Talanta 84 (2011) 651–658

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Comparative study on sample stacking by moving reaction boundary formed with weak acid and weak or strong alkali in capillary electrophoresis
I. Theory

Chengxi Cao*, Wei Zhang, Liuyin Fan**, Jing Shao, Si Li

Laboratory of Analytical Biochemistry & Bio-separation, Key Laboratory of MOE for Microbial Metabolism and School of Life Science & Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China

ARTICLE INFO

Article history:
Received 14 October 2010
Received in revised form 14 January 2011
Accepted 21 January 2011
Available online 28 January 2011

Keywords:
Capillary electrophoresis
Electromigration
Moving reaction boundary
Sample stacking

ABSTRACT

The condensation of low abundance zwitterion substance, such as protein and peptide, has great significance to the study on proteomics. This paper develops the theory on design of online stacking conditions of zwitterion by a moving reaction boundary (MRB) in capillary electrophoresis (CE). This concerns the choice of running and sample buffers, velocity design of MRB, and salt effect on the stacking. The theoretical results unveil that: (1) the velocity of MRB formed with weak acidic buffer and strong alkali should be set between zero and the velocity of zwitterion in the alkali phase, or no stacking occurs; (2) if a strong alkali is used to prepare the sample, a much long front plug of strong base must be injected before the alkaline sample plug for complete stacking, whereas no such front plug is needed if a weak alkali with enough high concentration and pH value is used to prepare the sample buffer; (3) the existence of salt in sample matrix has a weak effect on the stacking of zwitterion if sample is prepared with weak alkaline buffer, while has a dramatic effect on the same stacking if with a strong base buffer. In addition, the concentration of weak alkali used for preparation of sample should be set at the point, at which the velocity of MRB is as much as possible close to that of negative zwitterion. The developed theory and its computation are quantitatively proved by the experiments of zwitterion stacking by the MRB as shown in the previous and the accompanying papers. The proposed theoretic results hold obvious significances on-column stacking of low abundance zwitterions, such as amino acid, or peptides or proteins, in CE.

1. Introduction

On-column sample stacking has become a simple but powerful technique to improve the sensitivity of capillary electrophoresis (CE). The electro-stacking or the field-amplified sample injection (FASI) stacking was first recognized by Haglund and Tiselius [1] in 1950. And the theory was systemically advanced during 1991–1992[2–6]. Using FASI, Chien and Burgi [7] concentrated analytes 1000-fold. In 2000, Zhang et al. [8] achieved 10,000-fold FASI-based stacking. The theory of isotachophoresis (ITP)-based stacking for protein in polyacrylamide gel electrophoresis (PAGE) was developed by Ornstein [9] and Davis [10] in 1964 from the concept of MBS [11–17]. ITP also remains a popular preconcentration technique in CE [18–23]. During the last two decades, the theory of ITP stacking has been well developed for sample condensation by Boček and co-workers [21] and Shihabi [24,25]. The stacking mode of pH-mediated was advanced by Lunte's group [26–28]. Based up on their experiments, they further investigated the mechanism of base stacking [29]. A selective focusing for the analytes can be achieved by a pH junction [30–38]. Recently, Britz-McKibbin and co-workers combined the stacking with the 'sample sweeping', developed the online stacking strategies for trace analysis of metabolites [36,37] and the computer simulation for the dynamical mechanism of stacking [38]. To concentrate neutral analytes, Quirino et al. [39–43] and Palmer et al. [44–47] advanced a novel sweeping method in micellar electrokinetic chromatography (MEKC).

The theory of MRB [48,49] was developed from the pioneer ideas of precipitate reactive front evolved by Deman and Rigole [50,51] and stationary neutralization boundary by Pospichal et al. [52]. The theory of MRB opened new horizons for the investigation of electrophoresis. For example, it has been successfully used for the dynamic study of isoelectric focusing (IEF) by Svensson [53,54], as revealed by Cao et al. [48,49]. The MRB method has also been employed for the improvement of sensitivity of analyte in high salt matrix in CE [55,56] and for the illumination on the mechanism of EDTA-based sample sweeping [57–60] greatly contributed

---

* Corresponding author. Tel.: +86 21 34205820; fax: +86 21 34205820.
** Corresponding author. Tel.: +86 21 34205682.
E-mail addresses: cxcao@sjtu.edu.cn (C. Cao), lyfan@sjtu.edu.cn (L. Fan).

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.01.059

Nomenclature

a	the degree of ionization of weak electrolyte
c	the equivalent concentration (equiv m^{-3}). The subscripts + and − indicate the positive and negative reaction ions respectively and the superscripts α and β imply phase α and β respectively. Signed quantity, positive if the ion carries net positive charge (s), and negative if net negative charge(s), as has been treated by numerous scientists [11–18,48–52,57–60,63]
$\bar{c}$	the constituent concentration (equiv m^{-3}) which indicates that it do not apply to an ion but to the equilibrium mixture of all subspecies of a constituent
E	the electric field strength (V m^{-1}), the superscripts α and β imply phase α and β respectively
i	the electric current intensity (A m^{-2}) in capillary
L_0	the plug length of alkaline zwitterion matrix injected into the capillary (m)
L_x	the plug length (m) of front alkaline solution (without the zwitterion) injected into the capillary in the front of the sample plug. Signed quantity like c
$L_{c,x}$	the critical length of front alkaline plug of L_x (m) that produces complete stacking. Signed quantity like c
L_B	the distance (m) of boundary movement from the original point of boundary to the finish point of boundary after the end of MRB. Signed quantity like c
m	the mobility (m^2 V^{-1} s^{-1}). Signed quantity like c
$\bar{m}$	the constituent mobility (m^2 V^{-1} s^{-1})
t_B	the time (s) of boundary movement from the original point of boundary to the end of MRB
V_{EOF}	the velocity of EOF (m s^{-1}) moving towards the cathode normally. The subscript of β or γ implies the EOF velocity in capillary filled with only a single buffer
$\bar{V}_{\text{EOF}}$	the average velocity of EOF (m s^{-1}) in capillary filled with two different solutions
$V^{\alpha\beta}$	the velocity (m s^{-1}) of MRB. The subscripts “strong” and “weak” mean the strong or weak electrolytes respectively. Signed quantity like c
$V^{\beta}_{\text{OH}^-}$	the velocity of hydroxyl ion (m s^{-1}) in phase β, viz., the alkaline sample matrix, as shown in Fig. 1. The definition see Eq. (12).
$\bar{V}^{\beta}_{\text{OH}^-}$	the constituent velocity of hydroxyl ion (m s^{-1}) in phase β defined in Eq. (25). Always negative values.
$\bar{V}^{\alpha}_{z^+}$	the constituent velocity of zwitterion (m s^{-1}) in phase α as shown in expression (19)
$\bar{V}^{\beta}_{z^-}$	the consituent velocity of zwitterion (m s^{-1}) in phase β as given in experessions (7), (8) and (11)
k	the specific conductivity of a phase in Figs. 1 and 2 (S m^{-1})
ρ	the specific resistivity (Ω m^{-1}) of a phase

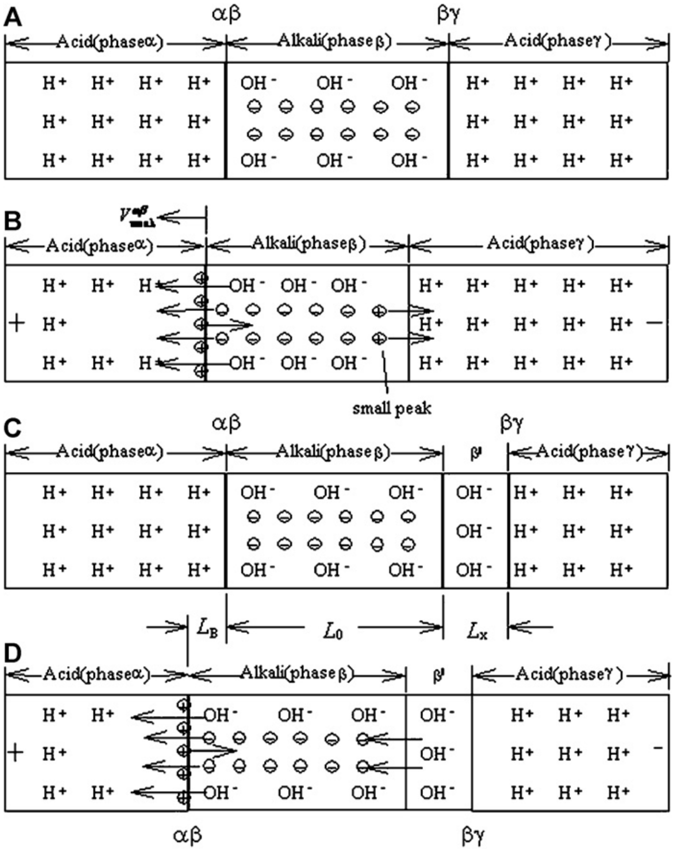

Fig. 1. The mechanism of stacking zwitterion with MRB moving towards the anode. (A) The initial state of the acidic buffer zones (phase α and γ) and sample zone of zwitterion (phase β) dissolved in the alkaline buffer and carried negative charges under the high pH condition, (B) the formation of MRB between phase α and β, which result in the stacking of most zwitterion in phase β and the creation of of low pH zone near phase γ owing to the fast electromigration of OH$^-$, leading to the positive charge carried by zwitterion and leaking of zwitterion near phase γ (this induces a small peak of zwitterions like His, Fig. 5 in Ref. [64]) after turn-on the electric field, (C) the injection of the front alkaline plug of phase β without the zwitterion before the injection of zwitterion, (D) the good stacking of zwitterion by the MRB and the front alkaline solution of phase β. The arrows indicate the directions of zwitterion and MRB, the symbols of ‘+’ and ‘−’ imply the anode and cathode respectively.

by Quirino and Terabe and co-workers [40–47,43,61] and Palmer et al. [44–47].

It was observed that the high concentration salt in the sample matrix has no effect on the MRB-based stacking [49,55], the stacking becomes good and much stable if the sample matrixes were prepared with weak electrolyte(s) (such as ammonia used in Section 3.1.3 [62] and sodium formate [55]). The relevant mechanism why the MRB-based stacking can condense the analyte(s) in high salt sample matrix was revealed recently [63]. However, if the sample matrix were prepared with strong electrolyte of NaOH, the stacking turns poor and much unstable, as will be shown in Ref. [64]. The relevant mechanism is still unclear. Furthermore, the comparison of MRB-based stacking with weak base (e.g., ammonia) and sodium hydroxide has not been performed yet.

Thus, the purposes herein are to perform the theoretical study on the MRB-based stacking, compare the MRB-based stacking of different sample matrixes prepared with weak and strong alkalis, and reveal the relevant mechanism of stacking under the different conditions in which strong or weak alkali was used as the preparation of sample matrix. The relevant experiments are shown in the accompanying paper [63] as well as the previous papers [55,56,61,62].

2. Theory

2.1. Velocity of MRB

Fig. 1A shows that the left solution in capillary is phase α, *viz.*, the running buffer, containing hydrogen ion, the middle is phase β holding hydroxyl ion and zwitterionic sample, e.g., amino acid, the right is the running buffer. Obviously, a MRB is created between phase α and β, when the electric field is applied. The boundary velocity can be computed with the following equations, as have been formulated [49,56,63],

$$V^{\alpha\beta}_{\mathrm{strong}} = \left(\frac{m^{\alpha}_{\mathrm{H^+}} c^{\alpha}_{\mathrm{H^+}}}{\kappa^{\alpha}} - \frac{m^{\beta}_{\mathrm{OH^-}} c^{\beta}_{\mathrm{OH^-}}}{\kappa^{\beta}} \right) \frac{i}{c^{\alpha}_{\mathrm{H^+}} - c^{\beta}_{\mathrm{OH^-}}} \tag{1}$$

$$V^{\alpha\beta}_{\mathrm{weak}} = \left(\frac{\bar{m}^{\alpha}_{\mathrm{H^+}} \bar{c}^{\alpha}_{\mathrm{H^+}}}{\kappa^{\alpha}} - \frac{\bar{m}^{\beta}_{\mathrm{OH^-}} \bar{c}^{\beta}_{\mathrm{OH^-}}}{\kappa^{\beta}} \right) \frac{i}{\bar{c}^{\alpha}_{\mathrm{H^+}} - \bar{c}^{\beta}_{\mathrm{OH^-}}} \tag{2}$$

Eq. (1) is used for the system of MRB created with strong electrolytes, e.g., HCl and NaOH [49], while Eq. (2) for the system of weak electrolytes, such as CH_3COOH and NH_3H_2O [49,56,63].

2.2. General conditions of stacking zwitterion by MRB

For simplity and convenience, amino acids are used as the zwitterion examples for the theoretic investigation of the stakcing. In addition, the absolute mobility of monovalence negative amino acid is approximately considered to be equal to that of the positive amino acid, as have been proved by Svensson [53,54] and will be further used herein and in the accompanying paper [64].

In the arrangement of Fig. 1A, the condition of pH values of phase α and β, which produce the stacking effect to a zwitterion, is the following inequality [49,56]

$$\mathrm{pH}^{a} < pI < \mathrm{pH}^{\beta} \tag{3}$$

In the alkaline solution of phase β in Fig. 1, if the pH value of solution is over 2 pH units than the $p\mathrm{K}_2$ of an amino acid, then over 99% of the zwitterion carries a negative charge, this results in the near maximum mobility of the amino acid towards the anode. And in the acidic solution of phase α in Fig. 1, if the pH value of solution is less 2 pH units than the $p\mathrm{K}_1$, about 99% of the zwitterion is ionized as a positively charged molecular, this leads to the near maximum mobility of the amino acid towards the cathode. Therefore, for near maximum stacking of amino acids, inequality (3) should be re-expressed as the much better conditional inequality of pH values of phase α and β, as given below [49,56]

$$\mathrm{pH}^{a} + 2 \le p\mathrm{K}_1 < \mathrm{pI} < p\mathrm{K}_2 \le \mathrm{pH}^{\beta} - 2 \tag{4a}$$

But for these amino acids of Lys, Arg and His, which have an ionizable side group in their molecule, inequality (4a) should be expressed as [49,56]

$$\mathrm{pH}^{a} + 2 \le p\mathrm{K}_{\mathrm{R}} < \mathrm{pI} < p\mathrm{K}_2 \le \mathrm{pH}^{\beta} - 2 \tag{4b}$$

Inequality (3) and (4) indicate the relations not only between amino acid stacked and the pH values of electrolytic soltions forming a MRB, but also between the pH values of phase α and β.

2.3. Stacking by MRB formed by weak acid and strong alkali

Assume the alkali in phase β is a strong base of NaOH, the boundary migrates towards the anode and there is a zwitterion in phase β in Fig. 1B. When the electric field was employed, the hydrogen ions in phase α were continuously neutralized by the hydroxy ions moving from phase β. Evidently, the condition, which can yield stacking effect to the zwitterion in phase β, is according adjusted as

$$\left| V^{\alpha\beta}_{\mathrm{w-s}} \right| < \left| \bar{V}^{\beta}_{\mathrm{z^-}} \right| = \left| \bar{m}^{\beta}_{\mathrm{z^-}} E^{\beta} \right| \tag{5a}$$

$$\bar{m}_{z^-} = a\, m_{z^-} \tag{5b}$$

Expression (5) implies that if the velocity of MRB in Fig. 1B is equal to or over that of the zwitterion in phase β, there is no any stacking efficiency to the zwitterion, conversely if the absolute velocity of MRB is less than that of the zwitterion, there exists a stacking of the zwitterion, and the slower the boundary moves towards the anode and the faster the zwitterion migrates towards the anode, the better the stacking becomes. The conclusion of Eq. (5) is well proved by the comparisons of His and Trp stacking in Table 2 in the accompanying paper [64].

In addition, the absolute velocity of MRB must be less than the velocity of electroosmotic flow (EOF) in the two buffer system moving towards the cathode generally, *viz.*,

$$\left| V^{\alpha\beta}_{\mathrm{w-s}} \right| < \bar{V}_{\mathrm{EOF}} \tag{6}$$

or, the zwitterion may migrate out of capillary and into the anodic vessel, even the MRB cannot be formed due to the absence of expression (6). As given by Chien and Burgi [5], the average EOF velocity in the two-buffer system in Fig. 1 is given as

$$\bar{V}_{\mathrm{EOF}} = \frac{V_{\mathrm{EOF},\gamma}(1-x)\rho^{\gamma}}{\rho^{\gamma}(1-x)+\rho^{\beta}x} + \frac{V_{\mathrm{EOF},\beta}\,x\rho^{\beta}}{\rho^{\gamma}(1-x)+\rho^{\beta}x} \tag{7}$$

Eq. (7) shows that the EOF velocity is not only weighted over the filled lenghts of their components, but also weighted over their partial resistances. It should be noted here that the parameter x is not a constant, but a variable quantity. With the reaction between phase α and β progresses, the length of phase β becomes shorter and shorter, conversely phase γ (or α) gets longer.

The velocities of zwitterion and hydroxyl ion in phase β are respectively

$$\bar{V}^{\beta}_{\mathrm{z^-}} = \bar{m}^{\beta}_{\mathrm{z^-}} E^{\beta} \tag{8}$$

$$V^{\beta}_{\mathrm{OH^-}} = m^{\beta}_{\mathrm{OH^-}} E^{\beta} \tag{9}$$

Because the velocity of zwitterion is always less than that of hydroxyl ion (the fastest anion [65]) in phase β, *viz.*,

$$\left| \bar{V}^{\gamma}_{\mathrm{z^-}} \right| < \left| V^{\gamma}_{\mathrm{OH^-}} \right| \tag{10}$$

Hence, the fastest electro-migration of hydroxyl ion near phase γ results in the obvious decrease of pH value in original phase β near phase γ, as shown in Fig. 1B. The zwitterion at the front part of phase β will lose its partial negative charge (such as Trp with pI 5.89), or even carry partial positive charge (such as His with pI 7.59). Thus, the zwitterion in phase β near phase γ cannot be stacked well. This leads to the existence of shoulder near the stacked peak of amino acid (see Figs. 4 and 8 of Ref. [64]) and even small peak before the partially stacked peak of amino acid (see Figs. 5 and 6 in Ref. [64]).

How to stack the shoulder and the small peak near the stacked peak of amino acid? Two methods can solve the problem. The first method is to use weak alkali with proper concentration and pH value as the sample buffer in displacement with the strong base of NaOH (see the sodium formate [55,56,63], ammonia used in Section 3.1.3 [62] and Section 3.5 herein). The second one is to inject a front plug of the same strong alkali NaOH without zwitterion, *viz.*, L_{x}, as shown in Fig. 1C. The second method is also an efficient one indeed. For a complete stacking of zwitterion in sample matrix, L_{x} ought to meet the following relations

$$\frac{L_0 - L_{\mathrm{B}}}{\bar{V}^{\beta}_{\mathrm{z^-}}} \le \frac{L_0 - L_{\mathrm{B}} + L_{\mathrm{x}}}{V^{\beta}_{\mathrm{OH^-}}} \tag{11}$$

$$\frac{L_0 - L_{\mathrm{B}}}{\bar{V}^{\beta}_{\mathrm{z^-}}} \le \frac{-L_{\mathrm{B}}}{V^{\alpha\beta}_{\mathrm{w-s}}} \tag{12}$$

$$\frac{-L_{\mathrm{B}}}{V^{\alpha\beta}_{\mathrm{w-s}}} = \frac{L_0 - L_{\mathrm{B}} + L_{\mathrm{x}}}{V^{\beta}_{\mathrm{OH^-}}} \tag{13}$$

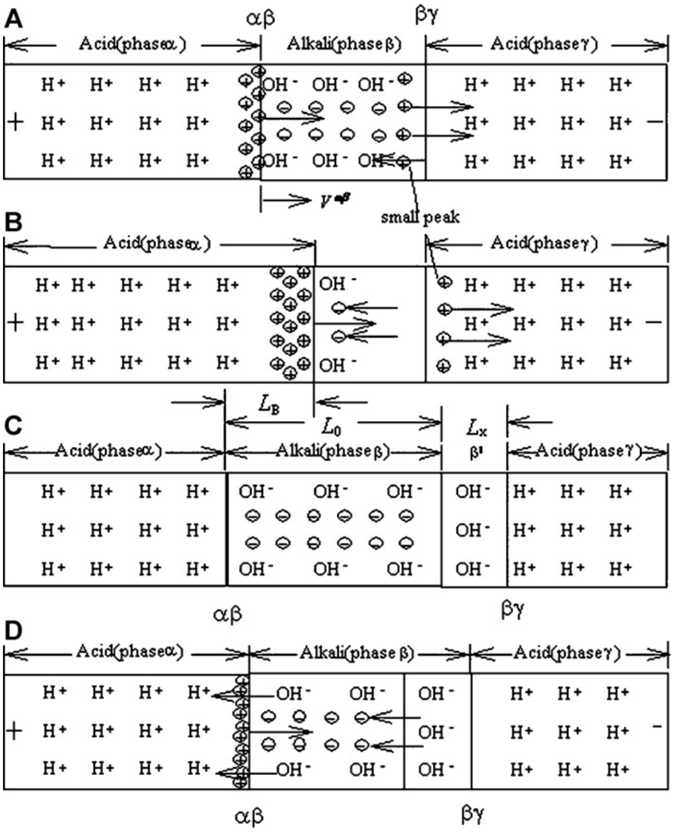

Fig. 2. The mechanism of stacking zwitterion by MRB towards the cathode. The initial state of stacking see Fig. 1A. (A) The formation of MRB towards the cathode, which result in the stacking of most zwitterion and leaking of negative zwitterion near phase γ, (B) partial stacking of zwitterion at the left side of sample plug due to $V^{\alpha\beta} > V^{\alpha}_{z^+}$ and the formation of small peak of zwitterion like His, (C) the front alkaline plug of phase β in the front of the alkaline zwitterion plug, (D) the well stacking of zwitterion by the MRB and the front alkaline phase β after use of electric field. The other symbols see Fig. 1.

Combing with Eqs. (11)–(13) one gets expression (14) for completely stacking zwitterion near phase γ,

$$L_{c,x} = \frac{V^{\beta}_{OH^-} - \bar{V}^{\beta}_{z^-}}{\bar{V}^{\beta}_{z^-} - V^{\alpha\beta}_{w-s}} L_0 \tag{14a}$$

$$V^{\alpha\beta}_{w-s} = \left(\frac{\bar{m}^{\alpha}_{H^+}\bar{c}^{\alpha}_{H^+}}{\kappa^{\alpha}} - \frac{m^{\beta}_{OH^-}c^{\beta}_{OH^-}}{\kappa^{\beta}} \right) \frac{i}{\bar{c}^{\alpha}_{H^+} - c^{\beta}_{OH^-}} \tag{14b}$$

Eq. (14) indicates that L_x must be injected up to a critical length ($L_{c,x}$) for the complete stacking of zwitterion injected with a given time (or length) L_0. Or the alkaline plug cannot be stacked completely, and under this condition of $L_x < L_{c.x,}$ a shoulder (see Figs. 4B, 4D, 6A–6B and 9A–9B in Ref. [64]), even double peaks (see Figs. 2A, 4A and 10A in Ref. [64]), can be observed. The significance of Eq. (14) is given in detail in Section 3.2.

Fig. 2A shows the boundary migrating towards the cathode. Manifestly, if the boundary velocity exceed that of the zwitterion in phase α, *viz.*,

$$V^{\alpha\beta}_{w-s} > \bar{V}^{\alpha}_{z^+} = \bar{m}^{\alpha}_{z^+} E^{\alpha} \tag{15a}$$

$$\bar{m}_{z^+} = a\, m_{z^+} \tag{15b}$$

the positive zwitterion in phase α near the MRB cannot catch up with the boundary moving towards the cathode, so can be only partially stacked as shown in Fig. 2B. The larger the difference of $V^{\alpha\beta}_{w-s} - \bar{V}^{\alpha}_{z^+}$ is, the poorer the stacking of the zwitterion by the MRB becomes.

In order to maximumly stack the zwitterion at the left side of the sample plug injected, evidently the velocity of MRB ought to meet to the following inequality

$$0 < V^{\alpha\beta}_{w-s} \le \bar{V}^{\alpha}_{z^+} = \bar{m}^{\alpha}_{z^+} E^{\alpha} \tag{16}$$

The predictions of expression (15) and (16) will be confirmed by the direct comparisons of His and Trp velocities in No. 1, 2, 3 and 5 of Table 2 in Ref. [64] and the related experiments, and also by the indirect results in Fig. 11 in Ref. [64] as well as the experiments in Refs. [56,63].

Next, we will address the stacking of zwitterion in phase β near the boundary β in Fig. 2A again. If there is no another alkaline plug in the front of the sample plug, we cannot completely stack the zwitterion near boundary βγ as analysed in Eq. (14) Hence, we ought to inject another front alkaline plug without the zwitterion, the critical length of $L_{c,x}$ is given as Eqs. (17a) and (17b) if there is no salt in the MRB [49]. Or, $L_{c,x}$ is given as Eqs. (17a) and (17c) if there is high salt like NaCl in the MRB [49].

$$L_{c,x} = -\frac{V^{\beta}_{OH^-} + \bar{V}^{\alpha}_{z^+}}{\bar{V}^{\alpha}_{z^+} + V^{\alpha\beta}_{w-s}} L_0 \tag{17a}$$

$$V^{\alpha\beta}_{w-s} = \left(\frac{\bar{m}^{\alpha}_{H^+}\bar{c}^{\alpha}_{H^+}}{\kappa^{\alpha}} - \frac{m^{\beta}_{OH^-}c^{\beta}_{OH^-}}{\kappa^{\beta}} \right) \frac{i}{\bar{c}^{\alpha}_{H^+} - c^{\beta}_{OH^-}} \tag{17b}$$

$$V^{\alpha\beta}_{w-s} = \left(\frac{m^{\alpha}_{H^+}c^{\alpha}_{H^+}}{\kappa^{\alpha}} - \frac{m^{\beta}_{OH^-}c^{\beta}_{OH^-}}{\kappa^{\beta}} \right) \frac{i}{c^{\alpha}_{H^+} - c^{\beta}_{OH^-}} \tag{17c}$$

The significances of the equations are the similar to those of inequality (14) (details see Section 3.2 herein). Paying careful attentions to the comparison of Eqs. (14b) and (17c), you can find that the constituent concentration and mobility of hydrogen ion in Eq. (14b) are changed as the concentration and mobility of free hydrogen ion in Eq. (17c), if there is a high salt in the MRB system [49].

2.4. Stacking by MRB formed with weak acid and alkali

Assume a weak alkali exists in phase β of Figs. 1 and 2 and the boundary moves towards the anode, then expressions (5) and (6) should be simply changed as(18)$\left|V^{\alpha\beta}_{weak}\right| < \left|\bar{V}^{\beta}_{z^-}\right|$

$$\left|V^{\alpha\beta}_{weak}\right| < \bar{V}_{EOF} \tag{19}$$

The significances of inequality (18) and (19) are similar to those of expression (5) and (6). Furthermore, under the conditions for MRB formed with weak acidic buffer and alkali, Eq. (14) should be re-expressed as Eqs. (20a) and (20b), if no background salt like NaCl exists in the system of MRB [49],

$$L_{c,x} = \frac{\bar{V}^{\beta}_{OH^-} - \bar{V}^{\beta}_{z^+}}{\bar{V}^{\beta}_{z^-} - V^{\alpha\beta}_{weak}} L_0 \tag{20a}$$

$$V^{\alpha\beta}_{weak} = \left(\frac{\bar{m}^{\alpha}_{H^+}\bar{c}^{\alpha}_{H^+}}{\kappa^{\alpha}} - \frac{\bar{m}^{\beta}_{OH^-}\bar{c}^{\beta}_{OH^-}}{\kappa^{\beta}} \right) \frac{i}{\bar{c}^{\alpha}_{H^+} - \bar{c}^{\beta}_{OH^-}} \tag{20b}$$

$$V^{\alpha\beta}_{weak} = \left(\frac{m^{\alpha}_{H^+}c^{\alpha}_{H^+}}{\kappa^{\alpha}} - \frac{m^{\beta}_{OH^-}c^{\beta}_{OH^-}}{\kappa^{\beta}} \right) \frac{i}{c^{\alpha}_{H^+} - c^{\beta}_{OH^-}} \tag{20c}$$

or as Eqs. (20a) and (20c) if there is high concentration salt NaCl in phase β [49]. In Eq. (20), the constituent velocity of hydroxyl ion is given as

$$\bar{V}^{\beta}_{OH^-} = \bar{m}^{\beta}_{OH^-} E^{\beta} \tag{21}$$

Table 1

The comparisons among the free hydroxyl ion mobility, the constituent mobilities of Trp, His and hydroxyl in NH_3H_2O ($\times 10^{-8}$ $m^2\,V^{-1}\,s^{-1}$).

m_{OH^-}	$\bar{m}_{z^-}$ (in 10 mM NH_3H_2O)		$\bar{m}_{OH^-}$ (in NH_3H_2O)[c]					
	Trp	His	1.0 mM	2.0 mM	5.0 mM	10 mM	15 mM	20 mM
–20.5[a]	–1.75[b]	–2.58[b]	–2.52	–1.74	–1.16	–0.85	–0.68	0.59

[a] The parameter is cited from Ref. [68].

[b] The data are calculated from the experiments of Fig. 1A and Table 1 in Ref. [64].

[c] The data are computed with the absolute mobility of hydroxyl ion and dissociation constant of NH_3H_2O at 25 °C (=1.79×10^{-5}) [66].

Assume the boundary moves in the direction of the cathode. Similarly, the velocity conditions of complete stacking of the zwitterion near phase α is the following expression,

$$0 < V_{\text{weak}}^{\alpha\beta} \leq \bar{V}_{z+}^{\alpha} = \bar{m}_{z+}^{\alpha} E^{\alpha} \tag{22}$$

The prediction of inequality (22) has been proved by the calculations of Fig. 5 and results in Refs. [56,63].

Clearly, to stack the zwitterion in phase β near the boundary βγ in Fig. 2A, we may inject a front alkaline plug (without the zwitterion) in the front of the sample plug, the critical length of $L_{c,x}$ is given as Eqs. (23a) and (23b), if there is no background salt like NaCl in MRB [56].

$$L_{c,x} = -\frac{\bar{V}_{OH^-}^{\beta} + \bar{V}_{z^+}^{\alpha}}{\bar{V}_{z^+}^{\alpha} + V_{\text{weak}}^{\alpha\beta}} L_0 \tag{23a}$$

$$V_{\text{weak}}^{\alpha\beta} = \left(\frac{\bar{m}_{H^+}^{\alpha} \bar{c}_{H^+}^{\alpha}}{\kappa^{\alpha}} - \frac{\bar{m}_{OH^-}^{\beta} \bar{c}_{OH^-}^{\beta}}{\kappa^{\beta}} \right) \frac{i}{\bar{c}_{H^+}^{\alpha} - \bar{c}_{OH^-}^{\beta}} \tag{23b}$$

$$V_{\text{weak}}^{\alpha\beta} = \left(\frac{m_{H^+}^{\alpha} c_{H^+}^{\alpha}}{\kappa^{\alpha}} - \frac{\bar{m}_{OH^-}^{\beta} \bar{c}_{OH^-}^{\beta}}{\kappa^{\beta}} \right) \frac{i}{c_{H^+}^{\alpha} - \bar{c}_{OH^-}^{\beta}} \tag{23c}$$

Or, $L_{c,x}$ is given corrected as Eqs. (23a) and (23c) as has been demonstrated in Ref. [56], if high salt exists in MRB. It should be noted that the constituent concentration of hydrogen in phase α existing in the denominator of Eq. (20c) is eliminated in that of Eq. (23c), while the concentration of hydroxyl ion in Eq. (20) becomes the constituent concentration of hydroxyl ion in Eq. (23c).

3. Results and discussion

3.1. Key conditions for stacking by MRB of weak acid and weak/strong alkali

The velocity of MRB holds a key importance to the design of MRB stacking of zwitterion and should be properly chosen. Eq. (5a) or (15a) indicates that if the absolute velocity of MRB towards the anode is faster than that of the negative zwitterion in phase β (moving towards the anode), we cannot stack the zwitterion any more. Inequality (6) implies that when MRB velocity is larger than that of EOF which usually moves towards the cathode, the sample plug at left end of capillary will migrates into the anodic vial and even no MRB occurs in the capillary. Inequality (16) and (22) imply that if MRB velocity in the direction of the cathode is faster than that of zwitterion in phase α (in the direction of cathode), no one can maximally focus the zwitterion by a MRB stacking. Integrating the results of discussions above together, one may obtain the following key condition for the MRB-based stacking in CE,

$$0 \leq V_{\text{weak}}^{\alpha\beta} (\text{or } V_{\text{w-s}}^{\alpha\beta}) \leq \bar{V}_{z+}^{\alpha} \tag{24}$$

The MRB designed in accordance with inequality (24) can lead to a good stacking of zwitterion, as compared with other conditions of boundary velocity. This theoretical analysis will be quantitatively demonstrated by the results of No. 1, 2, 3, and 5 in Table 2 and Fig. 11 in [64] (details see Section 3.2 in [64]).

3.2. Front alkali plug is needed for complete stacking if strong alkali is used to form MRB

An enough length of front alkali plug, *viz.*, $L_{c,x}$, is needed for the complete stacking of zwitterion, if zwitterion is prepared with a strong alkali. Eqs. (14) and (17) reveal that to well concentrate the zwitterion in phase β near boundary βγ, L_x should be increased up to a critical value, $L_{c,x}$. Eq. (14) also indicates that (1) the higher the value of $\left| V_{OH^-}^{\beta} - \bar{V}_{z^-}^{\beta} \right|$ is, the longer the injected plug of NaOH gets; and (2) conversely, the shorter the front plug of alkali becomes. The conclusions are well revealed by the predictions in Table 2 here and are further proved by the experiments of Figs. 9–10 in Ref. [64] (see Section 3.3 in Ref. [64]).

Eq. (14) further implies that if there exist $V_{OH^-}^{\beta} - \bar{V}_{z^-}^{\beta} \neq 0$ and $\bar{V}_{z^-}^{\beta} - V_{\text{w-s}}^{\alpha\beta} \to 0$, you can have $L_{c,x} \to \infty$. This situation means the possibility of any stacking to the same sample zone, if the free hydroxyl ion is continuously supplied.

3.3. No front alkali plug is needed for stacking if weak alkali is used to form MRB

For preparation of a sample, a weak alkali is of much superiority to a strong alkali. This will be proved by the following theoretical analyses and comparisons. In a weak alkali with enough high concentration, such as 5–20 mM NH_3H_2O (see Section 3.1.3 in Ref. [62] and Table 1 herein), there is always,

$$\left| \bar{m}_{OH^-}^{\beta} \right| \leq \left| \bar{m}_{z^-}^{\beta} \right| \tag{25}$$

While for a strong alkaline NaOH, there exists

$$\left| \bar{m}_{z^-}^{\beta} \right| < \left| m_{OH^-}^{\beta} \right| \tag{26}$$

Owing for the existence of inequality (24), for Eq. (17) or (23) there is always

$$\bar{V}_{z^+}^{\alpha} + V_{\text{weak}}^{\alpha\beta} \left(V_{\text{w-s}}^{\alpha\beta} \right) > 0 \tag{27}$$

Inequality (27) means that the result of inequality (17a) used for the system of weak acidic buffer (+, α) || strong alkali NaOH (–, β) is

$$L_{c,x} = -\frac{V_{OH^-}^{\beta} + \bar{V}_{z+}^{\alpha}}{\bar{V}_{z^+}^{\alpha} + V_{\text{w-s}}^{\alpha\beta}} L_0 > 0 \tag{28}$$

which indicates that a front alkaline plug needs to be injected in the front of the sample plug, in order to completely stack the zwitterion in the sample plug L_0. The analyses are proved by the computation in Table 2 here and further proved by the experiments of Figs. 9–10 in Ref. [64]. Obviously, the design with strong base as sample buffer is unwise. Since more partial of capillary is filled with the strong base rather than the alkaline sample, and less partial of capillary is used for the separation of zwitterion.

Table 2
The comparisons between the front plugs of strong and weak alkalis needed for complete stacking of zwitterion by MRB in CE.

	In 0.4 mM NaOH[a]		His in NH_3H_2O[b]		Trp in sodium formate[c]	
	His	Trp	5.0 mM	10 mM	60 mM	100 Mm
$L_{c,x}$[d]	301 s	555 s	−27 s	−60 s	−1.9 s	−3.3 s

[a] In: 40 mM pH 3.50 acetic buffer + 20 mM NaCl (+, α)||0.4 mM NaOH + 20 mM NaCl (−, β).

[b] In: 40 mM pH 3.50 acetic buffer + 10 mM NaCl (+, α)||5.0 or 10 mM NH_3H_2O + 20 mM NaCl (−, β).

[c] In: 40 mM pH 3.50 acetic buffer (+, α)||60 or 100 mM sodium formate + 20 mM NaCl (−, β).

[d] The plug of L_0 is set at 120 s (excluding 60 s sample injection for Trp in 0.4 mM NaOH. It was computed that 555 s 0.4 mM NaOH injection is needed if 120 sample of Trp is used. It is impossible to inject 555 s 0.4 mM NaOH).

While Eq. (23) and inequality (25) imply the existence of expressions (29)

$$L_{c,\mathrm{x}} = -\frac{\bar{V}^{\beta}_{\mathrm{OH}^-} + \bar{V}^{\alpha}_{\mathrm{z}^+}}{\bar{V}^{\alpha}_{\mathrm{z}^+} + V^{\alpha\beta}_{\mathrm{weak}}} L_0 \le 0 \tag{29}$$

Expression (29) shows that no front alkaline plug needs to be injected, if a weak alkali with enough high concentration and pH value (e.g., sodium formate used in Refs. [55,56,63], 5.0–15 mM ammonia in Section 3.1.3 of Ref. [62] and Table 1 herein) is used for the preparation of sample. This means that more partial of capillary can be filled with the weak alkaline sample and much higher sensitivity of CE can be achieved, if a weak base is used to prepare sample. The analyses of expression (29) are well proved by the results in Ref. [62] and Table 2 herein and the comparisons between Figs. 9–10 and 11–12 in Ref. [64] (details see Section 3.4 in Ref. [64]).

Obviously one can conclude that a weak alkali used as sample buffer is of superiority to a strong alkali. The comparisons between Eqs. (14) and (20) also result in the similar conclusions given above.

3.4. Dramatic influence of salt on stacking by MRB of weak acid and strong alkali

The presence of salt in MRB system has very weak effect on the stacking of zwitterion by MRB, if MRB is formed with weak electrolytic solutions. Fig. 3 manifests that the salt added into the pH 3.50 40 mM acetic buffer + 10 mM NaCl (+, α) || 10 mM ammonia (−, β) can slightly effect the boundary and zwitterion velocities. Hence, the salt does not significantly influence the stacking mechanism of MRB if a proper concentration of ammonia is chosen. This prediction is remarkably proved by the experiments in Figs. 11 (with 20 mM NaCl) and 12 (with 80 mM NaCl) in Ref. [64], and the results in Refs. [55,63]. However, if the salt is added into the MRB formed with 40 mM pH 3.50 acetic buffer (+, α) || 0.4–12 mM NaOH (−, β), the dramatic effect of salt can be observed as compared between Figs. 5 and 6. The predictions in Fig. 4 are highly verified by the computation results in Table 2 and the relevant experiments in Ref. [64] (details see Section 3.4 in Ref. [64]).

Thus, the comparisons between the results in Figs. 3 and 4 evidently manifest that the existence of the salt has little effect on the MRB formed with weak electrolytic buffers, but has the dramatic effect on the boundary created with one strong electrolyte like NaOH, and further show that a weak electrolyte used as sample buffer holds a incomparable superiority to a strong

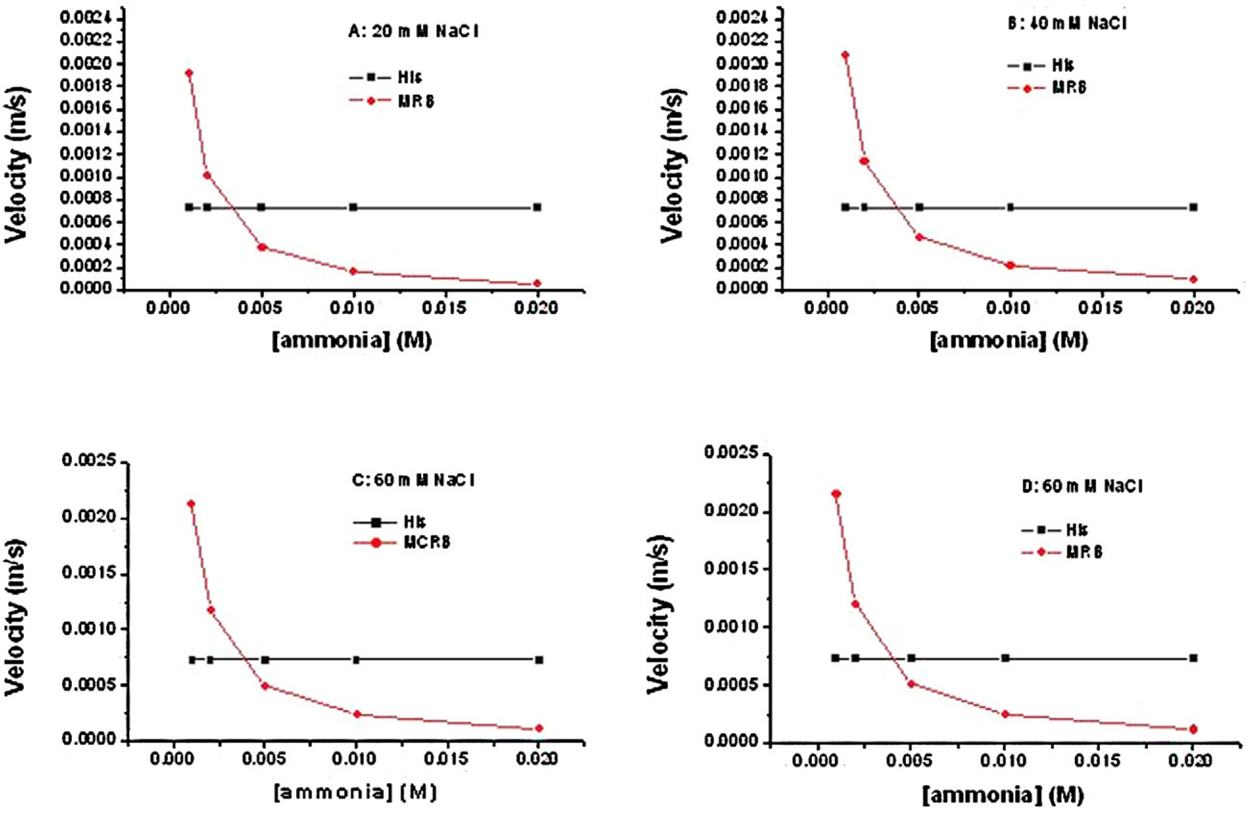

Fig. 3. The comparisons of His and MRB velocities in the system of pH 3.50 40 mM acetic buffer + 10 mM NaCl (+, α)||1.0–20 mM ammonia water + A: 20 mM, B: 40 mM, C: 60 mM and D: 80 mM NaCl (−, β). MRB velocity is computed with Eq. (25b) due to 20 mM NaCl in ammonia, His velocity with Eq. (16). Conditions: $i = 3822\ \mathrm{A/m^2}$, κ value of running buffer is given in Table 2 in Ref. [67], κ values of 1.0, 2.0, 5.0, 10, 15 and 20 mM ammonia equal 0.041, 0.043, 0.046, 0.049, 0.051 and 0.053 $\mathrm{S\,m^{-1}}$ respectively, κ values of 20, 40, 60 and 80 mM NaCl are respectively 0.193, 0.379, 0.561, 0.737 $\mathrm{S\,m^{-1}}$, κ value of phase β = the sigma of those of sodium formate and NaCl, $m_{\mathrm{H}^+} = 36.5$ and $m_{\mathrm{OH}^-} = 20.05 \times 10^{-8}\,\mathrm{m^2\,V^{-1}\,s^{-1}}$ in Ref. [65] which were corrected with the empirical equations in Refs. [67,68].

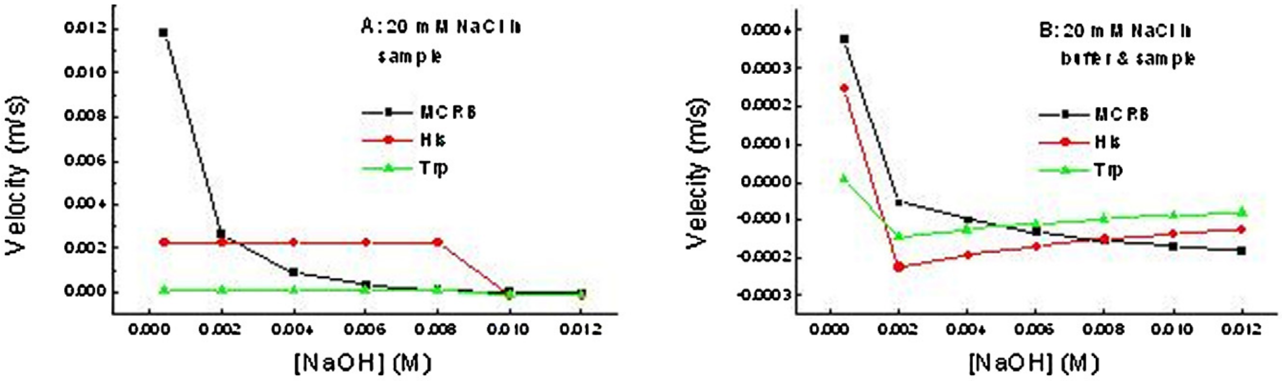

Fig. 4. The comparisons among the velocities of His, Trp and MRB. (A) The boundary of 40 mM pH 3.50 acetic buffer (+, α)||0.40–12 mM NaOH + 20 mM NaCl + 5 μg/ml His or 1–5 μg/ml Trp (−, β), (B) 40 mM pH 3.50 acetic buffer + 20 mM NaCl (+, α)||0.40–12 mM NaOH + 20 mM NaCl + 5 μg/ml His or 1–5 μg/ml Trp (−, β). MRB velocity computed with Eq. (1), His and Trp velocities with Eq. (16) if positive boundary velocity; MRB velocity with Eq. (15b), His and Trp velocities with Eq. (5b) if negative boundary velocity. Conditions: $i = 2256\,A/m^2$, κ values are given in Tables S2 and S3 in Ref. [64]. The other symbols see Fig. 3 here.

electrolyte, such as NaOH used herein and in the previous work [55,62,63] (Fig. 5).

3.5. *Balance concentration of weak alkali among MRB, OH⁻ and zwitterion velocities*

The above optimized concentration of the weak alkali should be balanced between the boundary velocity and the constituent velocity of hydroxyl ion. In order to efficiently condense the zwitterion in phase β near boundary βγ, the concentration of weak alkali in phase β should be increased up to the point, which leads to the existence of following inequality

$$\left|\bar{V}^{\beta}_{OH^-}\right| \le \left|\bar{V}^{\beta}_{z^-}\right| \tag{30}$$

$$\bar{m}^{\beta}_{OH^-} = a\ m^{\beta}_{OH^-} \tag{31}$$

Whereas, if the concentration of weak alkali in phase β is much high, the boundary velocity is very slow as proved by Eq. (23c), at the same time, the constituent mobility of hydroxyl ion is also as slow as shown by Eq. (31) and the results in Table 1, the reaction time of MRB for the neutralization between the weak alkali in phase β and the acidic buffer in phase α *viz.*,

$$t_B = \frac{L_0}{V^{\alpha\beta}_{weak} + \bar{V}^{\beta}_{OH^-}} \tag{32}$$

is prolonged. This situation is unexpected for our rapidly finish of stacking zwitterion by the MRB. More importantly, the situation may lead to the possibility that partially stacked zwitterions pass through the detector without separation due to much more time used for stacking. Hence, the concentration of weak alkali in phase β should be balanced among the requirements of inequality (24) and Eqs. (23c) and (32). In other words, the concentration of weak alkali should be balanced at the point, at which the boundary velocity is as much as possible close to the constituent velocity of zwitterion ionized as negative ion.

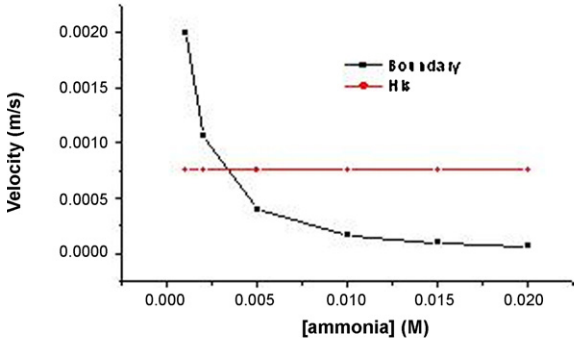

Fig. 5. The comparisons between the zwitterion and MRB velocities in the MRB system: 40 mM pH 3.50 acetic buffer + 10 mM NaCl (+, α)||1.0–20 mM ammonia water + 20 mM NaCl (−, β). MRB velocity with Eq. (25b), His velocity with Eq. (16). Conditions: $i = 3822\,A/m^2$, κ values are given in Tables S2 and S4 of Ref. [64]. Other symbols see Fig. 3.

4. Conclusion

From the above Section 3, one can achieve the following conclusions. At first, the kind of running buffer and sample buffer, velocity of MRB, and salt effect should be considered carefully in accordance with expression (3) and (4). Second, it would be better if the velocity of MRB ranges from zero to the velocity of zwitterion in the acidic running buffer. Third, a much long front plug of strong base is needed for the complete stacking of zwitterion, if a strong alkali is used to as the alkaline sample matrix. Fourth, if a weak alkali with enough high concentration and pH value is used as the sample buffer, no plug of weak base is needed. Fifth, the existence of salt in a sample matrix holds almost no obvious effect on the MRB stacking of zwitterion if the boundary is created with weak electrolytic solutions [55,66], but a dramatic effect on sample stacking is present if with a strong electrolyte, e.g., NaOH. Sixth, thus a weak alkali possesses incomparable superiority than a strong alkali when being used as the sample buffer. In addition, the concentration of weak alkali used for the preparation of sample matrix should be set at the point, at which the boundary velocity is as much as possible close to the constituent velocity of zwitterion ionized as negative ion. The theory developed can accurately predict the experimental conditions of stacking zwitterion.

Even these conclusions mentioned above are achieved with the sample mode of amino acid, their validities may concern some low abundance peptides and proteins faced in the study on proteomics.

Acknowledgements

The authors are grateful for the funding provided by the National Natural Scientific Found of China (No. 20821005, 20805031 and 21035004), National Key Basic Research Program (973 Program) of China (No. 2009CB118906), National Comprehensive Technology Platforms for Innovative Drug R&D (No. 2009ZX09301-007), Medicine and Engineering Interdisciplinary Fund of Shanghai Jiao Tong University (No. YG2010ZD$_2$09) and Shanghai Leading Academic Discipline Project (No. B203).

References

[1] H. Haglund, A. Tiselius, Acta Chem. Scand 4 (1950) 957–962.
[2] R.L. Chien, D.S. Burgi, J. Chromatogr. 559 (1991) 141–152.
[3] R.L. Chien, J.C. Helmer, Anal. Chem. 63 (1991) 1354–1361.
[4] R.L. Chien, D.S. Burgi, Anal. Chem. 63 (1991) 2042–2047.
[5] R.L. Chien, D.S. Burgi, Anal. Chem. 64 (1992) 489A–496A.
[6] R.L. Chien, D.S. Burgi, Anal. Chem. 64 (1992) 1046–1050.
[7] R.L. Chien, D.S. Burgi, J. Chromatogr. 559 (1991) 153–161.
[8] Y.K. Zhang, J. Zhu, L.H. Zhang, W.B. Zhang, Anal. Chem. 72 (2000) 5744–5747.
[9] L. Ornstein, Ann. N.Y. Acad. Sci. 121 (1964) 321–349.
[10] B.J. Davis, Ann. N.Y. Acad. Sci. 121 (1964) 404–427.
[11] D.A. MacInnes, L.G. Longsworth, Chem. Rev. 11 (1932) 171–230.
[12] V.P. Dole, J. Am. Chem. Soc. 67 (1945) 1119–1126.
[13] L.G. Longsworth, J. Am. Chem. Soc. 67 (1945) 1109–1119.
[14] H. Svensson, Acta Chem. Scand 2 (1948) 841–855.
[15] R.A. Alberty, J. Am. Chem. Soc. 72 (1950) 2361–2367.
[16] J.C. Nichol, J. Am. Chem. Soc. 72 (1950) 2367–2375.
[17] E.B. Dismukes, R.A. Alberty, J. Am. Chem. Soc. 76 (1954) 191–197.
[18] P. Gebauer, W. Thormann, P. Boček, Electrophoresis 16 (1995) 2039–2050.
[19] R. Weinberger, Am. Lab. 29 (1997) 49–51.
[20] E.K.M. Andersson, J. Chromatogr. A 846 (1999) 245–253.
[21] P. Gebauer, L. Křivánková, P. Pantucková, P. Boček, W. Thormann, Electrophoresis 21 (2000) 2797–2808.
[22] T. Hirokawa, H. Okamoto, N. Ikuta, Electrophoresis 22 (2001) 3483–3489.
[23] E.K.M. Andersson, I. Hagglund, J. Chromatogr. A 979 (2002) 11–25.
[24] Z.K. Shihabi, Electrophoresis 23 (2002) 1612–1617.
[25] Z.K. Shihabi, Electrophoresis 23 (2002) 1628–1632.
[26] M.E. Hadwiger, S.R. Torchia, S.R. Park, M.E. Biggin, C.E. Lunte, J. Chromatogr. B 681 (1996) 241–249.
[27] Y.P. Zhao, C.E. Lunte, Anal. Chem. 71 (1999) 3985–3991.
[28] E.M. Ward, M.R. Smyth, R. O'Kennedy, C.E. Lunte, J. Pharm. Biomed. 32 (2003) 813–822.
[29] S.D. Arnett, C.E. Lunte, Electrophoresis 24 (2003) 1745–1752.
[30] P. Britz-McKibbin, J. Wong, D.D.Y. Chen, J. Chromatogr. A 853 (1999) 535–540.
[31] P. Britz-McKibbin, D.D.Y. Chen, Anal. Chem. 72 (2000) 1242–1252.
[32] P. Britz-McKibbin, T. Ichihashi, K. Tsubota, D.D.Y. Chen, S. Terabe, J. Chromatogr. A 1013 (2003) 65–76.
[33] P. Britz-McKibbin, S. Terabe, Chem. Rec. 2 (2002) 397–404.
[34] P. Britz-McKibbin, K. Otsuka, S. Terabe, Anal. Chem. 74 (2002) 3736–3743.
[35] P. Britz-McKibbin, M.J. Markuszewski, T. Iyanagi, K. Matsuda, T. Nishioka, S. Terabe, Anal. Biochem. 313 (2003) 89–96.
[36] P. Britz-McKibbin, S. Terabe, J. Chromatogr. A 1000 (2003) 917–934.
[37] M.J. Markuszewski, P. Britz-McKibbin, S. Terabe, K. Matsuda, T. Nishioka, J. Chromatogr. A 989 (2003) 293–301.
[38] J.B. Kim, P. Britz-McKibbin, T. Hirokawa, S. Terabe, Anal. Chem. 75 (2003) 3986–3993.
[39] J.P. Quirino, S. Terabe, Science 282 (1998) 465–468.
[40] J.P. Quirino, S. Terabe, Anal. Chem. 72 (2000) 1023–1030.
[41] Y. Sera, N. Matsubara, K. Otsuka, S. Terabe, Electrophoresis 22 (2001) 3509–3513.
[42] M.R.N. Monton, J.P. Quirino, K. Otsuka, S. Terabe, J. Chromatogr. A 939 (2001) 99–108.
[43] J.P. Quirino, J.B. Kim, S. Terabe, J. Chromatogr. A 965 (2002) 357–373.
[44] J. Palmer, N.J. Munro, J.P. Landers, Anal. Chem. 71 (1999) 1679–1687.
[45] J. Palmer, J.P. Landers, Anal. Chem. 72 (2000) 1941–1943.
[46] J. Palmer, D.S. Burgi, N.J. Munro, J.P. Landers, Anal. Chem. 73 (2001) 725–731.
[47] J. Palmer, D.S. Burgi, J.P. Landers, Anal. Chem. 74 (2002) 632–638.
[48] C.X. Cao, J. Chromatogr. A 813 (1998) 153–171.
[49] C.X. Cao, L.Y. Fan, W. Zhang, Analyst 133 (2008) 1139–1157.
[50] J. Deman, W. Rigole, J. Phys. Chem. 74 (1970) 1122–1126.
[51] J. Deman, Anal. Chem. 42 (1970) 321–324.
[52] J. Pospichal, M. Deml, P. Boček, J. Chromatogr. 638 (1993) 179–186.
[53] H. Svensson, Acta Chem. Scand 15 (1961) 325–341.
[54] H. Svensson, Acta Chem. Scand 16 (1962) 456–466.
[55] C.X. Cao, Y.Z. He, M. Li, Y.T. Qian, S.L. Zhou, L. Yang, Q.S. Qu, Anal. Chem. 74 (2002) 4167–4174.
[56] C.X. Cao, W.Z. hang, W.H. Qin, S. Li, W. Liu, Anal. Chem. 77 (2005) 955–963.
[57] L.Y. Fan, C.J. Li, W. Zhang, C.X. Cao, P. Zhou, Z.X. Deng, Electrophoresis 29 (2008) 3989–3998.
[58] J. Jin, J. Shao, S. Li, W. Zhang, L.Y. Fan, C.X. Cao, J. Chromatogr. A 1216 (2009) 4913–4922.
[59] W. Zhang, J.F. Chen, L.Y. Fan, C.X. Cao, J.C. Ren, S. Li, J. Shao, Analyst 135 (2010) 140–148.
[60] W. Zhang, J. Jin, L.Y. Fan, S. Li, J. Shao, C.X. Cao, J. Sep. Sci. 32 (2009) 2123–2131.
[61] K. Isoo, S. Terabe, Anal. Chem. 75 (2003) 6789–6798.
[62] X. Wang, W. Zhang, L.Y. Fan, C.X. Cao, Anal. Chim. Acta 594 (2007) 290–296.
[63] W. Zhu, W. Zhang, L.Y. Fan, J. Shao, S. Li, J.L. Chen, C.X. Cao, Talanta 78 (2009) 1194–1200.
[64] W. Zhang, L.Y. Fan, J. Shao, S. Li, S. Li, C.X. Cao, Talanta (2011) doi:10.1016/j.talanta.2011.01.062.
[65] A.W. Adamson, A Textbook of Physical Chemistry, Academic Press Inc, New York, 1973.
[66] R.L. David, CRC Handbook of Chemistry and Physics, 73rd ed., CRC Press, Boca Raton, 1992–1993.
[67] J.C. Reijenga, E. Kenndler, J. Chromatogr. A 659 (1994) 403–415.
[68] C.X. Cao, J. Chromatogr. A 771 (1997) 374–378.