystallization from acetone, and used after drying. Et_4N^+-*p*-nitrophenolate and Et_4N^+-methanesulfonate ($Et_4NCH_3SO_3$) were synthesized by mixing Et_4NOH solution and a corresponding acid in methanol. The solvent was evaporated under reduced pressure, and then salts were recrystallized from an appropriate solvent.

3. Results and discussion

Phenols, carboxylic acids, and alcohols (HA) electrophoretically behave as anions in ACN in the presence of any anion tested (X^-=ClO_4^-, BF_4^-, NO_3^-, Br^-, $CH_3SO_3^-$, and Cl^-). The acidity of the conjugate acids (HX) of these anions is much higher than those of phenols and carboxylic acids; pK_a values in ACN for $HClO_4$, HBr, CH_3SO_3H, HNO_3, and HCl are 1.57, 5.5, 8.36, 8.9, and 8.9, whereas those of solutes range from 11 (picric acid) to 27.2 (phenol) or larger and mostly around 20 [19]. Judging from these values, the proton transfer

(shown by the following) from an acid to an anion does not take place except for relatively strong acids, such as picric acid, in the presence of relatively strong bases, e.g. NO_3^-, $CH_3SO_3^-$, or Cl^-.

$$X^- \cdots HA = XH + A^-$$

Electronic spectra were studied to verify that the phenomenon electrophoretically observed is due not to the proton transfer but to the heteroconjugated anion formation. The reaction of Cl^- with *p*-nitrophenol was selected because of very stable heteroconjugated anion formation. Fig. 1 shows electronic spectra of *p*-nitrophenol, *p*-nitrophenolate and *p*-nitrophenol–Cl^- systems. In ACN, the maximum wavelengths of *p*-nitrophenol and *p*-nitrophenolate are 310 nm and 430 nm, respectively. Though we changed balance between the phenol and the phenolate concentration, no homoconjugate absorption was observed because of low concentration; the shifts of absorption bands of *o*-nitrophenol [13] or 3,5-dinitrophenol [20] due to homoconjugation were reported (in both cases, the absorption bands of homoconjugated ions appeared between that of a phenol and that of a phenolate). When Cl^- is added to *p*-nitrophenol solution, a new absorption band at 320 nm emerges, which is due to the heteroconjugated anion, *p*-nitrophenol–Cl^-. During the addition of Cl^-, the absorption intensity at 430 nm due to *p*-nitrophenolate does not increase. Thus, we conclude that no proton transfer from *p*-nitrophenol to Cl^- takes place but the heteroconjugated anion is formed.

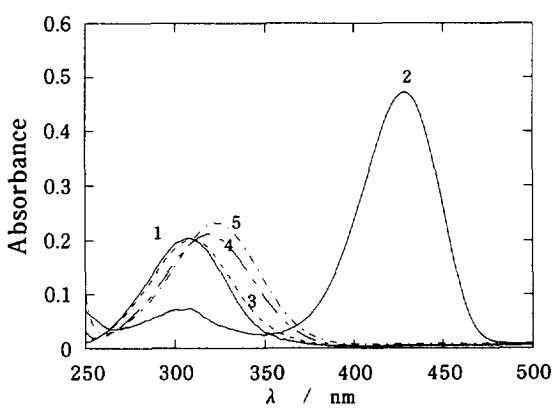

Fig. 1. Electronic spectra of *p*-nitrophenol, *p*-nitrophenolate and *p*-nitrophenol–Cl^- homoconjugated anion in ACN. (1) $2 \cdot 10^{-5}$ *M* *p*-nitrophenol. (2) $2 \cdot 10^{-5}$ *M* Et_4N^+ *p*-nitrophenolate. (3) $2 \cdot 10^{-5}$ *M* *p*-nitrophenol + 0.2 m*M* Et_4NCl. (4) $2 \cdot 10^{-5}$ *M* *p*-nitrophenol + 2 m*M* Et_4NCl. (5) $2 \cdot 10^{-5}$ *M* *p*-nitrophenol + 20 m*M* Et_4NCl.

We used typically 0.5 m*M* of solute as an injected sample and 10–100 m*M* running electrolytes. If the dilution in a capillary is taken into account, the concentration of an anion must be higher than that of a solute by 2–4 orders of magnitude. In the presence of Cl^-, ~3% of picric acid is dissociated if a concentration ratio ($[X^-]/[HA]$) is $1 \cdot 10^4$; in this case, the proton transfer contributes to the electrophoretic migration of the acids to a substantial extent. However, in other cases, differences in pK_a are much larger; e.g. only $3 \cdot 10^{-8}$ fraction of $1 \cdot 10^{-5}$ *M* *p*-nitrophenol ($pK_a = 20.7$ in ACN [19]) is dissociated in the presence of 0.1 *M* Cl^-; this must be negligible. In the following discussion, unless otherwise specified the dissociation due to the proton transfer is neglected and only heteroconjugation is taken into account.

3.1. Separation of phenols, carboxylic acids, and alcohols

Table 1 lists the apparent velocities of selected phenols when ClO_4^-, BF_4^-, NO_3^-, Br^-, $CH_3SO_3^-$, and Cl^- are added to the running solution. These values were determined at different concentration of a running electrolyte because of different abilities in heteroconjugated anion formation of these anions; some peaks of phenols were not detected with 0.05 *M* Cl^- solution, while no peaks were resolved with 0.015 *M* ClO_4^- solution. Therefore, though direct comparison is difficult, the order in the apparent velocities of a given phenol is as follows: $ClO_4^- < BF_4 < NO_3^- < Br^- = CH_3SO_3^- < Cl^-$, which correlates with the order of the ability of hydrogen bond formation, i.e. pK_a values of the conjugate acids (except for NO_3^-, see above), the ion-pair formation constants with a small cation [21], anion-exchange selectivity with anion-exchangers having $-NH_3^+$ as an ion-exchange site [22], and solvation energy in a solvent capable of donating hydrogen bonds [11]. It should be noted that the heteroconjugated anion formation is observed even in the presence of ClO_4^- which is usually regarded as inert. Further evidence for the heteroconjugation is that the addition of

Table 1
Electrophoretic velocity of phenols with various electrolytes

Phenols	Velocity (cm min^{-1})					
	Cl^{-} [a]	$CH_3SO_3^{-}$ [b]	Br^{-} [b]	NO_3^{-} [b]	BF_4^{-} [c]	ClO_4^{-} [c]
Phenol	6.36	3.46	3.41	2.32	0.19	0.17
p-Me	5.61	2.85	2.97	2.01	0.16	0.14
p-Et	5.26	2.81	2.71	1.78	0.18	0.14
p-*t*Bu	4.71	2.42	2.39	1.65	0.12	0.10
o-NO_2	1.47	0.57	0.55	0.30	– [d]	–
p-NO_2	–	–	–	4.65	0.39	0.42
p-Cl	6.97	4.48	–	3.00	0.27	0.24
m-OH	6.22	4.40	–	2.99	0.31	0.29
Picric	–	–	1.06	–	–	0.13
2,4-Di-NO_2	8.22	–	–	2.85	–	0.11

Velocity toward the anode under the electric field of 26 kV m^{-1}.
[a] Obtained with 0.015 *M* solution.
[b] Obtained with 0.03 *M* solution.
[c] Obtained with 0.05 *M* solution.
[d] Not detected.
Abbreviations, *p*-Me = *p*-cresol, *p*-Et = *p*-ethylphenol, *p*-*t*Bu = *p*-*tert.*-butylphenol, *o*-NO_2 = *o*-nitrophenol, *p*-NO_2 = *p*-nitrophenol, *p*-Cl = *p*-chlorophenol, *m*-OH = resorcinol, Picric = picric acid and 2,4-Di-NO_2 = 2,4-dinitrophenol.

methanol or water to the ACN running solution drastically reduces the migration of Brønsted acids toward the anode, and finally allows migration together with the flow marker. This is clearly due to the strong hydrogen bond formation ability water and methanol; i.e. these strongly solvate anions by hydrogen bonding, and also act as hydrogen bond acceptor toward phenols.

Figs. 2–5 show examples of electrophoretic separation of phenols, carboxylic acids, and alcohols. Fig. 2a and b show electropherograms of phenols with ClO_4^- as a running electrolyte. Though the heteroconjugation of phenols with ClO_4^- is very weak, the association does take place. Thus, as far as heteroconjugation in ACN is concerned, ClO_4^- is not inert but reactive. If ClO_4^- salts are used to adjust ionic strength of the ACN solution, it will be necessary to confirm whether the heteroconjugation of ClO_4^- disturbs solution equilibria or not.

The selectivity of separation is basically unchanged by varying an anion used as a running electrolyte as long as no proton transfer occurs. Some relatively strong acids such as picric acid and 2,4-dinitrophenol changed their peak positions; this must be due to proton transfer rather than to heteroconjugated anion formation. The electrophoretic migration of these nitrophenols to the anode is

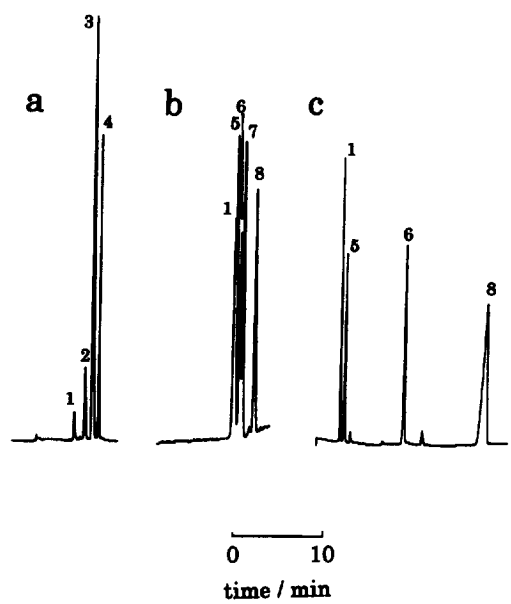

Fig. 2. Electrophoretic separation of phenols. (a, b) 0.1 *M* Et_4N ClO_4 in ACN; (c) 0.01 *M* Et_4NCl in ACN. Peak identification, (1) acetone, (2) *m*-aminophenol, (3) phenol, (4) *p*-hydroxybenzaldehyde, (5) *o*-nitrophenol, (6) 2,4-dinitrophenol, (7) picric acid and (8) *p*-nitrophenol. Detection at 265 nm. Other conditions are given in the text.

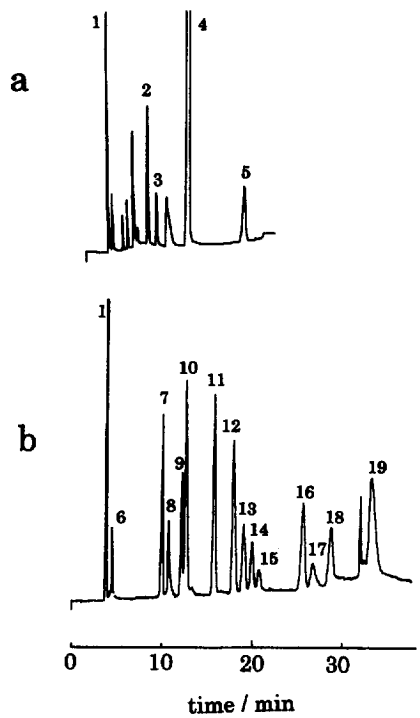

Fig. 3. Electrophoretic separation of phenols. (a) 0.01 *M* Et_4NCl in ACN; (b) 0.02 *M* in ACN. Peak identification, (1) acetone, (2) resorcinol, (3) *p*-chlorophenol, (4) *p*-hydroxybenzaldehyde, (5) *p*-nitrophenol, (6) *o*-nitrophenol, (7) *p*-isopropylphenol, (8) *p*-aminophenol, (9) *p-tert.*-butylphenol, (10) *m*-aminophenol, (11) *p*-ethylphenol, (12) *o*-cresol, (13) *m*-cresol, (14) *p*-cresol, (15) *p*-hydroxybenzylalcohol, (16) phenol, (17) *o*-aminophenol, (18) 2-naphthol and (19) 1-naphthol. Detection at 265 nm. Other conditions are given in the text.

slower than those of phenol, resorcinol and *p*-chlorophenol when ClO_4^- is used as a running electrolyte. In contrast, the electrophoretic migration of 2,4-dinitrophenol is faster than those of phenol, resorcinol, and *p*-chlorophenol, and the peak of picric acid is not detected with Cl^- solution as shown in Fig. 2c, where the sample was the same as used for Fig. 2b (four solute peaks in the order of *o*-nitrophenol, 2,4-dinitrophenol, picric acid and *p*-nitrophenol are seen). Peaks were missing for NO_3^-, and $CH_3SO_3^-$ running electrolytes. No proton transfer occurs in ClO_4^- solution because of the high acidity of $HClO_4$; therefore, migration order in this electrolyte system should reflect the heteroconjugated anion formation ability of phenols, i.e. the heteroconjugated anion formation ability of picric acid and 2,4-dinitrophenol is lower than that of e.g. phenol. Thus, the high electrophoretic migration of picric acid and 2,4-dinitrophenol in Cl^-, NO_3^- and $MeSO_3^-$ solutions is due to the proton transfer from these phenols to the anion. Both proton transfer and heteroconjugated anion formation should be taken into account for quantitative analyses of electrophoretic behaviors.

The overall migration order (corresponding to the order of electrophoretic migration, the higher electrophoretic migration rate results in the longer overall migration time) is *ortho-*<*meta-*<*para*-isomer for cresols and nitrophenols (though *m*-nitrophenol was not tested), and *para-*<*meta-*<*ortho*-isomer for aminophenols. Though the electrophoretic migration (or the extent of heteroconjugated anion formation) of *para-* and *meta*-isomers is explained by the electron density on an oxygen atom of a phenolic hydroxyl group as shown below, that of an *ortho*-isomer should be explained by taking other effects into account. In the cases of *o*-nitrophenol and *o*-cresol, steric effects and intramolecular hydrogen bond formation are involved in heteroconjugation. The *ortho*-substituted groups will sterically interfere with the heteroconjugation. In addition, the intramolecular hydrogen bond formation play an important role in some case. It is known that the homoconjugated anion formation constant of *o*-nitrophenol (log $K_{AHA}=2.11$ in ACN) is much smaller than that of *p*-nitrophenol (log $K_{AHA}=3.49$ in ACN) [11]. This is due to the intramolecular hydrogen bond formation between the hydroxyl group and the nitro group [11], which also weakens the heteroconjugation of *o*-nitrophenol. The lower acidity of *o*-nitrophenol compared to that of *p*-nitrophenol in ACN (pK_a values for *o-* and *p*-nitrophenol in ACN are reported to be 22.1 and 20.7) [18] is also due to the intramolecular hydrogen bond formation of the former. As discussed below, their intrinsic acidity in the absence of the intramolecular hydrogen bond formation is similar as seen in their pK_a values in water, 7.22 and 7.15 [23]. In contrast, intramolecular hydrogen bond formation is not associated with the heteroconjugated anion formation of aminophenols. It appears that the hydrogen bonding between an anion and the amino group contributes to the stabilization of the heteroconjugated anion formation of *o*-aminophenol.

Separation of geometrical isomers of carboxylic acids are incomplete, while the corresponding separation of phenols is feasible. As shown in Fig. 4, the separation of *ortho-* and *meta*-toluic acid or *ortho-* and *para*-nitrobenzoic acid was not possible under any conditions tested. The causes of these differences between phenols and carboxylic acids should be studied from viewpoint of structural chemistry.

The heteroconjugation of alcohols is also important in solution chemistry. Although the systematic investigation for aliphatic alcohols will be interesting, aryl-substituted alcohols are studied in this work because these can be detected with UV detection. As shown in Fig. 5, the separation based on the number of methylene groups between a benzene ring and a hydroxyl group was feasible. However, since effects of substituents on the benzene ring on the acidity of a compound or on the electron density of alcoholic hydroxyl groups are much weaker than for phenolic hydroxyl groups or carboxylic groups directly bonded on a benzene ring, the substitution of a weak electron donating group such as a methyl group on a benzene ring brought about only marginal modification in electrophoretic migration (or heteroconjugated anion formation). In contrast, the introduction of a nitro group on the benzene ring of benzyl alcohol resulted in enhanced electrophoretic migration to the anode.

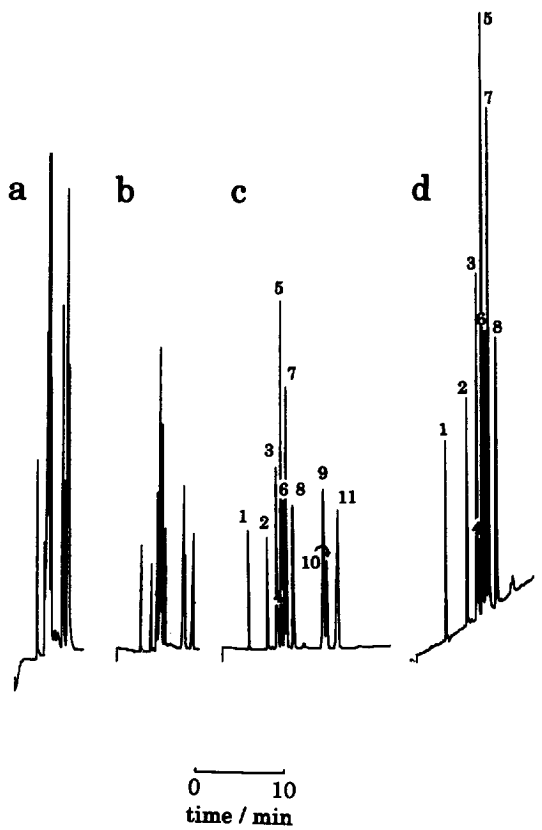

Fig. 4. Electrophoretic separation of carboxylic acids. (a) 0.005 *M* Et_4NCl; (b) 0.01 *M* Et_4NCl; (c) 0.015 *M* Et_4NCl; (d) 0.02 *M* Et_4NCl. Peak identification, (1) acetone, (2) *p*-aminobenzoic acid, (3) *p-tert.*-butylcarboxylic acid, (4) *m*-aminobenzoic acid, (5) *p*-ethylbenzoic acid, (6) *o-* and *m*-methylbenzoic acid, (7) *p*-methylbenzoic acid, (8) benzoic acid, (9) *m*-nitrobenzoic acid, (10) *o-* and *p*-nitrobenzoic acid and (11) phthalic acid. Detection at 225 nm. Other conditions are given in the text.

3.2. Determination of heteroconjugated anion formation

The following equation was derived to quantitatively interpret the electrophoretic behaviors of the Brønsted acids. Assuming 1:1 heteroconjugated anion formation,

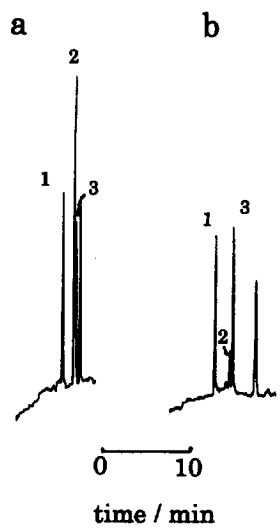

Fig. 5. Electrophoretic separation of alcohols. Running solution, 0.076 *M* $Et_4NCH_3SO_3$. Peak identification for (a), (1) acetone, (2) 3-phenylpropyl alcohol, (3) 2-phenylethyl alcohol, (4) benzyl alcohol; (b), (1) acetone, (2) *p*-methylbenzyl alcohol, (3) benzyl alcohol and (4) *p*-nitrobenzyl alcohol. Detection at 250 nm. Other conditions are given in the text.

$$v_s = v_{app} - v_f = L(1/t_{app} - 1/t_f) = \alpha v_{hetero}$$

Substituting $\alpha = (K_{HAX}C)/(K_{HAX}C+1)$ and $v_{hetero} = (E\lambda_{hetero})/F$ in this equation gives

$$1/(1/t_{app} - 1/t_f) = [1/(K_{HAX}C) + 1]LF/(E\lambda_{hetero}) \quad (1)$$

where v and t denote an electrophoretic velocity and the time of migration, subscripts, s, app, f, and hetero are the abbreviations of a solute, apparent, a flow, and a heteroconjugated anion, and α, K_{HAX}, C, L, E, F, and λ_{hetero} refer to the degree of heteroconjugated anion formation, a heteroconjugated anion formation constant, the concentration of an anion in running solution, the effective length of a capillary (36.8 cm in the present work), the strength of an electric field (15 kV was applied to the entire capillary, 57.8 cm), Faraday constant, and the molar ionic conductivity of a heteroconjugated anion, respectively. The last term was corrected according to Onsager's equation for an X^+Y^--type electrolyte [11,24].

$$\lambda = \lambda_0 - [e^2\lambda_0/\{24\pi\varepsilon_0\varepsilon kT(1+\sqrt{0.5})\} - F^2/(6\pi\eta N)]\kappa/(1+\kappa a)$$

$$\kappa = \{2N^2e^2/(\varepsilon_0\varepsilon RT)\}^{1/2}\sqrt{I}$$

where λ_0, e, ε_0, ε, k, η, N, a and I are the molar conductivity at infinite dilution, the elementary electric charge, the dielectric constant of vacuum, a specific dielectric constant, the Boltzmann constant, the viscosity of a solvent, Avogadro's number, the distance between ions, and molar ionic strength, respectively.

When the concentration of phenols or carboxylic acids is high, it is possible that two or more acid molecules simultaneously participate in the heteroconjugation with an anion [14]. However, under the present conditions where the concentration of an anion is much higher than that of an acid, such equilibria are negligible.

Though ionic strength should be adjusted by adding inert salts such as perchlorate salts, this procedure causes errors or unnecessary complication for the calculation of K_{HAX} because even ClO_4^- also forms heteroconjugated anions as shown above. The correction based on the Onsager's equation was therefore made instead of the adjustment of ionic strength to avoid this problem. According to Eq. (1), we can determine K_{HAX} from the dependence of migration times on the concentration of anions added as a running electrolyte. Fig. 6 shows example plots based on Eq. (1) for phenol–NO_3^- heteroconjugated anion formation; solid curves are drawn with fitted parameters. Table 2 summarizes some selected values K_{HAX} and λ_{hetero}. Though λ_{hetero} ranges 56–91 S cm^2 mol^{-1} (mostly 60–70 S cm^2 mol^{-1}), it will be reasonable that λ_{hetero} values should be almost the same because of the almost identical sizes of resulting heteroconjugated anions. Differences in λ_{hetero} listed in Table 2 are due to scattered data points; λ_{hetero} values listed are less reliable than K_{HAX}. Thus, it is reasonably concluded that a difference in λ_{hetero} makes negligible contribution to the separation of Brønsted acids. On the other hand, the difference in K_{HAX} is more associated with the separation of Brønsted acids by the developed meth-

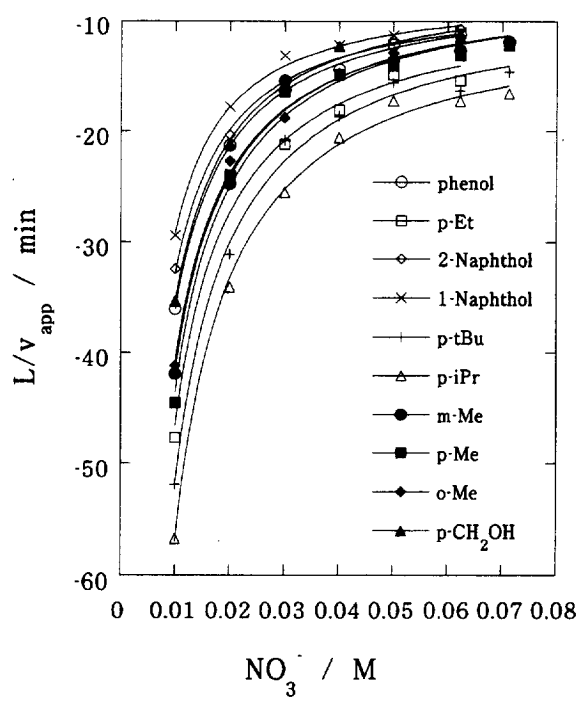

Fig. 6. Plots based on Eq. (1) for selected phenols with NO_3^- running electrolytes. Abbreviations: *p*-Et=*p*-ethylphenol, *o*-, *m*-, and *p*-Me=*o*-, *m*- and *p*-cresol, *p*-*t*Bu=*p*-*tert.*-butylphenol, *p*-iPr=*p*-isopropylphenol and *p*-CH_2OH=*p*-hydroxybenzylalcohol.

Table 2
K_{HAX} and λ_{hetero} for selected Brønsted acid with Cl^- and NO_3^-

	Cl^-		NO_3^-	
	$\log K_{HAX}$	λ_{hetero}/S cm^2 mol^{-1}	$\log K_{HAX}$	λ_{hetero}/S cm^2 mol^{-1}
Phenols				
Phenol	2.09(0.01)	66.5(0.6)	1.03(0.04)	70.2(2.1)
o-Me	1.72(0.13)	76.3(13.4)	0.96(0.11)	72.3(5.9)
m-Me	1.95(0.16)	65.2(11.4)	0.93(0.18)	75.0(9.9)
p-Me	1.87(0.15)	68.1(12.0)	0.88(0.17)	78.7(9.6)
p-Et	1.84(0.03)	69.2(2.2)	0.95(0.19)	65.0(8.6)
p-*t*Bu	1.79(0.04)	66.0(2.8)	0.89(0.12)	66.7(5.5)
o-NH_2	2.14(0.13)	60.3(6.6)	0.91(0.04)	75.3(4.6)
m-NH_2	1.81(0.04)	63.7(3.2)	0.65(0.09)	90.0(13.0)
p-NH_2	1.72(0.04)	63.6(3.4)	–[a]	–
o-NO_2	1.27(0.17)	55.5(15.9)	–	–
p-NO_2	2.69(0.00)	68.3(0.0)	–	–
p-CHO	2.43(0.16)	66.2(7.1)	–	–
p-Cl	2.17(0.03)	70.0(1.7)	1.05(0.13)	87.4(16.3)
p-CH_2OH	1.79(0.11)	72.0(10.9)	1.01(0.13)	74.3(6.9)
m-OH	2.02(0.05)	70.8(3.27)	1.03(0.04)	88.6(5.0)
Carboxylic acids				
Benzoic	1.77(0.07)	77.8(7.0)		
Phthalic	2.19(0.07)	68.5(4.7)		
o-Me	1.57((0.16)	86.0(19.8)		
m-Me	1.59(0.14)	83.6(16.9)		
p-Me	1.77(0.05)	70.2(4.2)		
p-Et	1.51(0.15)	90.6(20.3)		
p-*t*Bu	1.49(0.13)	88.1(17.4)		
o-NO_2	2.09(0.06)	70.5(4.5)		
m-NO_2	1.88(0.23)	83.1(22.4)		
p-NO_2	2.14(0.12)	68.6(8.5)		
m-NH_2	1.57(0.17)	78.9(19.1)		
p-NH_2	1.31(0.12)	90.9(19.4)		

[a] Not determined.

Standard deviations are given in parentheses.

Abbreviations for phenols: *o*-Me = *o*-cresol, *m*-Me = *m*-cresol, *p*-Me = *p*-cresol, *p*-Et = *p*-ethylphenol, *p*-*t*Bu = *p*-*tert.*-butylphenol, *o*-NH_2 = *o*-aminophenol, *m*-NH_2 = *m*-aminophenol, *p*-NH_2 = *p*-aminophenol, *o*-NO_2 = *o*-nitrophenol, *p*-NO_2 = *p*-nitrophenol, *p*-CHO = *p*-hydroxybenzaldehyde, *p*-Cl = *p*-chlorophenol, *p*-CH_2OH = *p*-hydroxybenzylalcohol and *m*-OH = resorcinol.

Abbreviations for benzoic acids: *o*-Me = *o*-toluic acid, *m*-Me = *m*-toluic acid, *p*-Me = *p*-toluic acid, *p*-Et = *p*-ethylbenzoic acid, *p*-*t*Bu = *p*-*tert.*-butylbenzoic acid, *o*-NO_2 = *o*-nitrobenzoic acid, *m*-NO_2 = *m*-nitrobenzoic acid, *p*-NO_2 = *p*-nitrobenzoic acid, *m*-NH_2 = *m*-aminobenzoic acid and *p*-NH_2 = *p*-aminobenzoic acid.

od. There is good agreement between the order of increasing K_{HAX} and the order of electrophoretic migration, albeit there are some exceptions.

Zundel and coworkers [25–29] have investigated heteroconjugated anion formation of phenols with some bases in aprotic solvents by IR spectrometry. According to their results, there are linear relations between the maximum absorption wave number and ΔpK_a (differences in pK_a in water between an acid and a base). In contrast, it has been reported that better relationship was observed between ΔG of heteroconjugation equilibria of phenols in a gas phase and pK_a values of the carboxylic acid having the same substituent as a phenol [30]. In this work, the authors stated that though the acidity of phenols is determined both by induction and resonance effects while that of carboxylic acids predominantly by the former, and that induction effects are more responsible for the heteroconjugated anion formation. In the present work, similar results were

obtained. Since available pK_a values in ACN are very few, those in water were used for discussion. Use of aqueous pK_a values for the discussion of phenomena in non-aqueous solution is reasonable because for a homologous series of compounds there are linear relationship between aqueous pK_a values and non-aqueous pK_a values [31,32]. Though there are apparently linear relations between log K_{HACl} (for phenol–Cl^- heteroconjugation) and pK_a values of either phenols or corresponding carboxylic acids, the correlation for the latter is better as shown in Fig. 7. Solid symbols represent *ortho*-substituted phenols, and open symbols *meta*- or *para*-substituted phenols. For *meta*- and *para*-substituted phenols, the linear relations are described by the following equations;

$$\log K_{HACl} = 4.53 - 0.26pK_a(\text{phenol}) \quad (r = 0.899)$$

$$\log K_{HACl} = 4.78 - 0.65pK_a(\text{carboxylic acid}) \quad (r = 0.923)$$

Also, there is a linear relation between K_{HACl} and Hammet σ [33].

$$\log K_{HACl} = 2.03 + 0.739\sigma \quad (r = 0.951)$$

Thus, K_{HAX} is basically explained by the electron density of the oxygen atom of the hydroxyl group of phenols.

For the heteroconjugation of *para*- and *meta*-substituted carboxylic acids, there is a similar relation between K_{HACl} and pK_a.

$$\log K_{HACl} = 3.50 - 0.431pK_a(\text{carboxylic acid}) \quad (r = 0.867)$$

The values for *o*-nitrobenzoic acid and *o*-toluic acid deviate from this line as well as in the case of *ortho*-substituted phenols.

In conclusion, since the separation in the present method can be predicted from the acidity of acids and the nature of the anion used as a running electrolyte, the optimization and the selection of running electrolytes is easy. Hence, this method offers further possibilities for CE separation, and can be a novel tool for studies of non-aqueous solution chemistry.

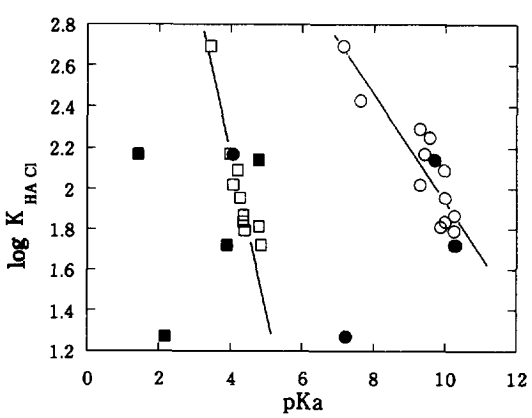

Fig. 7. Relation between log K_{HACl} and pK_a values. Circles, pK_a of phenols. Squares, pK_a of carboxylic acids having the same substitutes as phenols. Open symbols, *meta*- and *para*-substituted phenols. Solid symbols, *ortho*-substituted phenols.

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