

# SEPARATION OF AMINO ACIDS BY CONTINUOUS-FLOW ELECTROPHORESIS

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## Introduction

Currently, the application of electrophoresis to concentrate and/or separate biologically active compounds on an industrial scale is being investigated.<sup>1-4)</sup> We have presented a theoretical model describing a process to concentrate amino acid by a continuous-flow electrophoresis.<sup>6)</sup>

This paper extends our previous work to the separation of two amino acids. We conducted a numerical analysis of the theoretical model and verified its validity experimentally. A study was elaborated on the effect of buffer on the separation of two amino acids.

## 1. Theory

Amino acid, due to its amphoretic nature, can exist in a cationic, an anionic or a neutral molecular state, according to the pH of the environment. Hence, if a reversed pH gradient exists in an electric field, positive and negative ionic species are forced to migrate in reverse directions, respectively, toward a position referred to as the isoelectric pH point.

Because each amino acid has an inherent isoelectric point, the respective amino acids will concentrate at different positions and can be recovered separately under a mild pH gradient provided by adding a proper buffer such as weak acid and/or weak base.

Now consider a separation of *p*-amino benzoic

acid (*p*-ABA;  $A_1$ ) and histidine (His;  $A_2$ ) from their mixture in the presence of weak acid or base (B) as buffer and KCl as background electrolyte.

There are 13 species in the system, as follows:  $K^+$ ,  $Cl^-$ ,  $H^+$ ,  $OH^-$ ,  $A_1^+$ ,  $A_1^0$ ,  $A_1^-$ ,  $A_2^{2+}$ ,  $A_2^+$ ,  $A_2^0$ ,  $A_2^-$ ,  $B^0$ , and  $B^-$ .

The transport process of these species can be simulated numerically by use of the theoretical model proposed in our previous paper.<sup>6)</sup>

## 2. Experiment

The experimental apparatus and procedure were similar to those previously used.<sup>6)</sup> Concentrations of *p*-ABA and histidine in the samples were quantitatively analyzed with a reverse-phase liquid chromatograph.

Physical properties of the species are listed in **Table 1**. Most of cited references are the same as those in the previous paper.<sup>6)</sup> The diffusion coefficient of 2-(*N*-morpholino)ethanesulfonic acid (MES) was estimated by Wilke-Chang's correlation.<sup>5)</sup>

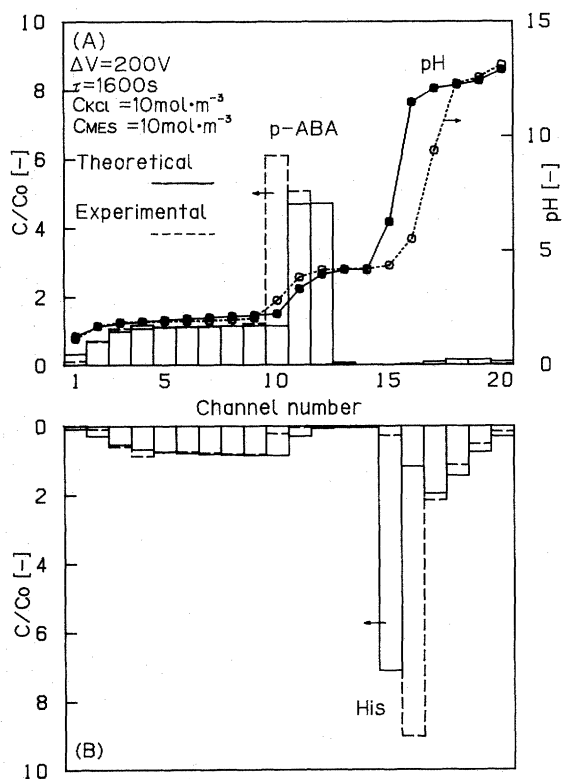
## 3. Results and Discussion

**Figure 1** shows theoretical and experimental results of the outlet concentration of the electrophoretic chamber at an applied voltage of 200V and a residence time of 1600 s. MES was added as a buffer in the solution. The concentrations of the amino acids were  $10^{-3} M$  and the concentrations of buffer and background electrolyte were  $10^{-2} M$  in the feed. The upper half of the figure represents the profiles of

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**Table 1.** Physical Properties at 298 K

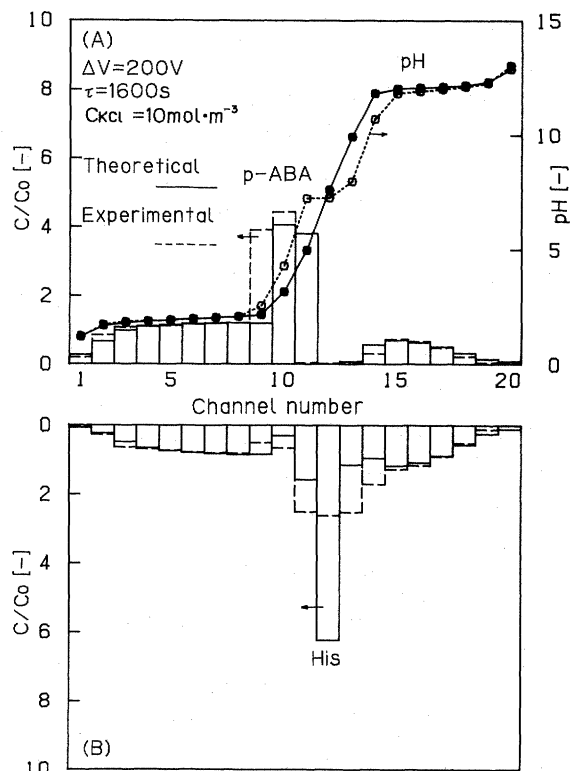
Species	Diffusion coef. [m <sup>2</sup> ·s <sup>-1</sup> ]	Dissociation const. [mol·m <sup>-3</sup> ]
K <sup>+</sup>	1.956 × 10 <sup>-9</sup>	—
Cl <sup>-</sup>	2.032 × 10 <sup>-9</sup>	—
H <sup>+</sup>	9.310 × 10 <sup>-9</sup>	—
OH <sup>-</sup>	5.277 × 10 <sup>-9</sup>	—
<i>p</i> -ABA <sup>(+)</sup>	8.425 × 10 <sup>-10</sup>	4.1687
<i>p</i> -ABA <sup>(0)</sup>	8.425 × 10 <sup>-10</sup>	—
<i>p</i> -ABA <sup>(-)</sup>	8.425 × 10 <sup>-10</sup>	1.288 × 10 <sup>-2</sup>
His <sup>(2+)</sup>	7.328 × 10 <sup>-10</sup>	15.14
His <sup>(+)</sup>	7.328 × 10 <sup>-10</sup>	9.12 × 10 <sup>-4</sup>
His <sup>(0)</sup>	7.328 × 10 <sup>-10</sup>	—
His <sup>(-)</sup>	7.328 × 10 <sup>-10</sup>	7.586 × 10 <sup>-7</sup>
HAc <sup>(0)</sup>	1.362 × 10 <sup>-9</sup>	—
HAc <sup>(-)</sup>	1.362 × 10 <sup>-9</sup>	1.754 × 10 <sup>-2</sup>
MES <sup>(0)</sup>	7.463 × 10 <sup>-10</sup>	—
MES <sup>(-)</sup>	7.463 × 10 <sup>-10</sup>	5.370 × 10 <sup>-4</sup>
H <sub>2</sub> O	—	1.009 × 10 <sup>-8</sup>



**Fig. 1.** Comparison of theoretical and experimental results for continuous electrophoretic separation of *p*-amino benzoic acid and histidine with added buffer, 2-(*N*-morpholino)ethanesulfonic acid

*p*-ABA concentration and pH and the lower half represents the profiles of histidine concentration.

**Figure 2** shows theoretical and experimental results at the same conditions as that of Fig. 1, but in the absence of buffer. In both figures, the theoretical concentration and pH profiles were in good agreement with those by experiment. This suggests that a quantitative estimation of the continuous electrophoretic process could be made by this kind of



**Fig. 2.** Comparison of theoretical and experimental results for continuous electrophoretic separation of *p*-amino benzoic acid and histidine without buffer

theoretical analysis. Slight differences in the pH distribution and in the position of the concentration peak of amino acids may be attributed to the difference of dissociation constants of the amino acids and the buffer between in the real solution and in an infinite dilute solution used in the theoretical analysis. Local ionic strength varies remarkably with position in the electrophoretic chamber.

Comparison of the two figures shows that added buffer plays a significant role in the separation of the two amino acids. To explore the effect of buffer addition more closely, the theoretical concentrations of amino acid and pH, together with the concentration of buffer at the outlet, are shown in **Figs. 3 and 4**.

MES is concentrated at the position between the concentration peaks of *p*-ABA and histidine in Fig. 3. The pH corresponding to the position of the MES concentration peak is about 4. The pH is in between the two isoelectric points of the amino acids.

MES acts not only as a buffer but also as a spacer of the two amino acids.

On the other hand, in Fig. 4, a steeper pH distribution is formed due to a rapid neutralization reaction between the hydrogen and hydroxyl ions. Hence the amino acids are concentrated at the narrow region, the concentration peaks of the two amino acids lying very close to each other. A perfect separation cannot be achieved in this system. It is found that the

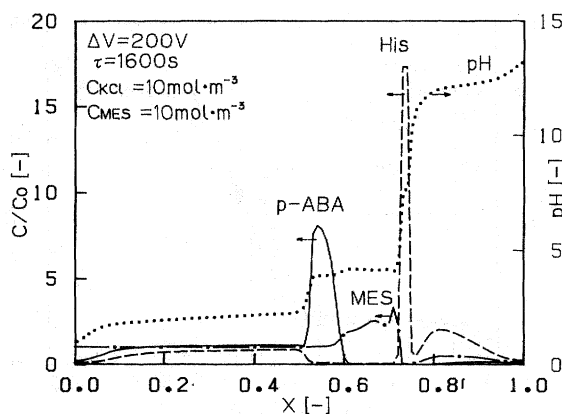


Fig. 3. Theoretical concentration distributions of components at outlet of electrophoretic chamber with 2-(N-morpholino)ethanesulfonic acid as buffer

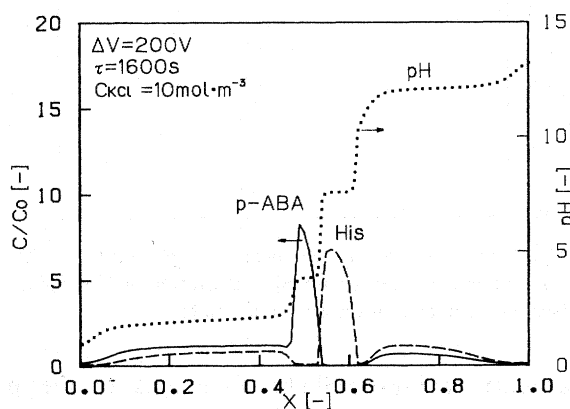


Fig. 4. Theoretical concentration distributions of components at outlet of electrophoretic chamber without buffer

theoretical peak height of *p*-ABA is higher than that of histidine in Fig. 4, the reverse of their relation in Fig. 2. This is because an integral average value was adopted in Fig. 2 and was not adopted in Fig. 4.

Figure 5 shows another calculated result, using acetic acid (HAc) as buffer. Acetic acid is found not to work as a good spacer, because the concentration peak of acetic acid shifts more closely to the anode than that of MES. The pH corresponding to the peak of acetic acid is about 3.4, which is less than the isoelectric point of *p*-ABA ( $pI=3.63$ ).

It is reasonable to assume that only  $B^-$  and  $H^+$  exist at the position of buffer peak. Then, using the electroneutrality condition,  $C_{B^-} = C_{H^+}$ , the equilibrium relation of the chemical dissociation reaction can be written as

$$pH = (pK_B - \log C_{B^0})/2 \quad (1)$$

Substituting the value of buffer concentration,  $C_B^0$  ( $\cong 10^{-2}$  M), and the dissociation constant into Eq. (1), pH for acetic acid and that for MES were estimated as 3.37 and 4.13. Then the following inequality equations can be obtained.

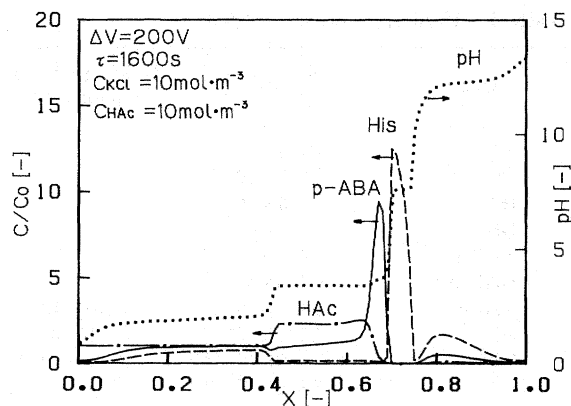


Fig. 5. Theoretical concentration distributions of components at outlet of electrophoretic chamber with acetic acid as buffer

$$\left. \begin{aligned} pI_{p-ABA} &< pH_{MES} < pI_{His} \\ pH_{HAc} &< pI_{p-ABA} < pI_{His} \end{aligned} \right\} \quad (2)$$

It can be concluded that MES works as a spacer and acetic acid does not in this system.

## Conclusions

The continuous electrophoretic separation of two amino acids has been examined theoretically and experimentally.

It was concluded that a proper buffer, which works as a spacer, is required to separate two amino acids. The buffer must well be chosen so that pH at its concentration peak can be in between the isoelectric point of the two amino acids to be separated.

## Nomenclature

$C$	= concentration	[mol·m <sup>-3</sup> ]
$pI$	= isoelectric point	[-]
$pK$	= dissociation constant	[-]
$V$	= applied voltage	[V]
$X$	= dimensionless $x$ coordinate	[-]
$x$	= coordinate normal to flow direction	[m]
$Y$	= dimensionless $y$ coordinate	[-]
$y$	= coordinate parallel to flow direction	[m]
$\tau$	= residence time	[s]

## <Subscripts>

$B$	= buffer
$0$	= inlet

## <Superscripts>

$+, -, 0$	= cationic, anionic and neutral species respectively
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## Literature Cited

- 1) Carmichael, G. R., H. L. Zingher, R. Datta and R. A. Yoshisato: *J. Chem. Tech. Biotechnol.*, **41**, 207 (1988).
- 2) Gobie, W. A. and C. F. Ivory: *AIChE J.*, **34**, 474 (1988).
- 3) Kuhn, R., H. Wagner, R. A. Mosher and W. Thormann: *Electrophoresis*, **8**, 503 (1987).

- 4) Thomson, A. R.: *J. Chem. Tech. Biotechnol.*, **34B**, 197 (1984).
- 5) Wilke, C. R. and P. Chang: *AIChE J.*, **1**, 264 (1955).
- 6) Zheng, S. N., J. H. Egocheaga, T. Sato, T. Yonemoto and T. Tadaki: *J. Chem. Eng. Japan*, **22**, 247 (1989).

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