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Study on mechanism of stacking of zwitterion in highly saline biologic sample by transient moving reaction boundary created by formic buffer and conjugate base in capillary electrophoresis

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ABSTRACT

The reason why a moving reaction boundary (MRB) can stack analyte in highly saline sample in capillary electrophoresis [C.X. Cao, Y.Z. He, M. Li, Y.T. Qian, S.L. Zhou, L. Yang, Q.S. Qu, Anal. Chem. 74 (2002) 4167] is still unclear. To illuminate the mechanism of such stacking, three MRBs formed by formic acid-NaOH buffer and sodium formate as well as 40, 80 and 120 mmol/L sodium chloride in matrixes were studied. The computation with MRB theory shows that sodium chloride in matrix has weak effect on the stacking efficiency, whether the concentration of sodium chloride is set at 40, or 80, or 120 mmol/L. The conclusion has been highly manifested by numerous experiments. Furthermore, the computer simulation and theoretical analyses depict that this kind of stacking is induced by the mechanism of MRB, rather than that of electrostacking or isotachophoresis (ITP) under the given electrolytic system. Finally, the application of the sample condensation was achieved for the stacking of analyte(s) in highly saline biological sample of *skeletonema costarum*' culture with up to 527 mmol/L total salt and health human urine with 150–320 mmol/L inorganic ions (Cl^-, Na^+, K^+, PO_4^{3-}, etc.). The results herein have a clear significance to the design on stacking of analyte in highly saline biological sample.

1. Introduction

Numerous biological sample matrixes, such as serum, urine and zymosis, contain high salt. The salt in these matrixes ought to be removed, otherwise it brings about some harmful action to sample stacking in capillary electrophoresis (CE). For example, salt in a matrix breaks down sample pre-concentration by electrostacking [1–3] or field-amplified sample injection (FASI) [4–7] in CE. The removal of salt from matrix is troublesome. Thus, it is great interesting that sample with high salt can be directly stacked to achieve good improvement of detection sensitivity without loss of separative efficiency of CE.

Numerous methods have been developed for stacking of saline sample in CE. The first method is isotachophoresis (ITP) induced sample stacking. In the last two decades [8–10], the theoretical and experimental studies on transient ITP were widely performed for stacking analytes in saline matrix. The second method is the "acetonitrile addition" idealized by Shihabi [11], the relevant mechanism is considered to be a transient ITP/or FASI. The existence of acetonitrile in sample matrix can reduce the ionization of salt and overcome the harmful effect of the salt on sample stacking by FASI.

The third is the "pH-mediated-induced sample concentration" described mainly by Lunte's group [12–14]. In this method, the sample is dissolved in a weakly acidic (or basic) buffer with salt; conversely the running buffer is just the conjugate base (or acid). A moving reaction boundary (MRB) is formed between the weak acid (or base) and the conjugate base (or acid), if an electric field is applied. The MRB results in a low conductivity zone in the original matrix. The zone further leads to a FASI. The fourth is the "dynamic-pH-junction-induced stacking" developed by Britz-Mckibbin et al. [15–17]. In the method, the analyte velocity is greatly regulated by pH value, but the velocity of the unwanted analyte cannot be adjusted due to its insensitivity to pH. Hence, a selective preconcentration can be achieved in this kind of stacking. The computer simulation revealed that the pH junction-induced stacking was relied on ITP mechanism [17]. The fifth is the sweeping technique for analyte in salt matrix in micellar electrokinetic chromatography [18–22]. The sweeping method cannot only stack neutral analyte, but also condensate ionic solutes in highly saline matrix.

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Table 1
pK_a values and some mobilities of some ions in sample matrix and 32.8 mmol/L pH 2.85 buffer.

Ions	pK_a	Mobility (10^{-8} m^2 V^{-1} s^{-1})		
		Phase α (formic buffer)	Phase β (matrix)	Phase γ (formic buffer)
Hydrogen	–	36.5	–	36.5
Sodium	–	5.19	5.19	5.19
Hydroxyl	–	–	−20.9	–
Chloride	–	–	−7.91	–
Formic acid	3.75	−0.63	−5.66	−0.63
Trp	9.13 (pK_2)	1.22[a]	−0.32[b]	1.22[a]

[a] The mobility was computed from the pK_1 (Trp) = 2.83 and Trp mobility (+2.49 × 10^{-8} m^2 v^{-1} s^{-1}) carrying one positive charge [29].
[b] The computation procedure was given in the appendix.

The sixth is the stacking method induced by MRB developed mainly by Cao et al. [23–25]. The stacking could be used for the condensation of analytes in highly saline matrix [23]. Recently, the theoretical procedure was developed for the quantitative design on stacking condition and selective stacking by using the theory of MRB [24,25]. Furthermore, the relevant MRB theory, methods and relevant applications have been reviewed [25]. Even so, the reason why MRB-based stacking can condense analyte in highly saline biological matrix is still unclear.

Thus, the purposes herein are to (i) show the theoretic prediction of weak impact of salt on the MRB-based stacking, (ii) report experiments proving the prediction, (iii) show simulation results revealing non-ITP or non-FASI mechanism in the MRB-based stacking system, and (iv) unveil reason why MRB can stack analyte in highly saline biological matrix without loss of stacking efficiency. In addition, the application of the method was briefly tested for some solute(s) in highly saline culture liquor and health human urine.

2. Theoretical computation

The mechanism of sample stacking by the MRB system of formic buffer and sodium formate without salt has been well investigated in the previous work [24,25]. Hence, the paper studies three kinds of MRBs with high salt in the sample matrix. Below are the three MRBs:

Boundary 1: 32.8 mmol/L pH 2.85 formate buffer (+, α) || [→] 8.2–82.5 mmol/L sodium formate + 40 mmol/L NaCl (−, β).
Boundary 2: 32.8 mmol/L pH 2.85 formate buffer (+, α) || [→] 8.2–82.5 mmol/L sodium formate + 80 mmol/L NaCl (−, β).
Boundary 3: 32.8 mmol/L pH 2.85 formate buffer (+, α) || [→] 8.2–82.5 mmol/L sodium formate + 120 mmol/L NaCl (−, β).

where "||" implies a boundary, the symbol of '[→]' indicates the direction of MRB, "+" and "−" imply the anode and cathode, respectively, and "α" and "β" mean phase α and β, respectively. The three MRBs have been used for the stacking of analytes in saline matrix [23]. In the three MRBs, the boundary velocity should be computed with the following equation [24,25]

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

where c is the equivalent concentration (equiv. m^{-3}), the bar "–" over c means the constituent concentration, the subscripts H+ and OH− indicate the hydrogen and hdyroxyl ions, respectively, the superscripts α and β imply phase α and β, respectively; m is the mobility (m^2 V^{-1} s^{-1}), the bar "–" over m indicates the constituent mobility; i is the electric current intensity (A m^{-2}) in capillary; $V^{\alpha\beta}$ is the velocity (m s^{-1}) of MRB; κ is the specific conductivity (S m^{-1}). The consituent mobility and concentration are, respectively, defined as [24,25]

$$\bar{m} = \sum a_i m_i \quad (2)$$

$$\bar{c} = \sum c_i \quad (3)$$

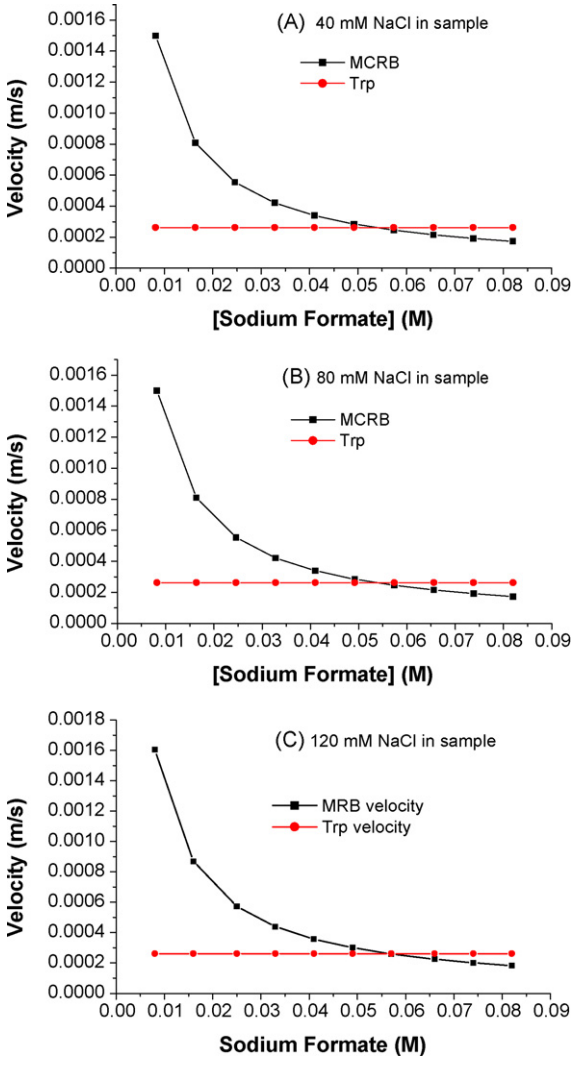

Fig. 1. Velocities of Trp and MRB of 32.8 mmol/L pH 2.85 formate buffer (+, α) || 8.2–82.5 mmol/L sodium formate + A: 40 mmol/L, B: 80 mmol/L and C: 120 mmol/L NaCl (−, β). *Conditions*: Current density −2265 A/m^2, κ values in sodium formate solutions with different concentration sodium chloride are given in Table S1.