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Mathematical model and computer simulation on moving precipitate boundary electrophoresis for offline sample pre- concentration of heavy metal ion

Jiang Chang [a], Jie Zhang [a], Hou-Yu Wang [a], Liu-Yin Fan [a], Yin-Ping Fan [a,b], Shan Li [b,]**, Cheng-Xi Cao [a,]*

[a] Laboratory of Analytical Biochemistry and Bio-separation, State Key Laboratory of Microbial Metabolism, School of Life Science and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China

[b] School of Bioscience and Bioengineering, South China University of Technology, Guangzhou 510006, China

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ABSTRACT

In this paper, a mathematical model and computer simulator were developed for offline sample pretreatment of heavy metal ion based on moving precipitate boundary (MPB) electrophoresis. The simulation indicates that (i) the program can easily accomplish numerical computations, such as the velocities of MPB and elution boundary (EB), and enrichment factor (EF) etc; (ii) the simulator can vividly imitate the dynamics of MPB, EB, precipitate zone, and precipitate-elution; and (iii) the software may simply optimized experimental conditions via the influence factors (e.g., voltage, hydroxyl, hydrogen and metal ions) on the EF. As a proof of concept, copper ion and its precipitate with definite blue color were, respectively chosen as mode heavy metal ion and alkaline precipitate for the relevant experiments of MPB-based sample preconcentration of heavy metal ion in large tube. All of the experimental results manifest the validity of developed mathematical model and the relevant simulation results. The model and simulator advanced herein are of clear significance to the optimization of experimental conditions and understanding of offline MPB- based sample condensation of heavy metal ion.

1. Introduction

Off-line sample condensation or pretreatment has significant uses in analytical, biochemistry and environmental sciences. For example, liquid–liquid or liquid–solid extraction has been used as a normal pretreatment procedure of analyte from numerous biosamples, e.g., plasma, urine, hair and zymotic fluid [1,2]. Solid phase extraction (SPE) has been widely used in LC/LC–MS and GC/GC–MS [3,4]. Liquid phase micrextraction (LPM) has been developed as a green analytical and sample pretreatment procedures hyphenated with capillary electrophoresis (CE) [5,6].

Electrophoretic technique has been well designed as a series of online sample stacking methods for sensitive increase of CE, e.g., electrostacking (ES) [7], field-amplified sample injection (FASI) [8], transient isotachophoresis (ITP) [9], acetonitrile salt mixtures [10], pH mediated sample concentration [11], velocity-difference-induced focusing by dynamic pH junction [12], and SDS-based or EDTA-based sample sweeping [13,14]. However, electrophoretic technique and its sample stacking were rarely reported as an offline sample condensation procedure for sensitive enhancement of instrumental analysis, even the concept of precipitate reaction front (PRF) [15,16], having potential to become a off-line sample preconcentration, has been advanced for over 40 years.

As early as in 1970, Deman and Rigole [15,16] advanced the important ideal of PRF (viz., the protype of moving reaction boundary (MRB)), performed the experiments of PRF in U-shape electrophoretic tube with agar gel, and observed the separation of metal ions and the condensation phenomenon of metal ions in the PRF system. At the same time, they advanced the Deman–Rigole's equations accounting for the movement of PRF. In 1997–2008, Cao et al. [17–19] developed the theory of MRB from the model of MPB, demonstrated the validity of MRB theory with the experiments of MPB, and used the concept of MPB for the preparation of stable colloid of inorganic precipitate in agarose gel. However, the idea of MPB has not been used for the purposes of analytical chemistry yet, even Deman–Rigole's experiments implied the potential of MPB-based preconcentration of heavy metal ions for application of analytical chemistry [15,16].

The concept of MPB can not be easily designed as an online sample stacking technique for sensitive increase of capillary electrophoresis (CE) thanks to precipitate-induced blocking

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* Corresponding author. Fax: +86 21 3420 5820.
** Corresponding author.
E-mail addresses: lishan@scut.edu.cn (S. Li), cxcao@sjtu.edu.cn (C.-X. Cao).

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in separation microcolumn. However, it can be theoretically developed as an offline sample condensation method of heavy metal ion joined by various analytical instruments, e.g., atomic absorption spectroscopy (AAS), inductively coupled plasma–mass spectrometry (ICP–MS) and ion chromatography (IC). Recently, the concepts of MPB and moving neutralization boundary (MNB) were combined together as an offline sample pretreatment of heavy metal ion for sensitive increase of instrumental analysis (therein, CE was chosen as a model analytical instrument) [19]. In the run of offline MRP-based preconcentration of heavy metal ions, over one hundred fold enrichment factor (EF) was achieved for the analysis of environmental samples with five heavy metal ions. However, it was observed that the optimization of experimental conditions was very complex and longtime consumption. To solve the two issues, two improvement pathways of MRB-based sample pretreatment ought to be accomplished. The first pathway is to construct a multi-channels apparatus (e.g., 96 channels) for offline condensation of heavy metal ion. This kind of apparatus can greatly reduce the average offline preconcentration time from 160 min to about 1.6 min for each run, very fast as compared with the current apparatus [17,19] Such work is under its way in our laboratory.

The second pathway is to perform the computer simulation on the experimental conditions of MPB-based sample preconcentration, because it becomes an economical, vivid and effective tool optimizing different electrophoretic conditions and providing profound understanding on the separation dynamics of analytes. A series of computer simulators of electrophoretic techniques have developed based on different modes, such as isotachophoresis (ITP) [20–22], isoelectric focusing (IEF) [23–25], capillary zone electrophoresis (CZE) [26–29] and online sample condensation [30,31]. However, there have not been a mathematical model and simulation software imitating the offline MPB-based sample preconcentration of heavy metal ion.

For better application and understanding of the offline sample preconcentration of heavy metal ion, herein we developed a novel mathematical model and simulation software based on MPB model, predicted the results of MPB enrichment of heavy metal ions via the numerical computation, optimized the experiment conditions, and finally performed the relevant experiments demonstrating the validity of the developed model and software. As a proof of concept, copper ion and its alkaline precipitate with definite blue color were herein chosen as mode heavy metal ion and alkali for the relevant experiments, respectively. Below are the relevant model, the simulator, the numerical calculation and simulation results, and the demonstration of experiments.

2. Theoretical

2.1. Mode of MPB without elution ion

The concept of a MPB has been discussed in detail in the previous work [17]. Fig. 1 shows the brief model of a MPB, copper ions and hydroxyl ions are used as example here. Panel A1 presents the initial state MPB formed with hydroxyl and copper ions. The positive reaction ion of Cu(II) is present in phase α and the negative reaction ion of OH^- exists in phase β. As an electric field is supplied across the whole electrophoretic tube (see Panel A2), the copper and hydroxyl ions would electromigrate in opposite direction and react with each other when they meet. The relevant boundary reaction is

$$\mathrm{Cu(II)}\ (\text{phase } \alpha,\ +) + 2\mathrm{OH}^-\ (\text{phase } \beta,\ -) \rightarrow \mathrm{Cu(OH)_2}\downarrow \qquad \text{(a)}$$

where, the symbols of ‘α’ and ‘β’ imply phase α and β, respectively, the symbols of ‘+’ and ‘−’ mean the anode and the cathode, respectively. At the same time, a precipitate boundary created between phase α and phase β

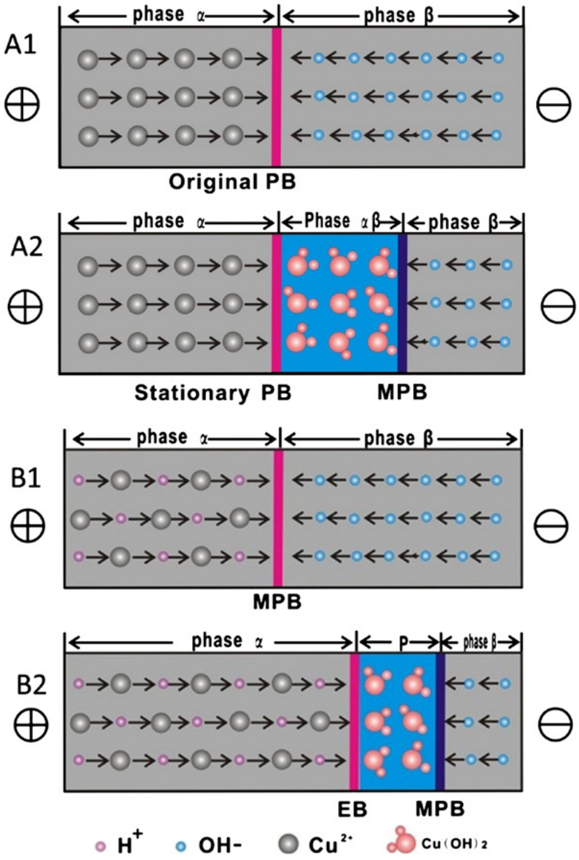


Fig. 1. Diagram of initial precipitate boundary (PB) formed with copper ion in phase α and hydroxyl ion in phase β after electric field supply (A1); Movement of MPB and produced precipitation zone between phases α and β (A2). Diagram of initial preconcentration of copper ion by the MPB with elution hydrogen ion (B1); copper ions ceaselessly preconcentration via the elution boundary (EB) and the precipitation zone ‘P’ extend gradually (B2). The symbols of ‘+’ and ‘−’ indicate the anode and cathode, respectively. Other symbols see the figure and context.

$$\mathrm{Cu(II),\ KCl}\ (\text{phase } \alpha,\ +) \| [\rightarrow]\ \mathrm{OH}^-,\ \mathrm{KCl}\ (\text{phase } \beta,\ -) \qquad \text{(i)}$$

where, the symbol of ‘‖’ indicates a boundary created between phase α and β, the symbol of ‘[→]’ implies boundary motion direction which may be toward to the anode or the cathode, or be stationary. The moving direction of boundary can be vindicated by the judgment expression (JE) [17]. With processing of the running, Reaction (a) can produce homogeneous precipitation zone of $Cu(OH)_2$ in gel [18]. Thus, the velocity of MPB should be computed with [17]

$$V^{\alpha\beta}_{MPB,sim} = \frac{m^{\alpha}_{Cu(II)} c^{\alpha}_{Cu(II)} - m^{\beta}_{OH^-} c^{\beta}_{OH^-}}{c^{\alpha}_{Cu(II)} - c^{\beta}_{OH^-}} E \qquad (1)$$

where m is the ionic mobility ($m^2\,V^{-1}\,s^{-1}$), c is the equivalent concentration (equiv. m^{-3}), $V^{\alpha\beta}_{MPB,sim}$ is the velocities ($m\,s^{-1}$) of the MPB formed between phase α and phase β, E is the electric field strength ($V\,m^{-1}$) in the electrophoretic tube. The enrichment factor (EF) of Cu(II) in the MPB system can be defined as [17]

$$\mathrm{EF} = \left| \frac{\left(m^{\alpha}_{\mathrm{Cu(II)}} - m^{\beta}_{\mathrm{OH}^-} \right) c^{\beta}_{\mathrm{OH}^-}}{m^{\alpha}_{\mathrm{Cu(II)}} c^{\alpha}_{\mathrm{Cu(II)}} - m^{\beta}_{\mathrm{OH}^-} c^{\beta}_{\mathrm{OH}^-}} \right| \qquad (2)$$

2.2. Mode of MPB with elution ion

If an elution ion is added into the system mentioned above (Fig. 1B), such as hydrogen ion, the hydrogen and hydroxyl ions react with each other. Besides Reaction (a), there is also Reaction (b) within the initial precipitate boundary [17,18]

$$H^+ \text{ (phase } \alpha, +) + OH^- \text{ (phase } \beta, -) \rightarrow H_2O \tag{b}$$

Reaction (b) but not (a) occurs at first thanks to the rapid movement of hydrogen ion (36.3×10^{-8} m^2 V^{-1} s^{-1}) in contrast to the slow motion of copper ion (5.66×10^{-8} m^2 V^{-1} s^{-1}) [32]. However for a while, Reaction (a) becomes dominant and alkaline precipitate of $Cu(OH)_2$ is produced in gel, because the water ion product (1.0×10^{-14}) is extremely greater than the product of Cu(II) and OH^- (2.2×10^{-20}) [32]. The hydrogen ion neutralizes the alkaline precipitation and releases Cu(II) form the precipitate. Thus, the initial boundary Reaction (b) is transformed as elution or neutralization reaction

$$2H^+ \text{ (phase } \alpha, +) + Cu(OH)_2 \text{ (phase P)} \rightarrow 2H_2O + Cu(II) \tag{c}$$

where, the symbol of 'P' means the precipitation phase of $Cu(OH)_2$. The released copper ion migrates towards the cathode under the electric field and reacts with hydroxyl ion once again, leading to the re-precipitation of $Cu(OH)_2$ within the precipitate boundary. Hence, the initial boundary system in Panel B is isolated by the precipitate of $Cu(OH)_2$ and transformed as

$$H^+\text{, Cu(II), KCl (phase } \alpha, +)\|[\rightarrow]Cu(OH)_2\text{, Cu(II), KCl (phase P)}\| [\rightarrow]OH^-\text{, KCl (phase } \beta, -) \tag{ii}$$

Obviously, a new phase of precipitate is created during the elution reaction, and the new phase results in two kinds of boundaries, viz., the elution boundary (EB) and MPB in Panel B2. Boundary (ii) can also be considered as a MNB [17,19]. There are two cycles in Panel B2 or Boundary (ii). Namely, the copper ion cycles from the EB to the MPB, and the precipitate of $Cu(OH)_2$ does from the MPB to the EB. During the process of the two cycles, the concentration of copper ion in the precipitation zone is gradually increased, because velocity of copper ions is designed to be greater than the velocity of EB, and then copper ion is sequentially condensed within the very thin boundary which is invisible to naked eyes. After just a few minutes reaction, the concentration of copper ion within the thin boundary reaches to the limit, over which the copper concentration can not continue to increase. After then, the precipitation zone extends gradually as the copper ion continuously moves from the anode into the precipitate zone. If the precipitation zone extends to a certain extent, we can observe the blue precipitate by the naked eyes. Evidently, the whole system reaches an equilibrium steady-state, under which the velocity of EB is [17]

$$V_{\mathrm{EB,eq}} = \frac{m^{\alpha}_{H^+} c^{\alpha}_{H^+} - m^{\beta}_{OH^-} c^{\beta}_{OH^-}}{c^{\alpha}_{H^+} - c^{\beta}_{OH^-}} E \tag{3}$$

where, the subscript of 'eq' indicates an equilibrium steady-state. The enrichment factor ($\mathrm{EF_{eq}}$) of Cu(II) in a MPB-based system at steady-state can be defined as [17]

$$\mathrm{EF}_{eq} = \frac{P_{eq} + c^{\mathrm{P}}_{Cu(II),eq}}{c^{\alpha}_{Cu(II)}} \tag{4}$$

where P_{eq} is the concentration of the precipitate at steady-state of equilibrium, $c^{\mathrm{P}}_{Cu(II),eq}$ is copper ion concentration in the precipitation zone at the steady-state of equilibrium. P_{eq} and $c^{\mathrm{P}}_{Cu(II),eq}$ can be, respectively written as [17]

$$P_{eq} = \left| c^{\alpha}_{H^+} c^{\beta}_{OH^-} \frac{m^{\beta}_{OH^-} - m^{\alpha}_{H^-}}{m^{\alpha}_{H^-} c^{\alpha}_{H^-} - m^{\beta}_{OH^-} c^{\beta}_{OH^-}} \right| \tag{5}$$

$$c^{\mathrm{P}}_{Cu(II),eq} = c^{\alpha}_{H^+} c^{\beta}_{OH^-} \frac{m^{\beta}_{OH^+} - m^{\alpha}_{Cu(II)}}{\left(m^{\alpha}_{Cu(II)} - m^{\alpha}_{H^+}\right) c^{\alpha}_{H^+} - \left(m^{\alpha}_{Cu(II)} - m^{\beta}_{OH^-}\right) c^{\beta}_{OH^+}} \tag{6}$$

2.3. Mathematical principles for computer simulation

In order to complete mathematical model, besides the two MPB models without and with elution ion mentioned above, other important electrophoresis dynamics and electrochemical equations are needed herein. The electro-neutrality equation is the first condition used for computation of ionic concentrations in the entire MPB system [33]

$$\left|\sum c_+ z_+\right| = \left|\sum c_- z_-\right| \tag{7}$$

where z is the ionic charge(s). Second, Kohlrausch's regulating function has validity to the MPB system [33,34]

$$\sum \frac{c^{\alpha}_{+,i}}{m^{\alpha}_{+,i}} = \sum \frac{c^{\beta}_{-,i}}{m^{\beta}_{-,i}} = \sum \frac{c^{P}_{-,i}}{m^{P}_{-,i}} \tag{8}$$

Third, the current density of the whole MPB system is a constant, Namely, there is

$$\frac{\sum_{i=1}^{n} |z_i| c^{\alpha}_i m^{\alpha}_i}{\kappa^{\alpha}} = \frac{\sum_{i=1}^{n} |z_i| c^{\beta}_i m^{\beta}_i}{\kappa^{\beta}} = \frac{\sum_{i=1}^{n} |z_i| c^{P}_i m^{P}_i}{\kappa^{P}} \tag{9}$$

where, κ is the conductivity of phase (S m^{-1}). Fourth, K^+ always exists in the system as a background electrolyte and does not participate in the reaction. According to the classic jump boundaries conditions by Mosher et al. [33], we have

$$\left(c^{\alpha}_{K^+} - c^{P}_{K^+}\right) \frac{V_{\mathrm{EB,eq}}}{i} = \frac{c^{\alpha}_{K^+} m^{\alpha}_{K^+}}{\kappa^{\alpha}} - \frac{c^{P}_{K^+} m^{P}_{K^+}}{\kappa^{P}} \tag{10}$$

where i is the current intensity in electrophoretic tube (A m^{-2}). In the simulation, the velocity of an ion in the MPB system is computed with

$$V_i = m_i \frac{i}{\kappa} = m_i E \tag{11}$$

Due to the influcence of ionic strength on ionic mobility, the empirical equation [35] adjusts the absolute ionic mobility as

$$m_{i,\mathrm{act}} = m_i \exp\left(-\eta \sqrt{z_i I}\right) (\eta = 0.77 \quad \text{if} \quad z \geq 2; \quad \eta = 0.50 \quad \text{if} \quad z = 1) \tag{12}$$

where m_{act} and m_0 mean the actual and absolute mobility, respectively, η is the coefficient, z is the ionic valence, and I is the ionic strength. Note herein, the ionic strength should be always well controlled within 100 mM. In order to compute the conductivities in different phases, the conductivity of a phase is

$$\kappa = F\left(\sum_{i=1}^{n} |z_i| c_i m_i + c_{OH^-} m_{\mathrm{OH^-}} + c_{\mathrm{H^+}} m_{\mathrm{H^+}}\right) \tag{13}$$

where, F is the Faraday constant. At 25 °C, the product (κ_{w}) of water is 10^{-14}. With these equations mentioned above, we can compute the velocities of MPB and EB, EF and other ionic concentrations as well as the concentration of precipitation, and then predict the development of MPB and EB, and perform the relevant optimization of experimental conditions.

3. Experimental

3.1. Chemicals

Hydrochloric acid (HCl, Guaranteed Reagent grade, GR), sodium hydroxide (NaOH, GR), sodium acetate (NaAc, Analytical Reagent Grade, AR), potassium chloride (KCl, GR), ethylenediaminetetraacetic acid disodium salt (Na_2EDTA, AR), benzoic acid (C_6H_5COOH, AR), and potassium hydrogen phthalate ($COOHC_6H_4$-COOK, AR) as well as agarose (biological reagent) without electroosmotic flow (EOF) were purchased from Shanghai Chemical Reagents Co., Ltd. (Shanghai, China). Copper chloride ($CuCl_2 \cdot H_2O$, AR) was obtained from Sinapharm Chemical Reagent Co., Ltd. (Shanghai, China). Acetic acid (CH_3COOH, AR) was from Shanghai Lingfeng Reagent Factory (Shanghai, China).

3.2. Apparatuses

The experiments of MPB was performed via laboratory home-made apparatus of the large electrophoretic tube [17]. A glass tube (total length 15 cm, 3.6 mm I.D. and 6.0 mm O.D.) was the core component of the home-made apparatus, which was filled with 1.0% (w/v) agarose gel containing 100 mM ionic strength solution. Two ends of the gel tube were connected with the two rubber by the T-shape pipes, which were also joined with two peristaltic pumps (HL-2, Shanghai JiaPeng technology Co., Ltd. Shanghai, China), as well as two platinum electrodes interlinked with a power supply (DYY-4C, Beijing Liu-yi Scientific Instrument Factor, Beijing, China). Two peristaltic pumps continued to pump the anolyte and catholyte into their tubes. The tube was fixed on an operating-table (obtained from Shanghai Clinx Science Instruments Co., Ltd., Shanghai, China) together with the ruler. A digital camera (Model DX6490, Kodak Co. Ltd., US) was installed over the operating-table. The camera was used to record the movement of the boundary with the time and monitor the color change. Experiments were performed at ambient temperature.

The quantitation of copper ion sample obtained from the MPB run was conducted a P/ACE^{TM} MDQ Capillary Electrophoresis (CE) System (the Beckman Coulter Co., Fulliton, CA, USA). The system was equipped with a liquid circulation cooling temperature control system, 32 Karat Beckman Software subsystems, as well as UV detector. The system could also provide voltage up to 30 kV and reverse voltage. Uncoated fused-silica capillaries used here possessed total length of 60.2 cm, effective length of 50.6 cm, inner diameter of 75 μm (Yongnian Optical Fiber Factory, Hebei, China). A pH meter (320, Mettler-Toledo Instruments Ltd., Shanghai, China) could be used for adjusting the pH values of buffers. A pure water system (Ultra Clear Basic, SG Wasseraufbereitung und Regenerierstation Gmbh, Germany) was used to produce ultrapure water.

3.3. Solution, gel and sample

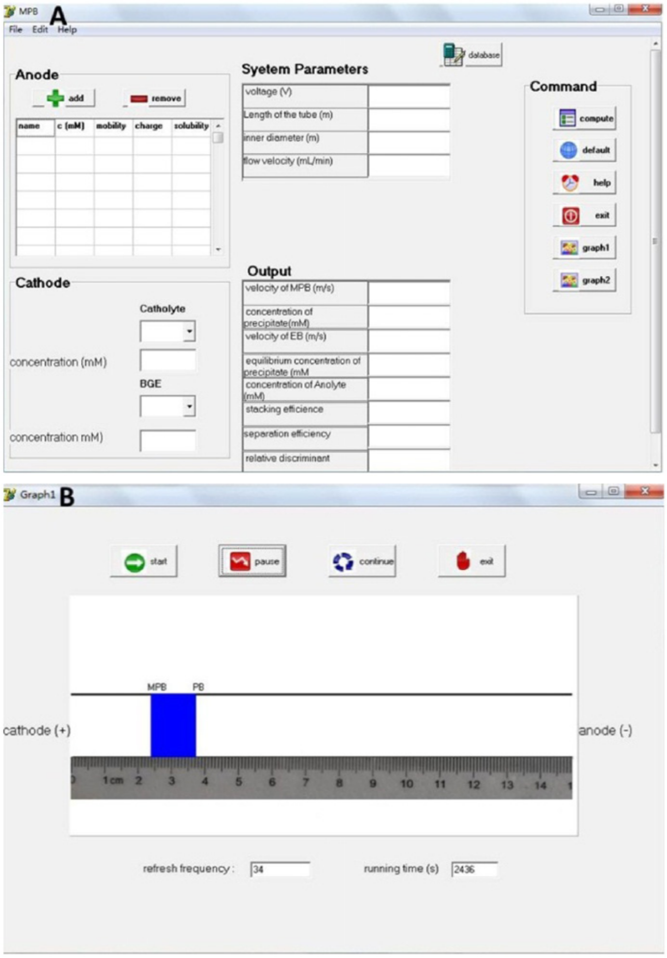
Four stock solutions of titrated NaOH, titrated HCl, 50 mM $CuCl_2$ and 2.0 M KCl were prepared at first for the run of MPB in the large tube. Classic acid-base titration was used for accurately determining the concentration of dilutions of NaOH and HCl. To maintain consistency and stabilization in the MPB system, the ionic strength of all solutions was adjusted to 100 mM via 1 M KCl. The titrated NaOH and 2.0 M KCl were used for the preparation of the cathodic solution within 100 mM ionic strength. The anodic solution was consisted of the dilution of the titrated HCl solution, the stock $CuCl_2$ solution and KCl solution within 100 mM ionic strength.

CE was used to determine the concentration of copper ion in different MPB samples. According to the previous work [17,34], 40 mM pH 5.0 HAc–NaAc solution with 30 mM EDTA was used as the running buffer of CE analysis of copper ion sample. For establishing the calibration curve of $[Cu\text{-}EDTA]^{2-}$, the stock solution with 100 mM pH 5.0 HAc–NaAc buffer plus 5.0 mM $[Cu\text{-}EDTA]^{2-}$ was prepared ahead of schedule. Then the stock solution was diluted with 100 mM pH 5.0 HAc–NaAc buffer to obtain 1 mM, 2.5 mM, 5 mM, 7.5 mM, 10 mM, 12.5 mM $[Cu\text{-}EDTA]^{2-}$ standard solutions containing 0.2 mg/ml benzoic acid used as an internal standard (IS).

3.4. Procedures

3.4.1. Procedure of computer simulation

On the basis of the mathematical model described in Section 2, we compiled a computer software with Borland-Delphi 7 (Fig. 2). The software was consisted of two windows, the first one was used for the input of parameters and the calculation of MPB experimental conditions (Fig. 2A). The second one was used to show the dynamic process of offline MPB-based preconcentration of metal ion (Fig. 2B). In the first window, the component, concentration, absolute mobility, charge and solubility of heavy metal ion could be input in the anode frame, and other analytes such as elution ions also could enter in this frame; while the component and concentration of NaOH could be set in the cathode frame. The experimental conditions could be adjusted in the system parameters frame such as voltage as well as concentrations of hydrogen, hydroxyl and metal ions. The software could output the velocities of MPB and EB, the concentrations of precipitate and metal ions, the enrichment factor



refresh frequency : 34

running time (s) 2436

Fig. 2. Main window of anode frame, cathode frame, system parameters frame and output frame in the developed simulation software (A); window of dynamics simulation of MPB-based preconcentration (B).

and the migration rate of analyte as well as JE value. The second window could display a vivid simulation on the dynamic process of MPB in the large tube. In the simulation herein, the following physico-chemical parameters were used for the computation: copper ion mobility ($m_0 = 56.6 \times 10^{-9}\ \mathrm{m^2\,V^{-1}\,s^{-1}}$), potassium ion mobility ($m_0 = 76.2 \times 10^{-9}\ \mathrm{m^2\,V^{-1}\,s^{-1}}$), chlorine ion mobility ($m_0 = 67.0 \times 10^{-9}\ \mathrm{m^2\,V^{-1}\,s^{-1}}$), hydrogen ion mobility ($m_0 = 362.0 \times 10^{-9}\ \mathrm{m^2\,V^{-1}\,s^{-1}}$), and hydroxyl ion mobility ($m_0 = 205.0 \times 10^{-9}\ \mathrm{m^2\,V^{-1}\,s^{-1}}$) [32].

3.4.2. Procedure of MPB

The method for preparing the agarose gel in the large electrophoretic tube was similar with that in the previous procedure [17]. The glass tube was filled with 1.0% (w/v) agarose gel and 100 mM ionic strength solution KCl as background electrolyte. Each two ends of the gel-filled tube were joint with the cathode and anode, respectively by the rubber pipes, and two peristaltic pumps continuously pumped the anolyte and catholyte into the gel-filled tube during the whole process. After the power supplied, it could easily position the movement of the MPB by using a digital camera. The experimental velocity of MPB is computed via

$$V_{MPB,exp} = \frac{D_{MPB,exp}}{t} \tag{14}$$

where, $V_{\mathrm{MPB,exp}}$ is the experimental velocity of MPB ($\mathrm{m\,s^{-1}}$), $D_{\mathrm{MPB,exp}}$ is the experimental ovement of MPB within its given time *t*. In each run, the experiment was repeat three times under the same conditions.

3.4.3. Procedure of CE

Before use, a new capillary should be rinsed with 1.0 M NaOH for 10 min, ultrapure water for 5 min, 1.0 M HCl for 10 min and ultrapure water for 5 min in order, followed by running buffer (40 mM pH 5.0 HAc–NaAc) for 10 min. At the end of each run, the capillary should be flushed with ultrapure water for 2 min and with running buffer for 3 min. 0.5 psi 10 s was used for hydrodynamical sample injection. The voltage applied across the capillary was −30 kV and the absorbance wavelength was set at 254 nm.

3.4.4. Determination of copper ion in MPB system via CE

After a run of MPB-based preconcentration, the power supply was turned off, the gel was removed from the glass tube at once, and cut off the blue gel strip containing a large number of copper ion and the precipitate of $Cu(OH)_2$. This step should be very careful. If blue strip contained the blank gel, it would dilute the concentration of the copper that would make experiment results smaller. After then, the gel with the preconcentrated copper was put into an eppendorf tube, and weighed as soon as possible, added with the running buffer of CE, and heat up until the gel has totally dissolved before its determination via CE. The volume of gel did not change before and after the dissolution. This ensured preciseness of the calculation of concentration of Cu(II) in the tube. Before a determination, 0.2 mg/ml internal standard of benzoic acid was added into the eppendorf tube to form the final volumes of sample solution up to 1000 μL. After that, the prepared samples were injected into the capillary and the relevant concentration of metal ion was detected with CE. After a CE run, it could obtain the peak area of copper ion, then we could work out the copper concentration in accordance with the calibration curve,

$$c_{Cu} = \frac{M}{1000\rho} \times f\left(\frac{A}{B}\right) \tag{15}$$

In Eq. (15), *M* is the mass of gel after cutting off (kg), ρ is density of agarose gel ($\mathrm{kg\,m^3}$), *f*(*A*/B) is the calibration curve.

4. Results and discussion

4.1. Numerical computation of MPB

By numerical computation via the developed software, we could predict the tendency of the boundary movement before the experiment based on the JE value (no show herein) which was an important parameter in MPB system, at the same time boundary velocity was also the same function. Boundary velocity of MPB was the most significant parameters influencing the EF in the process. It was convenient to make a better design of experiments. Table 1 showed the calculation results of MPB velocity and relevant experimental results in the large tube. The MPB system was formed with 4 mM NaOH+96 mM KCl in phase β and 5 mM, 7.5 mM, 10 mM, 15 mM, 20 mM, 25 mM Cu(II)+90 mM, 85 mM, 80 mM, 70 mM, 60 mM, 50 mM KCl in phase α. The data in Table 1 clearly revealed that if concentration of Cu(II) was less than 10 mM, the MPB migrated towards the anode, namely the velocity of MPB was a negative value. Table 1 further revealed that the velocity decreased from $-8.7 \times 10^{-5}\ \mathrm{m\,s^{-1}}$ to $-1.34 \times 10^{-5}\ \mathrm{m\,s^{-1}}$ as the concentration of copper ion was increased, while the relevant experimental velocity of MPB was decreased form $-8.4 \times 10^{-5}\ \mathrm{m\,s^{-1}}$ to $-1.62 \times 10^{-5}\ \mathrm{m\,s^{-1}}$. Clearly, the experiments demonstrated the numerical computation of MPB velocity. If the metal ion concentration was further increased, both the numerical computation and experiment boundary motions were towards the cathode. Correspondently, the simulation velocity of MPB was increased form $0.04 \times 10^{-5}\ \mathrm{m\,s^{-1}}$ to $1.52 \times 10^{-5}\ \mathrm{m\,s^{-1}}$, the experimental velocity of MPB was also increased form $0.06 \times 10^{-5}\ \mathrm{m\,s^{-1}}$ to $1.59 \times 10^{-5}\ \mathrm{m\,s^{-1}}$, and the ratios of the simulated and the experimental velocities drifted from 0.83 to 1.03, demonstrating a fair coincidence of MPB velocity between the numeral computations and the experiments.

The numerical calculations of EF values, EB velocity, and optimization of condition would be directly and indirectly given from Section 4.2 to Section 4.6.

4.2. Simulation of MPB without elution ion

The simulator could vividly imitate the dynamic process of MPB in large tube, visually predict the evolution of MPB and accurately compute ionic concentration. Fig. 3A displayed the dynamic process of MPB system was formed with 10 mM Cu(II)+80 mM KCl in phase α and 8.0 mM OH^-+92 mM KCl in phase β. The computation results showed that: (i) the MPB almost migrated as a constant velocity, while the velocity of PB nearly

Table 1

Comparisons between simulation and experimental boundary velocities in MRB (without elution ion) formed with 4 mM NaOH+96 mM KCl in phase β and 5–25 mM Cu(II)+90–50 mM KCl in phase α.

Cu (mM)	NaOH (mM)	V_{MPB} ($\mathrm{m\,s^{-1}}$) (Sim)[a]	V_{MPB} ($\mathrm{m\,s^{-1}}$) (Exp)[a]	Ratio (Sim/Exp)[a]
5	4	-8.70×10^{-5}	-8.40×10^{-5}	1.03
7.5	4	-1.34×10^{-5}	-1.62×10^{-5}	0.83
10	4	0.05×10^{-5}	0.06×10^{-5}	0.83
15	4	0.99×10^{-5}	1.11×10^{-5}	0.89
20	4	1.34×10^{-5}	1.49×10^{-5}	0.90
25	4	1.52×10^{-5}	1.59×10^{-5}	0.95

Experimental conditions: 5 mM, 7.5 mM, 10 mM, 15 mM, 20 mM, 25 mM Cu(II)+90 mM, 85 mM, 80 mM, 70 mM, 60 mM, 50 mM KCl in phase α. The other conditions are the same as those in Fig. 3.

[a] Sim means computation value via the developed simulator, Exp indicates the experimental results.

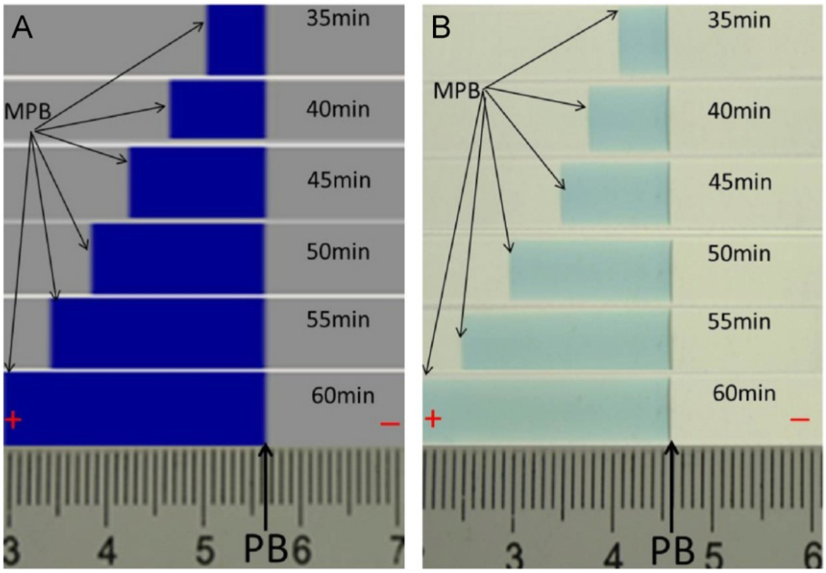


Fig. 3. Comparisons of computer simulation (A) and experiment (B) on MPB (without elution ion) formed with 10.0 mM copper ion+80 mM KCl and 8.0 mM NaOH +92 mM KCl under different running times. Experimental conditions: 200 V; 1.0 mL min^{-1} flow rate of the anolyte and catholyte; 1.0% agarose gel with 100 mM KCl in the tube; I.D. 3.6 mm and length 150 mm glass tube. 'PB' and 'MPB' imply 'precipitate boundary' and 'moving precipitate boundary', respectively, the symbol '+' and '−' indicate the anode and the cathode, respectively.

was zero according to Eq. (6), and (ii) the concentration of precipitate $Cu(OH)_2$ was uniform distribution in precipitation zone created by MPB.

The predicted results were proved by the relevant experiment below. Fig. 3B showed the experiments of a MPB originally was formed with 10 mM Cu(II)+80 mM KCl in phase α and 8.0 mM OH-+92 mM KCl in phase β in a gel-filled large tube. The experiments of Fig. 3B demonstrated that (i) the velocity of MPB nearly kept as a constant value, because boundary moved within the same distance within a unite time; (ii) the PB stayed motionless; and (iii) the color of precipitation zone was deeply consistent, indicating even concentration distribution of precipitate $Cu(OH)_2$ within the gel [19]. The experiments in Fig. 3B commendably confirmed the simulation results in Fig. 3A.

4.3. Copper preconcentration via MPB without elution ion

Enrichment factor (EF) was the most important experimental parameters in the MPB system. Table 2 gave the simulation and experimental values of EF in the MPB system without elution ion. The MPB system was formed with 4.0 mM OH^-+92 mM KCl in phase β and 1.5 mM, 1.0 mM, 0.5 mM, 0.1 mM Cu(II)+97 mM, 98 mM, 99 mM, 100 mM KCl in phase α. It could be observed from Table 2 that the EF value increased with the decrease of copper concentration under the condition of the same concentration of NaOH. When the concentration of copper ion decreased from 1.5 mM to 0.1 mM, the simulating EF values varied from 2.9-fold to 69.1-fold. In the meantime, the experimental EF values altered from 2.5-fold to 53.4-fold. As the concentration of copper ion was more than 0.5 mM, the EF value was just 8.8-fold. However, if the concentration of copper ion decreased to 0.1 mM, the EF value was in rapid growth. These results indicated that low concentration of copper ion could obtain a better enrichment via the MPB-based system of offline preconcentration. Evidently, the experimental results manifested the simulation results. However, the MRB-based preconcentration system without elution ion was generally poorer than that with elution ion, as revealed in Section 4.5.

Table 2
Comparison between simulation and the experimental values of EF in MRB system (without elution ion) with 10 mM Cu(II)+80 mM KCl in phase α and 8.0 mM OH^-+92 mM KCl in phase β.

Cu (mM)	NaOH (mM)	EF (Sim) [a]	EF (Exp) [a]	Ratio (Sim/Exp) [a]
1.5	4	2.77	2.51	1.10
1	4	4.22	4.13	1.02
0.5	4	8.81	8.43	1.05
0.1	4	69.1	53.4	1.29

The experimental conditions are the same as those in Fig. 3.

[a] Sim means computation value via the developed simulator, Exp indicates the experimental results.

4.4. Simulation of MPB with elution ion

Fig. 4A showed the simulation results of the MPB with the elution ions, which was formed with 1.5 mM $CuCl_2$+2.1 mM HCl+94 mM KCl in phase α and 4 mM NaOH+96 mM KCl in phase β. It is displayed from Fig. 4A and the relevant computation results that: (i) the velocity of EB between phase α and precipitate zone was kept a constant one rather than zero; and (ii) the velocity of MPB between precipitate zone and phase β was a little faster than that of EB, namely, the length of the precipitation region continuously increased tardily during the run of MPB-based preconcentration of copper ion.

These simulation results were verified by the following experiment. Fig. 4B exhibited the corresponding experimental results under the exact conditions used in the simulation mentioned above. It was demonstrated in Fig. 4B that: (i) the EB began to move, and almost was in uniform motion type since it moved the same distance within the same time; and (ii) MPB moved a little faster than EB, which led to the precipitation region in between EB and MPB increased gradually. These experiments were basically consistent with the results of computer simulation.

The brown precipitate was observed during the experiment of MPB with elution ion (Fig. 4B). The main reason was that some trace metal ions such as Ca(II), Fe(III), Mg(II) and Ba(II) exist in the background electrolyte of potassium chloride and other chemical reagents. These trace metal ions obtained a good enrichment via

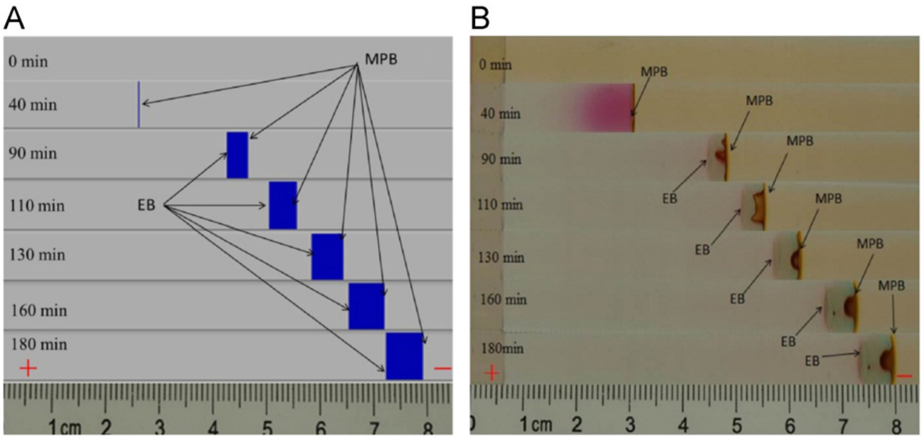


Fig. 4. Comparisons of simulation (A) and experiment (B) on MPB (with elution ion) formed with 1.5 mM Cu(II)+2.1 mM HCl+94 mM KCl in analyte and 4 mM NaOH+96 mM KCl in catholyte. "EB" means "elution boundary". The experimental conditions and symbols are the same as those in Fig. 3.

Table 3
Comparison between simulation and the experimental values of EF in MPB system formed with 4.0 mM OH^-+92 mM KCl in phase β as well as 1.5, 1.0, 0.5, 0.1 mM Cu(II)+97–100 mM KCl in phase α.

Cu (mM)	HCl (mM)	NaOH (mM)	EF (Sim)[a]	EF (Exp) [a]	Ration (Sim/Exp)[a]
1.5	2.1	4.0	20.6	19.5	1.06
1	2.1	4.0	30.9	31.5	0.98
0.5	2.1	4.0	61.8	55.2	1.12
0.1	2.1	4.0	308	123.2	2.5

The experimental conditions are the same as those in Fig. 3.

[a] Sim means computation value via the developed simulator, Exp indicates the experimental results.

the MPB system with the elution ion. These results illustrated MPB system with the elution ion had the strong EF of trace metal ions, in contrast to that without elution ion in Section 4.3.

4.5. *Copper preconcentration via MPB with elution ion*

Table 3 indicated the simulation results of EF_{eq} at the equilibrium state and the relevant experiments in the large tube. The MPB system was formed with 4.0 mM OH^-+92 mM KCl in phase β and 1.5 mM, 1.0 mM, 0.5 mM, 0.1 mM Cu(II)+2.1 mM H^++94 mM, 96 mM, 97 mM, 98 mM KCl in phase α. In Table 3, it was observed that the EF at steady-state was in a steady increase as the concentration of copper ions decreased in the same system. In other words, the lower the concentration of copper ion was, the better the enrichment of offline MPB method became. As the concentration of copper ion was decreased from 1.5 mM to 0.1 mM, the simulation EF_{eq} of MPB-based preconcentration system was increased form 20.6-fold to 308-fold. The corresponding experimental EF_{eq} value was also increased form 19.5-fold to 123.2-fold. Except the case of 0.1 mM copper ion, the ratio ranged form 1.06 to 1.12 indicating very fair consistency between the simulations and experiments.

The deviation between the simulating and experimental results could be easily understood as the concentration of copper ion was set at 0.1 mM. The lower concentration of copper ion produced a narrower precipitation zone strip in MPB-based system of preconcentration (0.1 mm width). Evidently, it was impossible to accurately cut off the exact copper-containing gel strip from the copper-free or low copper-containing gel [19]. This implied the unavoidable dilution of copper-containing gel by the copper-free gel [19]. As a result, the concentrations of copper ion measured in the experiment was often much lower than simulation one. The elution and elution-free MPB systems had different EF value to low concentration copper ion. As the concentration was set at 0.1 mM, the EF value of elution-free MPB system was 69.1, while it became 308 in the elution MPB system. The results revealed that low concentration metal ion in the elution system had a better EF value, as compared with that in the elution-free MPB system of preconcentration.

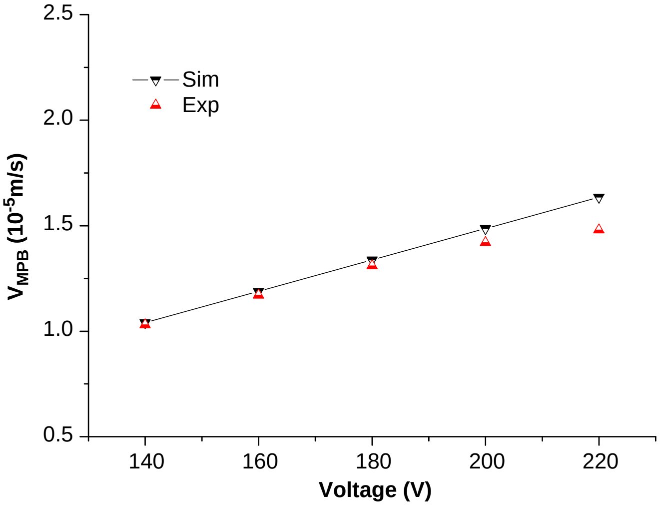


Fig. 5. Relationship between voltage and migration velocity of MPB formed with 10.0 mM copper ions+80 mM KCl in phase α and 8.0 mM hydroxyl ion+92 mM KCl in phase β under different voltages of 140 V, 160 V, 180 V, 200 V 220 V. The experimental conditions are the same as those in Fig. 3.

4.6. *Effect of voltage*

By numerical calculation and dynamic simulation, the software could be used to simply optimize the experimental conditions such as voltage, hydroxyl, hydrogen as well as metal ion as, had been discussed above. Herein, we further used voltage as an example to illustrate the relevant optimization of conditions. Fig. 5 exhibited the simulating and experimental results of MPB system formed with 10.0 mM Cu(II)+80 mM KCl in phase α and 8.0 mM hydroxyl ion+92 mM KCl in phase β under different voltages. Fig. 5 showed that both the simulation and the experimental demonstrated that the velocities of MPB increased with the enhancement of the voltage, both of them had a fairly agreement. According to Eq. (1), the velocity was in proportion to the voltage/current density under the same condition, this was

Table 4
Comparison between simulation and the experimental values of EF in MPB system (without elution ion) formed with 10.0 mM Cu(II)+80 mM KCl in phase α and 8.0 mM OH^1+92 mM KCl in phase β under different voltages.

Voltage (V)	Cu (mM)	NaOH (mM)	EF (Sim) [a]	EF (Exp) [a]	Ration (Sim/Exp) [a]
140	10	8	11.0	12.3	0.90
160	10	8	11.0	11.9	0.93
180	10	8	11.0	10.9	1.01
200	10	8	11.0	12.7	0.88
220	10	8	11.0	18.9	0.58

Experimental conditions: 140 V, 160 V, 180 V, 200 V and 220 V. The other conditions are the same as those in Fig. 3.

[a] Sim means computation value via the developed simulator, Exp indicates the experimental results.

reason why V_{MPB} had an approximate linear relation with the voltage as showed in Fig. 5. However, it was obviously found that if the voltage exceeded 220 V, the experimental values have a relatively large deviation from the simulation results. It could be observed that the boundary distorted at 5 min after boundary appearing (no show herein). This was caused by Joule heating. As the voltage increased, the system would produce a large amount of Joule heating; if the Joule heating was increased a certain extent in the glass tube, it would seriously affected the movement of the boundary.

We also made a quantitative comparison of EF results of computer simulation and experimental results under different voltages/current density. Table 4 showed that different voltages had no influence on EF of the MPB in simulation, and the corresponding experiments proved the result of simulation. Based on Eq. (2), it was obvious that voltage and EF did not matter, as had been certified by results of simulations and relevant experiments. It could be further observed that if the voltage exceeded 220 V, a large quantities of metal aggregated in the tortuous boundary, there was quite deviation between experimental and simulated values. The reason was the same as the interpretation given just above. That was a further evidence of the need for software setting a limit of the used voltage.

4.7. Deviation analysis

It could be observed that the results of computer simulation and experiment had a high degree of agreement, but there were still some deviations between them. The deviations were mainly originated from the following reasons. At first, the simulation errors were mainly from the derivation of the equations themselves which carried out in the ideal state. Hence, some deviations were always present in calculating the ionic strength and the conductance. These simulated deviations were inevitable. Second, in the experiment, the precipitate continuously generated in the process of reaction, the conductance and ionic strength of the entire tube would be changed, and thus these would affect the experimental results. Third, in the experiment of FE, copper ion mainly enriched at boundary, it was difficult to accurately cut off gel strip from the interface, constantly cut off the next blank gel in which the reaction solution existed, and afterwards it would produce errors.

5. Conclusions

From the above results and discussions, it was available that a mathematical mode of MPB was developed for computer simulation on offline sample pretreatment of heavy metal ion via MPB created with copper ion and sodium hydroxide in gel-filled tube. The mode concerned the boundary velocity equations of MPB, EB, EF and other electrophoresis dynamics and electrochemical equations (e.g., Kohlrausch' regulating function, electro-conditional equation and jump boundary equation, etc). To demonstrate the validity of the developed model and simulator, the relevant experiments were performed relied on a home-made apparatus of MPB. The comparative investigations indicated that: (i) the developed software could quantitatively compute the velocity and EF of MPB; (ii) the computer program could vividly imitate a dynamic evolution of MPB with/without elution ion; and (iii) the experimental results and relevant simulation results were in a good agreement. The developed software could precisely predict the results of the MPB-based preconcentration, conveniently optimize the experiment conditions of the MPB, and provide a good understanding to the offline heavy metal ion preconcentration.

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