Short communication

# Bidirectional isotachophoresis
# II. Fifteen electrolyte systems covering the pH range 3.5–10

Takeshi Hirokawa

*Applied Physics and Chemistry, Faculty of Engineering, Hiroshima University, Kagamiyama 1, Higashi-Hiroshima 724, Japan*

## Abstract

Fifteen electrolyte systems useful for bidirectional isotachophoresis were proposed covering the pH range 3.5–10. The pH dependence on the $R_L$ values of the terminating zones was simulated for all the electrolyte systems proposed, together with the isotachophoretic velocities. A 10 m$M$ HCl solution was the anionic leading electrolyte and a 10 m$M$ KOH solution the cationic one, of which the pH was adjusted by adding ten kinds of pH buffers. The present simulation might be conveniently used to select an appropriate bidirectional electrolyte system for the samples of interest.

## 1. Introduction

In our previous paper [1], it was demonstrated that an isotachophoretic (ITP) stacking configuration could be formed in a separation tube both for anions and cations of a sample, when the anolyte was a leading electrolyte for anions and the catholyte was one for cations and the sample was injected to the interface in a separation tube. Obviously, such a bidirectional electrolyte system is useful for the simultaneous analysis of anions and cations in a sample when a dual-detector system is available. Even when such detection system is not available, the electrolyte system can be conveniently used for successive analysis of anions and cations.

The pH difference between the anolyte and the catholyte of the bidirectional electrolyte system is not arbitrary, because the pH-buffering ions in each electrolyte simultaneously play the role of terminating ions, and the suitability of the buffers as terminator depends on the pH of the leading electrolyte used in combination.

In this paper, fifteen electrolyte systems suitable for bidirectional ITP were proposed covering the pH range of 3.5–10. The pH dependence of the $R_L$ values of the terminators was simulated for convenient selection of the operational system ($R_E$ is one of the qualitative indices of ITP [2]). The ITP velocities were also simulated to show the difference of the migration time of cationic and anionic zones.

## 2. Theoretical

### 2.1. Operational electrolyte system for bidirectional ITP

Fig. 1 shows a typical bidirectional electrolyte system. The anolyte is a HCl solution buffered by adding $\beta$-alanine to pH 3.6 [abbreviated as
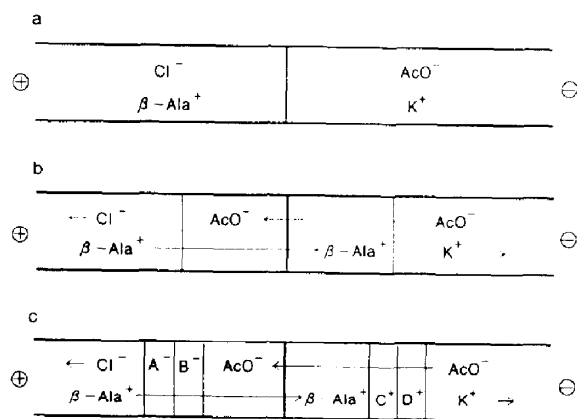
Fig. 1. Schematic diagrams of capillary bidirectional ITP. The anolyte (an anionic leading electrolyte) is a HCl solution buffered by $\beta$-alanine and the catholyte (a cationic leading electrolyte) is a KOH solution buffered by acetic acid (electrolyte system a in Table 1). A⁻, B⁻, C⁺ and D⁺ are sample components. (a) Before migration, (b) blank run, (c) steady state of sample components.

HCl/$\beta$-Ala (pH 3.6)]. The catholyte is a KOH solution buffered by adding acetic acid to pH 4.8 [abbreviated as KOH/acetic acid (pH 4.8)]. After electrophoretic migration started, as shown in Fig. 1b, acetic acid buffer can be a suitable terminator for anions, and $\beta$-alanine plays the role of a terminator for cations. When a sample mixture is injected, as shown in Fig. 1c, the anionic and cationic components migrate isotachophoretically toward electrodes. As is obvious from Fig. 1b, the constituents of two terminating zones developed are the same (acetic acid and $\beta$-alanine), but the concentrations and the pH values were different from each other [1].

## 2.2. Restriction in the selection of bidirectional electrolyte system

Several leading electrolytes for cationic analysis and anionic analysis have been proposed [2–4]. The pH of the solution ranges from 3 to 10. As discussed previously [1], these leading electrolytes cannot be combined arbitrarily. That is, the pH difference between the anolyte and the catholyte should be approximately in the range defined as follows:

$$0.5 \leqslant pH_C - pH_A \leqslant 1.5 \tag{1}$$

where $pH_C$ and $pH_A$ are the pH values of the catholyte and the anolyte, respectively. Consequently, the pH of the catholyte should be appropriately higher than that of the anolyte. In the derivation of Eq. 1, it was assumed that the anolyte and the catholyte of the bidirectional electrolyte system have the maximum buffering ability, that is the pH value of each electrolyte solution is equal to the $pK_a$ of the pH-buffering counter ion used.

When the difference $pH_C - pH_A$ is too small or too large, the effective mobility of the terminators might become inappropriately large or small. Fig. 2a shows a typical bidirectional elec-
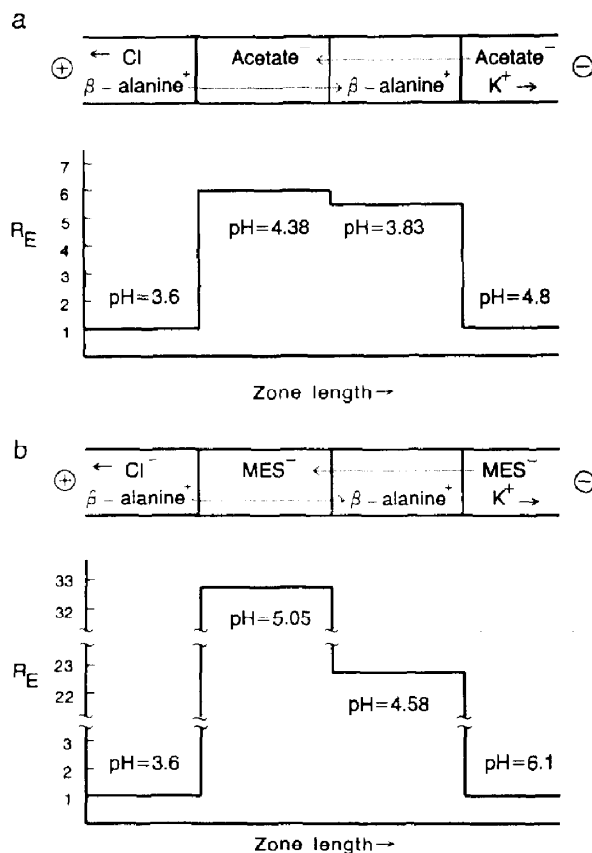


Fig. 2. Simulated $R_E$ profile of bidirectional ITP. (a) HCl/$\beta$-alanine (pH$_C$ 3.6)–KOH/acetic acid (pH$_A$ 4.8) system, (b) HCl/$\beta$-alanine (pH$_C$ 3.6)–KOH/MES (pH$_A$ 6.1). The leading anion was 10 mM Cl⁻ and the leading cation was 10 mM K⁺.

trolyte systems of HCl/$\beta$-alanine (pH$_C$ 3.6)–KOH/acetic acid (pH$_A$ 4.8). Obviously, this electrolyte system fulfills Eq. 1. According to our simulation, the effective mobility and $R_E$ value of terminating acetate ion were $12.5 \cdot 10^{-5}$ cm$^2$ V$^{-1}$ s$^{-1}$ and 5.99, respectively, at the steady state. These values for the other terminator $\beta$-alanine were $12.5 \cdot 10^{-5}$ cm$^2$ V$^{-1}$ s$^{-1}$ and 5.73, respectively. Simulated effective mobility suggested acetic acid and $\beta$-alanine are suitable as the terminators. The effective mobility simulated for the leading ion ($\bar{m}_1$) was $74.7 \cdot 10^{-5}$ cm$^2$ V$^{-1}$ s$^{-1}$ for Cl$^-$ and $71.4 \cdot 10^{-5}$ cm$^2$ V$^{-1}$ s$^{-1}$ for K$^+$.

On the other hand, if a leading electrolyte KOH/MES(pH$_A$ 6.1) is combined with that of HCl/$\beta$-alanine (pH$_C$ 3.6), this system does not fulfill Eq. 1. In such case, as shown in Fig. 2b, $R_E$ values of MES and $\beta$-alanine become too large or effective mobilities become too small according to our simulation. The effective mobility and $R_E$ value of terminating MES ion were $1.95 \cdot 10^{-5}$ cm$^2$ V$^{-1}$ s$^{-1}$ and 38.3, and those of $\beta$-alanine were $3.15 \cdot 10^{-5}$ cm$^2$ V$^{-1}$ s$^{-1}$ and 22.7, respectively. These values suggest that the

electrolyte system shown in Fig. 2b is practically of no analytical use.

For the above bidirectional simulation of the steady state of bidirectional ITP, a simulation program for isotachophoretic separation (SIPS) program was used [2] on a personal computer Model PC-9801RA2 (NEC, Tokyo, Japan). It should be noted that the simulation of bidirectional ITP separation can be carried out as the combination of independent simulations for anions and cations, because there is no essential difference between the steady state of bidirectional ITP and that of unidirectional ITP. Simulational procedures and the examples were detailed in the Refs. [4] and [2], respectively.

### 2.3. Fifteen electrolyte systems for bidirectional ITP

Taking into account the limitation on the pH difference described by Eq. 1, fifteen kinds of electrolyte systems were proposed as shown in Table 1. Eight kinds of anionic buffers and nine kinds of cationic buffers were used to cover the

Table 1
Electrolyte systems for bidirectional ITP

| Anolyte: 10 mM HCl + buffer | | | | Catholyte: 10 mM KOH + buffer | | | |
|---|---|---|---|---|---|---|---|
| Cationic buffer | $m_0^*$ ($\times 10^{-5}$/cm V$^{-1}$ s$^{-1}$) | p$K_a$ | pH range | Anionic buffer | $m_0$ ($\times 10^{-5}$/cm V$^{-1}$ s$^{-1}$) | p$K_a$ | pH range |
| (a) $\beta$-Alanine | 36.7 | 3.552 | 3.0–4.2 | Ac | 42.4 | 4.756 | 4.2–5.6 |
| (b) $\epsilon$-AMC | 28.8 | 4.373 | 3.6–5.2 | Ac | | | 4.2–5.6 |
| (c) $\beta$-Alanine | 36.7 | 3.552 | 3.0–4.2 | SUC | 33.0 | 4.207 | 4.8–6.2 |
| | | | | | 60.9 | 5.637 | |
| (d) $\epsilon$-AMC | 28.8 | 4.373 | 3.6–5.2 | SUC | | | 4.6–6.0 |
| (e) Creatinine | 37.2 | 4.828 | 4.4–5.4 | MES | 28.0 | 6.095 | 5.4–6.6 |
| (f) Histidine | 29.6 | 6.040 | 5.2–6.6 | MES | | | 5.4–6.6 |
| (g) Histidine | | | 5.6–6.8 | MOPS | 24.4 | 7.15 | 6.6–7.8 |
| (h) Imidazole | 52.0 | 7.150 | 6.4–7.6 | GlyGly | 31.5 | 8.400 | 7.8–9.0 |
| (i) Tris | 29.5 | 8.076 | 7.2–8.6 | GlyGly | | | 7.8–9.0 |
| (j) Tris | | | 7.4–8.6 | Histidine | 28.8 | 9.342 | 8.8–10.2 |
| (k) Ammediol | 32.0 | 8.780 | 8.0–9.4 | Histidine | | | 8.8–10.0 |
| (l) Ammediol | | | 8.0–9.4 | Valine | 28.4 | 9.710 | 9.2–10.4 |
| (m) Ammediol | | | 8.2–9.4 | $\beta$-Alanine | 30.8 | 10.24 | 9.6–10.6 |
| (n) Ethanolamine | 44.3 | 9.498 | 8.8–10.2 | $\beta$-Alanine | | | 9.6–10.8 |
| (o) Ethanolamine | | | 8.8–10.0 | Proline | 29.0 | 10.64 | 10.0–11.2 |

Ac = Acetic acid; $\epsilon$-AMC = $\epsilon$-aminocaproic acid; SUC = succinic acid; MES = N-morpholinoethanesulfonic acid; MOPS = 3-morpholinopropanesulfonic acid; GlyGly = glycylglycine; Tris = tris(hydroxymethyl)aminomethane.
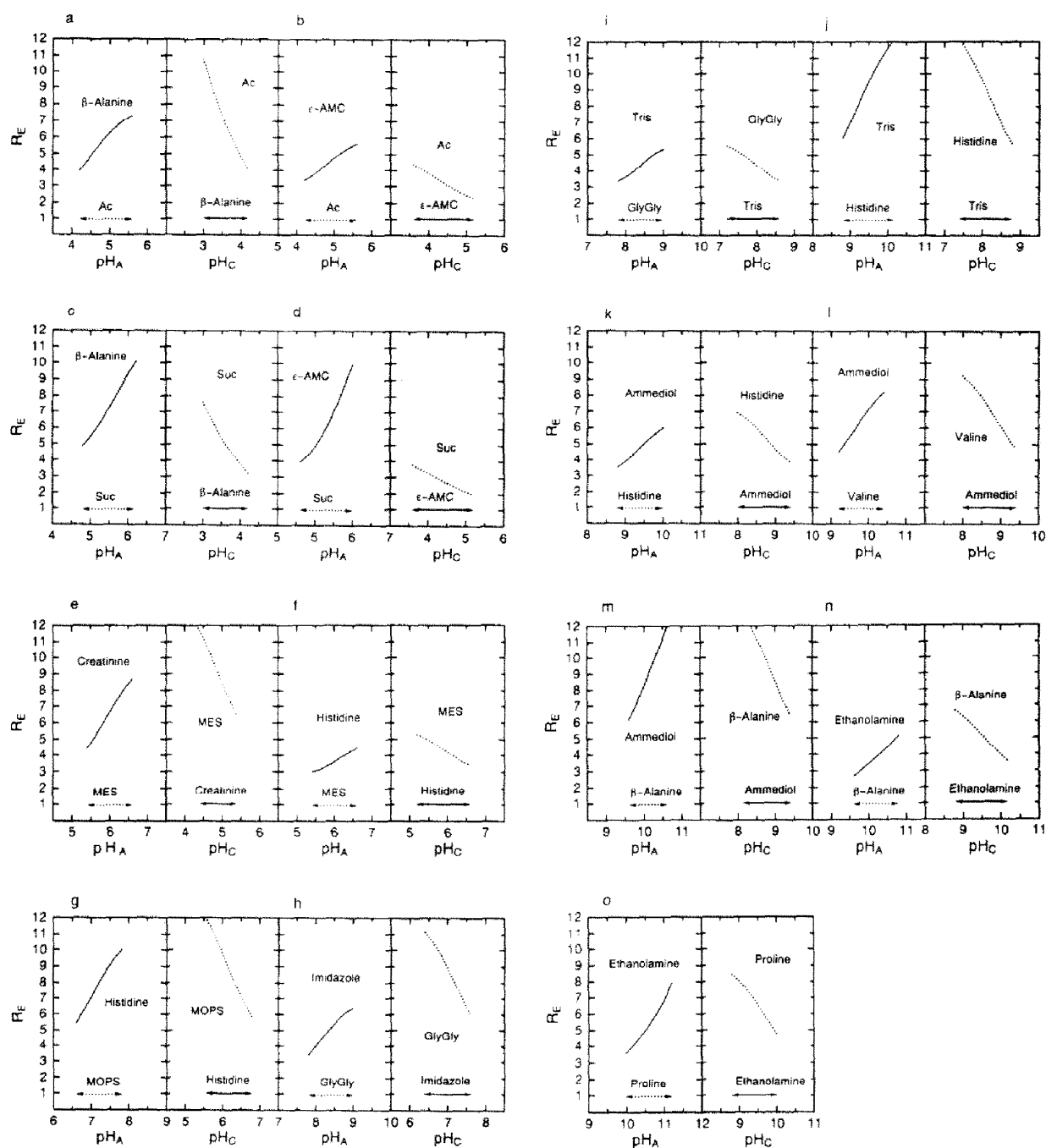
Fig. 3. pH dependence on $R_i$ values of the terminators for bidirectional electrolyte systems listed in Table 1. Solid curves = terminating cations; broken curves = terminating anions. (a) HCl/$\beta$-alanine–KOH/acetic acid system, (b) HCl/$\epsilon$-AMC–KOH/ acetic acid system, (c) HCl/$\beta$-alanine–KOH/succinic acid system, (d) HCl/$\epsilon$-AMC–KOH/succinic acid system, (e) HCl/ creatinine–KOH/MES system, (f) HCl/histidine–KOH/MES system, (g) HCl/histidine–KOH/MOPS system, (h) HCl/imida-zole–KOH/glycylglycine system, (i) HCl/Tris–KOH/glycylglycine system, (j) HCl/Tris–KOH/histidine system, (k) HCl/ ammediol–KOH/histidine system, (l) HCl/ammediol–KOH/valine system, (m) HCl/ammediol–KOH/$\beta$-alanine system, (n) HCl/ethanolamine–KOH/$\beta$-alanine system, (o) HCl/ethanolamine–KOH/proline system.

pH range 3.5–10. The leading anion was 10 mM Cl⁻ and the leading cation was 10 mM K⁺. The mobilities and $pK_a$ values of the buffers at 25°C used for simulation were also listed in Table 1.


## 3. Results and discussion

Using bidirectional electrolyte systems shown in Table 1, the $R_E$ values of the anionic and cationic terminators were simulated and the pH dependence profiles were shown in Fig. 3a–o, where a–o corresponds to electrolyte systems a–o in Table 1. The pH values of the leading electrolyte was varied in the range $pK_a \pm 0.5$ keeping $R_E < 12$ (effective mobility $> 1.95 \cdot 10^{-5}$ cm² V⁻¹ s⁻¹).

In Fig. 3a, for example, the left solid curve shows the $R_E$ values of the cationic terminator ($\beta$-alanine), where the pH of the cationic leading electrolyte (pH of anolyte, $pH_A$) is varied in the range 4.2–5.6 by adding acetic acid to 10 mM KOH. The right broken curve in Fig. 3a shows the $R_E$ values of the anionic terminator (acetic acid), where the pH of the anionic leading

electrolyte (pH of catholyte, $pH_C$) is varied in the pH range 3.0–4.2 by adding $\beta$-alanine to 10 mM HCl. By using Fig. 3, one can select bidirectional ITP electrolyte system suitable for the sample components of interest. The method is detailed in Refs. [2]–[4].

Table 2 summarizes the ITP velocities simulated for bidirectional electrolyte systems listed in Table 1, when the inner diameter of the separation tubes was 0.5 mm and the migration current was 100 μA. The velocities of anions were shown with negative signs. The tabulated velocities ($v$) can be used to estimate the values with different inner diameters ($d$ mm) and currents ($I$) on the assumption of no temperature change in the separation tube:

$$v \ (\text{cm/min}) =$$
$$\text{tabulated velocity} \cdot (0.25/d^2) \cdot I/100 \qquad (2)$$

Obviously from Table 2, the ITP velocities of the electrolyte systems proposed were approximately the same with each other (2 cm/min) both for anions and cations. Therefore, the length of the separation tube can be adjusted to coincide the starting time of the anionic sample

Table 2
ITP velocities for the bidirectional electrolyte systems

| System[a] | $pH_A$ | Anionic velocity (cm/min) | $pH_C$ | Cationic velocity (cm/min) |
|---|---|---|---|---|
| a | 3.6 | − 2.04 | 4.8 | 2.04 |
| b | 4.4 | − 2.33 | 4.8 | 2.04 |
| c | 3.6 | − 2.04 | 5.6 | 1.92 |
| d | 4.4 | − 2.33 | 5.6 | 1.92 |
| e | 4.8 | − 2.17 | 6.0 | 2.35 |
| f | 6.0 | − 2.34 | 6.0 | 2.35 |
| g | 6.0 | − 2.34 | 7.2 | 2.44 |
| h | 7.2 | − 1.93 | 8.4 | 2.27 |
| i | 8.0 | − 2.35 | 8.4 | 2.27 |
| j | 8.0 | − 2.35 | 9.4 | 2.32 |
| k | 8.8 | − 2.29 | 9.4 | 2.32 |
| l | 8.8 | − 2.29 | 9.8 | 2.32 |
| m | 8.8 | − 2.29 | 10.2 | 2.23 |
| n | 9.4 | − 2.04 | 10.2 | 2.23 |
| o | 9.4 | − 2.04 | 10.6 | 2.18 |

Anolyte: 10 mM HCl + buffer; catholyte: 10 mM KOH + buffer.
[a] Bidirectional electrolyte systems listed in Table 1.

zones and cationic ones, or to delay the detection.

To detect both cations and anions by using the single detector system without switching, the zones should be driven to the detector by a suitable hydrostatic flow. An electroosmotic flow in a fused-silica capillary tube may be useful for the purpose because it is an ideal plug flow. However, since the electroosmotic flow is generally not so high compared with the ITP velocity, an additional pressure-driven flow is necessary.

In conclusion, ITP can be bidirectional as capillary zone electrophoresis by using an appropriate bidirectional electrolyte system, which consists of a leading electrolyte for anions (anolyte) and that for cations (catholyte). The fifteen electrolyte systems in Table 1 will be useful to achieve bidirectional ITP.

## References

[1] T. Hirokawa, K. Watanabe, Y. Yokota and Y. Kiso, J. Chromatogr., 633 (1993) 251.

[2] T. Hirokawa, M. Nishino, N. Aoki, Y. Kiso, Y. Sawamoto, T. Yagi and J. Akiyama, J. Chromatogr., 271 (1983) D1.

[3] F.M. Everaerts, J.L. Beckers and Th.P.E.M. Verheggen, Isotachophoresis —Theory, Instrumentation and Applications, Elsevier, Amsterdam, Oxford, New York, 1976, pp. 283–301.

[4] P. Boček, M. Deml, P. Gebauer and V. Dolnik, in B.J. Radola (Editor), Analytical Isotachophoresis, VCH, Basle, Cambridge, New York, 1988.