Experimental study on moving neutralization reaction boundary created with the strong reactive electrolytes of HCl and NaOH in agarose gel

Cheng-Xi Cao[a], Shu-Lin Zhou[b], You-Zhao He[a,*], Xiao-Yun Zheng[c], Wen-Kui Chen[b], Yi-Tai Qian[a]

[a]*Department of Chemistry, University of Science and Technology of China, 230026 Hefei, China*
[b]*Department of Parasitology, Wannan Medical College, 241001 Wuhu, China*
[c]*Department of Forensic Medicine, Wannan Medical College, 241001 Wuhu, China*

Abstract

In this paper, a moving neutralization reaction boundary (MNRB) is created with the strong reactive electrolytes of HCl and NaOH in agarose gel. The motions of the MNRB are investigated and compared with the predictions with the theory of the moving chemical reaction boundary (MCRB). The results show that, under appreciate experimental conditions, the experiments on the MNRB are exactly in coincidence with the predictions with the MCRB theory. Thus, the results excellently demonstrate that the MCRB theory is valid for the MNRB formed with the strong reactive electrolytes of HCl and NaOH. Additionally, it is, as discussed in this paper, imperative to develop a method to obtain ionic mobility at different temperatures and ionic strengths, in order to investigate the movements of the MCRB more efficiently. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Moving chemical reaction boundary; Isoelectric focusing; Hydrochloric acid; Sodium hydroxide

1. Introduction

In 1961–1962, Svensson advanced the famous theory of classic isoelectric focusing (IEF) [1,2]. According to Svensson's theory, Vesterberg [3] synthesized the carrier ampholyte, a very important substance for IEF. With the carrier ampholyte, IEF was gradually used in biochemistry and biomedicine [4–7]. However, in IEF, there still exist some phenomena, such as cathodic drift and plateau of pH gradient [4–12], which are not elucidated by Svensson's IEF theory and other hypotheses as reviewed in Refs. [6] and [12] (see p. 301 in Ref. [6] and the Introduction in Ref. [12]).

In order to unveil the mechanism of IEF, the authors [12–16] developed the concept of the moving chemical reaction boundary (MCRB) from the ideas of the precipitate reaction front by Demen and Rigole [17,18] and of the stationary neutralization

*Corresponding author. Tel.: +86-551-3607-072; fax: +86-551-3671-760.

E-mail addresses: cxcao@mail.ustc.edu.cn (C.-X. Cao), yzhe@ustc.edu.cn (Y.-Z. He).

reaction boundary by Pospichal et al. [19], formulated the moving chemical reaction boundary equations (MCRBEs) for both strong and weak reactive electrolytes, discovered the relations between IEF and the MCRB [12,20,21] and those between Kolhrausch's regulating function and the MCRBEs [22]. The MCRB theory is the counterpart of the moving boundary system (MBS) developed about 60 years ago by Longsworth [23], Dole [24], Svensson [25], Alberty [26] and Nichol [27].

The MCRB theory has been partially demonstrated by some experiments. The prediction with the MCRBEs (viz., Deman–Rigole's equation, see Eq. (2) in Ref. [18]) is qualitative coincidence with the experiments on precipitate reaction front by Deman and Rigole [17,18]. The calculations with the stationary neutralization reaction boundary equations (SNRBEs) deduced from the MCRBEs are in exact agreement with the experiments on the stationary neutralization boundary with electrically controlled electrofocusing apparatus by Pospichal et al. [19]. Recently, the authors manifested the predictions with the MCRB theory are in quantitative agreement with the experiments on the moving precipitate reaction boundary created with $CoCl_2$ and NaOH [28–30].

Is the MCRB theory valid for a MNRB created with the strong reactive electrolytes of acid and alkali like HCl and NaOH, or not? To the authors' knowledge, except for the experiments on the stationary neutralization reaction boundary by Pospichal et al. [19], there is still no systemic quantitative experimental data to prove the validity to a MNRB. Thus, it is necessary to test the validity of the theory for a MNRB, additionally to supply an inexpensive and uncomplicated, but efficient and convenient method for the study of MNRBs and electrofocusing.

2. Experimental

2.1. Reagents

The reagents used here are HCl, NaOH, KCl and bromphenol blue, the former three are AR grade [respectively, purchased from Tianjin Chemical Reagents Co. (Tianjinm, China), Shanghai Chemical Reagents Co. (Shanghai, China) and Nanjin Chemical Factory (Nanjin, China)], and the latter is of CR

grade (purchased from Shanghai Chemical Reagents). The agarose used as the anti-convection medium is a biochemical reagent (Shanghai Huang-Hua Pharmaceutical Factory, Shanghai, China). Note here, the agarose gel is better in contrast to the agar gel, since in agarose gel there is nearly complete absence of electroosmostic flow (EOF) [6,31,32].

2.2. Apparatus

The apparatus was made in the laboratory and developed from our previous apparatus [30]. As shown in Fig. 1, a glass tube is filled with 0.5–2.0% (w/v) agarose gel containing the alkali NaOH, 0.1 mol/l background electrolyte KCl and 0.1% (w/v) bromphenol blue. The tube, together with the ruler, is fixed on a small performing-table, over which an adjustable camera (Model SD345, Minolta Co. Ltd., Japan) is fixed. The camera is used to record the movement of the MNRB with the time. With the two rubber-tubes, the two ends of the glass tube are connected to two three-way-pipes, which are joined with two peristaltic pumps (viz., pumps 1 and 2, Model HL-2, Shanghai Luxi Biochemical Instrument Factory, Shanghai, China) and two platinum electrodes (viz., the anode and cathode). The flows of anolyte and catholyte are pushed by the two pumps. A power supply (Model DYY-III4, constant voltage 0–600 or 0–1200 V, constant current 0–25 or 0–100 mA, Beijing Luyi Instrument Factory, Beijing, China) is used to yield the direct current. At the two ends of the glass tube, two fine electrodes inserted through the rubber tubes are connected with a recorder (XWD2-213, Shanghai Automatic Instrument Factory No. 3, Shanghai, China), with which one can monitor the voltage between the two ends of the glass tube.

The laboratory-made apparatus is very efficient and convenient for the experimental studies on MNRBs created with strong reactive electrolytes of acid and alkali as will be shown in Fig. 2, but inexpensive and uncomplicated as compared to some commercial apparatus used for IEF [19,33].

2.3. Procedures

The preparation of gel is according to the methods described in Refs. [17] and [18].

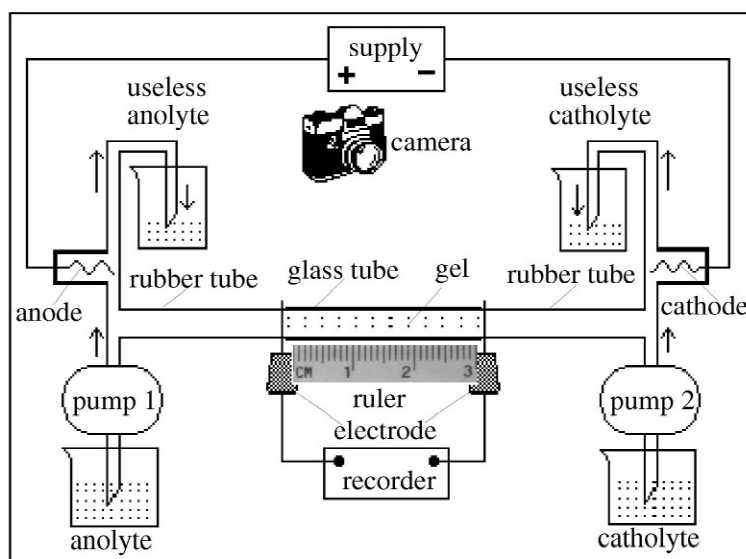Initially, the agarose gel in the glass tube contains

Fig. 1. Diagrams of the procedure and apparatus for experiments of the MNRB formed with strong reactive electrolytes of acid and alkali. The direct current is generalized with a power supply; the flows of anolyte and catholyte are pushed by pumps 1 and 2, respectively; the glass tube (including the ruler) is fixed on a small operating-table; over the table a camera is fixed to record the movement of the MNRB with time; the voltage between two ends of the glass tube is recorded with two electrodes connected with a recorder. The arrows indicate the flow directions of anolyte and catholyte. The symbols, $+$ and $-$, denote the anode and cathode, respectively.

0.004–0.014 mol/l NaOH, 0.1 mol/l background electrolyte KCl and 0.1% (w/v) bromphenol blue. The catholyte holds 0.004–0.014 mol/l NaOH and 0.1 mol/l KCl, and the anolyte comprises 0.004–0.014 mol/l HCl and 0.1 mol/l KCl. All the runs of MNRBs created with the strong acid and alkali here are designed to move towards the cathode.

Therefore, before each run, the overall color in the tube is blue due to the co-existence of the bromphenol blue and alkali of NaOH as shown in Fig. 2A. However, after the run begins, the left side of the tube becomes yellow as shown in Fig. 2B, because of the co-existence of the bromphenol and acid of HCl at the left side of tube while the acid of HCl displaces the alkali of NaOH gradually. As the displacement continues, the yellow zone comprising HCl becomes longer and longer, in contrast, the blue zone containing NaOH gradually becomes shorter (see Fig. 2B and C). A clear boundary created with HCl and NaOH can be observed during the displacement and electromigration reaction between H^+ and OH^-.

Here, the bromphenol blue is well used as the mark that marks the yellow zone holding HCl, the blue zone comprising NaOH and the boundary that separates the yellow and blue zones sharply as clearly displayed in Fig. 2. The length of the displacement of the MNRB is just that of yellow acidic zone, viz., neutralization reaction zone that is similar to the precipitate reaction zone [28–30], as shown in Fig. 2B and C.

In each experiment, two tubes under the same conditions were run individually. During each run, a series of photographs are, as shown in Fig. 2, taken at different times using the camera in Fig. 1. With those photographs, we can directly monitor the displacements of boundaries, viz., the neutralization reaction zone. The observed velocity of a MNRB can be calculated with:

$$\mu_{obs}^{\alpha\beta} = l_{obs}/t \qquad (1)$$

where, l_{obs}, is the displacement of boundary, signed quantity its positive and negative mean the cathodic and anodic movements, respectively, and its length is equal to the lengths of the yellow acidic zone which can be directly determined with compasses from the photograph given in Fig. 2, t is the run time and $\mu_{obs}^{\alpha\beta}$ is the observed boundary velocity.

The theoretical boundary velocity of the MNRB, as formulated in Ref. [28], is expressed as:
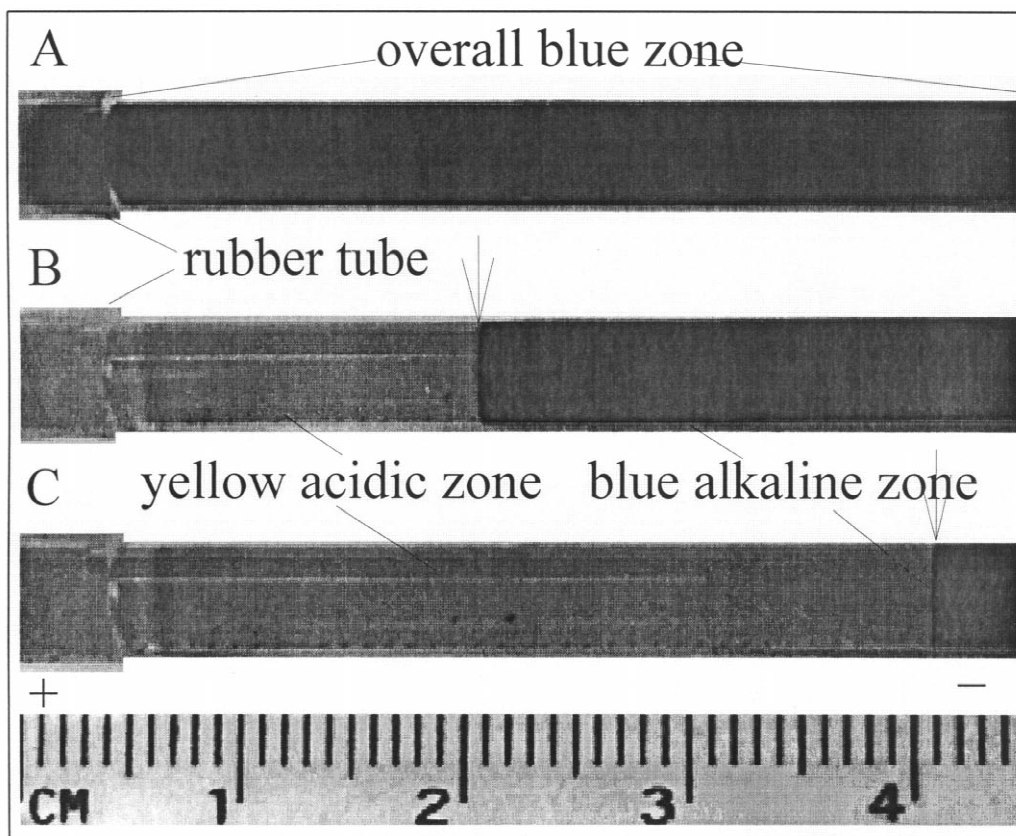
Fig. 2. The clear and sharp boundary during the run of the MNRB created with the strong reactive electrolytes of HCl and NaOH in agarose gel. (A) Just before the start of the run; (B) 5 min after the run; and (C) 10 min after the run. The arrows indicate the sharp boundaries formed between the yellow (HCl) and blue (NaOH) zones. The symbols $+$ and $-$ denote the anode and cathode, respectively. The yellow acidic zone, viz., the neutralization reaction zone – being similar to the precipitate reaction zone [28–30], ranges from the symbol $+$ to the boundary indicated by the arrow, and the blue alkaline zone occupies from the boundary to the symbol $-$. Conditions: 0.01 mol/l HCl, 0.01 mol/l NaOH, 1% (w/v) agarose gel, 0.1 mol/l background electrolyte KCl, 0.1% (w/v) bromphenol blue in gel, constant current intensity 1.2 mA/mm^2, 90 mm×4.4 mm I.D.×6.2 mm O.D., and temperature 18–20°C.

$$\mu_{the}^{\alpha\beta} = \frac{m_{H+}^{\alpha} c_{H+}^{\alpha} E^{\alpha} - m_{OH-}^{\beta} c_{OH-}^{\beta} E^{\beta}}{c_{H+}^{\alpha} - c_{OH-}^{\beta}} \qquad (2)$$

where, the superscripts, α and β, indicate phase α and β, respectively, the subscripts, H+ and OH−, mean positive and negative reacting ions like hydrogen and hydroxyl ions, respectively, m the mobility, c the equivalent concentration, E the electric field strength. Note here, m and c are positive if the ion carries net positive charge or negative if net negative charge, as have been defined by numerous scientists [12–19,23–28].

Owing to the large quantity of background elec-

trolyte KCl in the electrolyte system, the conductivity, as treated in Refs. [17–19] and [28–30], is considered uniform over a whole conductor approximately (the conductivity of solution containing 0.1 mol/l KCl is equal to 1.29 S/m at 25°C), viz.:

$$E^{\alpha} = E^{\beta} \qquad (3a)$$

$$I/q\kappa^{\alpha} = I/q\kappa^{\beta} \qquad (3b)$$

where, I is the current (mA), q is the cross-sectional area of tube (mm^2), κ the specific conductance of phase/or solution (S m^{-1}).

For the existence of high ionic strength, the mobilities of hydrogen and hydroxyl ions in Eq. (2) ought to be corrected by the empirical equation given by Reijenga and co-workers [34–36] and the authors [37,38]:

$$m_{\mathrm{act}} = m_0 \exp(-\eta \sqrt{z\mathrm{I}})$$
$$(\eta = 0.77 \text{ if } z = 2; \eta = 0.50 \text{ if } z = 1) \tag{4}$$

where, m_{act} and m_0 are the actual and absolute mobilities, respectively, the ionic strength (I) is:

$$\mathrm{I} = 0.5\sum c_i z_i^2 \tag{5}$$

where z is the ionic valence, and η the coefficient. Note here, the overall/or apparent mobilities of hydrogen and hydroxyl ions are equal to their actual mobilities owing to the complete ionization of the strong reaction electrolytes HCl and NaOH and the almost complete absence of EOF in agarose gel (see Refs. [6,31,32]).

The absolute mobilities of the hydroxyl and hydrogen ions at a temperature of 25°C, obtained from Ref. [39], are $20.5 \cdot 10^{-8}$ and $36.3 \cdot 10^{-8}$ m^2 V^{-1} s^{-1}, respectively.

For the reasons cited above, the theoretical velocity of the boundary should be calculated with Eq. (6):

$$\mu_{\mathrm{the}}^{\alpha\beta} = \frac{m_{\mathrm{act,H+}}^{\alpha} c_{\mathrm{H+}}^{\alpha} - m_{\mathrm{act,OH-}}^{\beta} c_{\mathrm{OH-}}^{\beta}}{(c_{\mathrm{H+}}^{\alpha} - c_{\mathrm{OH-}}^{\beta}) \cdot \kappa} \cdot i \tag{6}$$

where, i is the constant current intensity (mA/mm^2).

In the following experiments, monitoring the voltage between the two ends of run tube with the voltage recorder in Fig. 1, the authors found that, even when a constant voltage of power supply is used, the voltage between the two ends of the run tube decreases with the time. Since the cold anolyte and catholyte flow continuously while the electrolyte solution in the tube is gradually heated by Joule heating generated by the electric field, this leads to the decrease of the voltage between the two ends of tube, viz., the increase of the conductance of the solution in the tube, as compared with the voltages distributed to the flowing anolyte and catholyte. The tube was laboratory-made, its I.D. ranges from 4.2 mm to 4.6 mm. Because of this, constant current for different runs is impossible.

However, constant current intensity is possible for both each run and different runs. Hence, in the following experiments, we use the constant current intensity instead of the constant voltage or current used in general electrophoresis, as shown in Eq. (6).

After experiments on MNRBs, the treatment of experimental data, including the statistical analyses, is completed with Microsoft Exel (ver. 97, Microsoft, USA) and Origin (ver. 5.0, Microcal Software, USA) software.

3. Results and discussion

3.1. Sharp boundary and good repeatability

The boundary of a MNRB created with the strong reactive electrolytes of HCl and NaOH in agarose gel is, as exhibited in Fig. 2, very sharp and clear. Fig. 2A displays the overall blue tube containing the 1% (w/v) gel, 0.01 mol/l NaOH, 0.1 mol/l KCl and 0.1% (w/v) bromphenol blue just before the run; Fig. 2B presents the movement (21 mm toward the cathode) of the MNRB 5 min after the run; and Fig. 2C shows the movement (41 mm toward the cathode) of the MNRB 10 min after the run. Fig. 2 certifies that the boundaries are very sharp and clear and that the displacement of the MNRB increases as a linear function of time. Distinctly, the displacement of a MNRB can be directly determined with compasses from the photographs, the method is very convenient.

However, Fig. 3 proves that, if the same MNRB is created in gel-free solution, the boundary is badly unclear, confused and skewed. Fig. 3A exhibits the half-parabola-shape boundary indicated by the fine arrows, definitely the boundary is critically equivocal, confused and twisted as implied by the fine and hard arrows. If we rotate the tube gently, the half-parabola-shape boundary becomes a bullet-shape one as shown in Fig. 3B.

Thus, the comparisons between Figs. 2 and 3 obviously demonstrate the good anti-convection of the agarose gel and the necessity of gel for the formation of a stable, clear and sharp boundary of MNCR under the given experimental conditions.

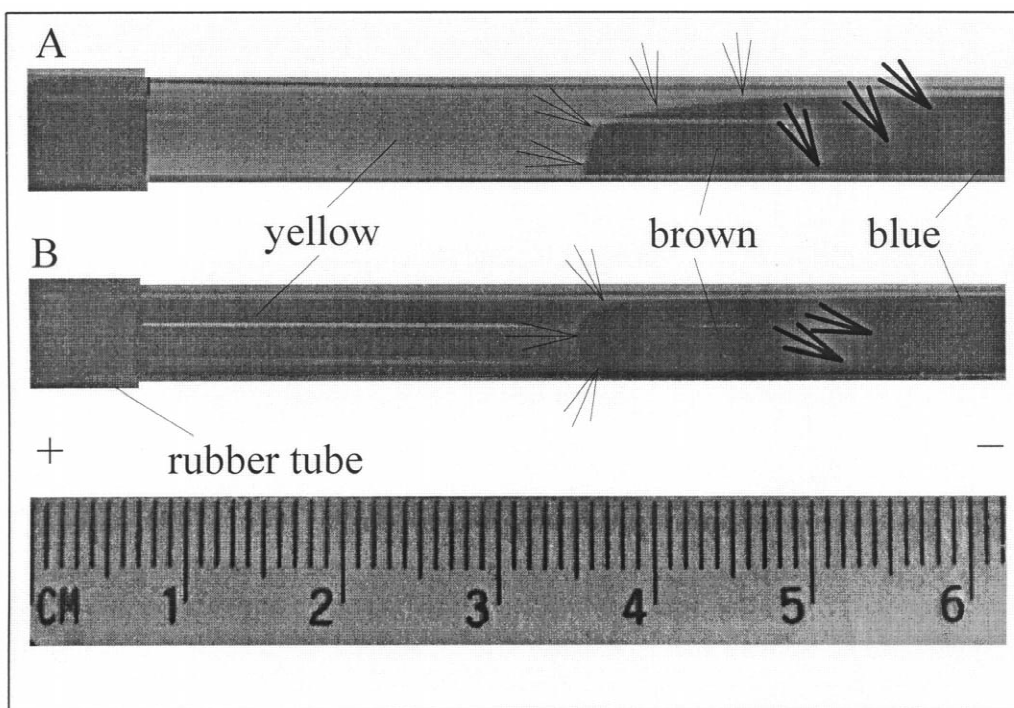In our experiments on MNRBs, we investigated the stability of the method described in Fig. 1. In the

Fig. 3. The badly confused and skew boundary of the MNRB created with HCl and NaOH in gel-free electrolyte solution. (A) The severely skewed boundary. (B) The bullet-shape boundary after the gentle rotation of the tube in A. The fine arrows indicate the clear boundaries formed between the yellow and brown zones. The directions of arrows are perpendicular to the boundary, the hard arrows imply the confused boundaries between the brown and blue solutions, from the fine arrows to the hard arrows the brown color becomes deeper and deeper. The symbols + and − denote the anode and cathode, respectively. Conditions: 0.01 mol/l HCl, 0.01 mol/l NaOH and constant current intensity 0.6 mA/mm^2. Other conditions as in Fig. 2.

investigation, we performed within-day and day-to-day experiments under the same experimental conditions. In both the within-day and the day-to-day experiments, the boundary displacements are determined, then statistical analyses are carried out, all of the results show that the averages of boundary displacement within 20 min are 37.1±1.67 ($n=5$) and 38.8±1.36 ($n=5$) mm for the former and latter, respectively, and the relative standard deviations (RSDs) for the former and latter are 4.5% and 3.5%, respectively, clearly very low values. Evidently, good repeatability can be obtained.

3.2. Boundary velocity – current intensity

In the investigation of the validity of MCRB theory for a MNRB created with the strong reactive electrolytes of HCl and NaOH, we first study the

relationship between the boundary velocity and current intensity. According to Eq. (6), the boundary velocity is proportional to the current intensity. Accordingly, the higher the current intensity is the faster the velocity becomes. The theoretical calculation is in quite good agreement with the experiment of a MNRB created with strong HCl and NaOH.

The experiments in Fig. 4A were performed under the range of current intensity 0.4–1.4 mA/mm^2 and temperature 18–20°C. As shown in Fig. 4A, the excellent coincidence is present between the theoretical and experimental velocities: the correlation coefficient (r) equals 0.9996 ($n=6$). The results exactly demonstrate the validity of the theory for a MNRB formed with strong reactive electrolytes of HCl and NaOH.

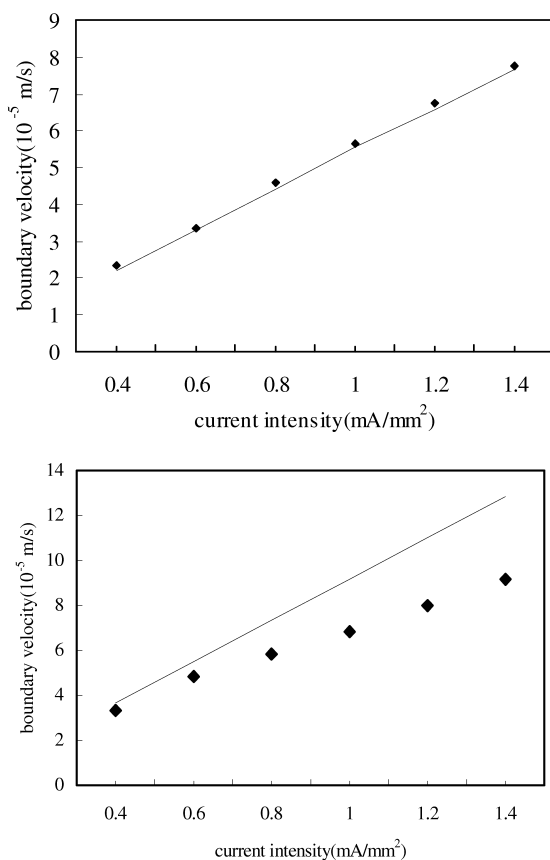The runs in Fig. 4B were carried out under the same range of current intensity but under obvious

Fig. 4. The boundary velocity at different current intensity. (A) Run under temperature 18–20°C. Conditions: 0.01 mol/l HCl and 0.01 mol/l NaOH, 4.2–4.6 mm I.D.×5.8–6.2 mm O.D. Other conditions as in Fig. 2. (B) Run under temperature 7–9°C. Conditions: 0.014 mol/l HCl, 0.01 mol/l NaOH, 1% (w/v) agarose gel, 0.1 mol/l KCl, 0.1% (w/v) bromphenol blue, flow-rate 0.6 ml/min, 90 mm×4.2–4.6 mm I.D.×5.8–6.2 mm O.D.

low temperature 7–9°C which is much lower than the standard temperature of 25°C. Comparing the results of Fig. 4A with those of Fig. 4B, clearly the results of Fig. 4B are poorer ($r = 0.9983$, $n = 6$) and the differences between the theoretical and observed data are apparent, but Fig. 4B, at least semi-quantitatively, demonstrates the agreement between the observed and the theoretical results.

The temperature plays strong influence on the movement of the MNRB created with the strong reactive electrolytes of HCl and NaOH. When the runs are performed at 18–20°C which is near to but lower than the standard temperature 25°C, all of the

observed values are sightly higher than the theoretical values as shown in Fig. 4A. Whereas if at 7–9°C, which is much lower than 25°C, all of the observed values are much lower than the theoretical values (see the details in Fig. 4B). Evidently, the strong influence of temperature is present in the runs. This will be further proved by Figs. 5 and 6.

For this reason, in our formal runs, we picked out those experiments performed at 18–20°C, which is lower than, but near to 25°C, and then operated the statistical treatment on them as will shown in Fig. 8.

3.3. Boundary velocity – [HCl] and [NaOH]

According to Eqs. (2) and (6), if one increases the concentrations of hydrogen and hydroxyl ions simultaneously, the boundary velocity is almost constant. This prediction is demonstrated by Fig. 5. In the runs in Fig. 5, as the concentrations of HCl and NaOH increase greatly from 0.004 to 0.014 mol/l, the velocity of the MNRB slightly decreases from 3.41 to $3.33 \cdot 10^{-5}$ m/s. Those changes fit the theoretical computation.

Therefore, the experiments in Fig. 5 distinctly manifest the validity of the theory. Fig. 5 also verifies that the observed values from five out of six runs are slightly over the theoretical data. This reveals the same temperature influence on the movement of MNRB as has been shown in Fig. 4A and will be discussed in Section 3.4 (see Fig. 6B).
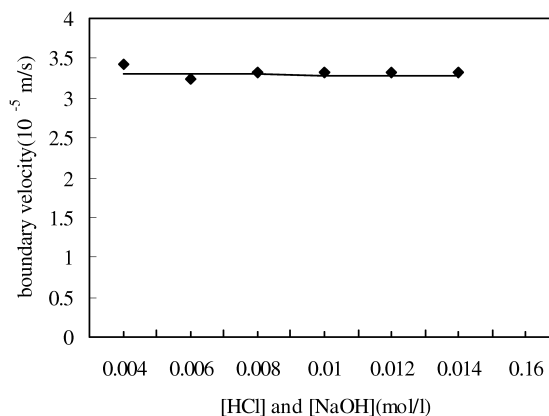

Fig. 5. The boundary velocity at different concentrations of HCl and NaOH ([HCl] = [NaOH]). Conditions: current intensity 0.6 mA/mm². Other conditions as in Fig. 4A.
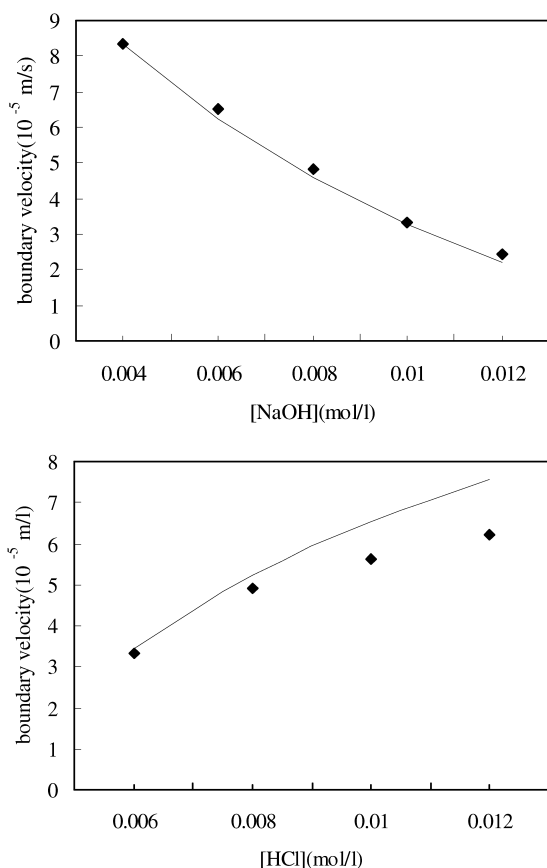
Fig. 6. The boundary velocity at different concentrations of NaOH/or HCl. (A) Run at different concentrations of NaOH and under the temperature 18–20°C. Conditions: 0.01 mol/l HCl and constant current intensity 0.6 mA/mm^2. Other conditions as in Fig. 4A. (B) Run at different concentrations of HCl and under the temperature 7–9°C. Conditions: 0.006 mol/l NaOH and constant current intensity 0.6 mA/mm^2. Other conditions as in Fig. 4B.

3.4. Boundary velocity – [HCl] or [NaOH]

The relations between the velocity and concentration of HCl or NaOH are taken into account and further compared with the theoretical predictions. Eqs. (2) and (6) reveal if the concentration of HCl is fixed at constant value, the increase of NaOH concentration leads to the decrease of the boundary velocity. According to this prediction, we performed the experiments of MNRBs, in which [HCl] is fixed at 0.01 mol/l, but the concentration of NaOH increases from 0.004 to 0.014 mol/l. All the results are given in Fig. 6A. It is shown that at 18–20°C, the

experimental results almost fit the theoretical prediction completely (the r value between theoretical and observed velocities equals 0.9988, $n=5$), this shows the conformity between the theoretical and experimental results. One can also observe the temperature influence that has been mentioned in Figs. 4A and 5.

In the experiments performed at 7–9°C shown in Fig. 6B, the concentration of NaOH is constant at 0.006 mol/l, while that of HCl is raised from 0.06 to 0.12 mol/l. This figure reveals that quite good agreements are present between the observed and calculated values. However, the agreements in Fig. 6B are not very good compared with those in Fig. 6A due to the low experimental temperature (7–9°C). The r value in Fig. 6B is equal to 0.9912, which is poor as compared with that in Fig. 6A.

Fig. 6B also obviously demonstrates the same temperature influence shown in Fig. 4B.

All of those comparisons between Fig. 4A and B and between Fig. 6A and B show the strong influence of temperature on the movement of the MNRB created with the strong reactive electrolytes of HCl and NaOH.

3.5. Boundary velocity – agarose gel

Additionally, we investigated the boundary velocity at different concentrations of agarose gel. Fig. 7 proves, the boundary velocity changes, to some extent, with the concentration of agarose gel. At low
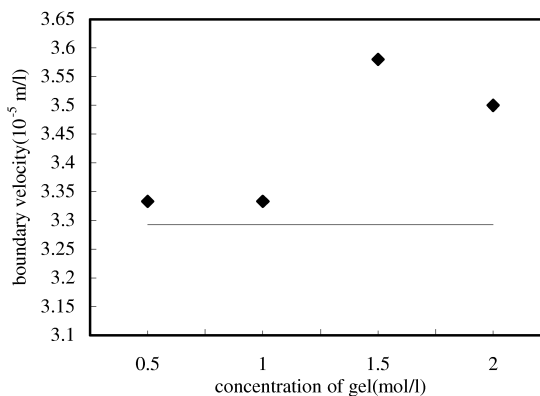

Fig. 7. The boundary velocity at different concentrations of agarose gel. Conditions: 0.01 mol/l HCl, 0.01 mol/l NaOH and current intensity 0.6 mA/mm^2. Other conditions as in Fig. 4A.

concentrations of 0.5–1.0% (w/v) agarose gel, the observed velocities are very close to the theoretical boundary velocity. Whereas, at high concentrations (1.5–2.0%), the agreement is poor (the relative difference between the theoretical and observed velocities is, as computed by the authors, near to 10%) as compared with that with 0.5–1% (w/v) gel used as anti-convection medium. The results imply the influence of agarose gel on the MNRB. Thus, in our most of runs, 1% gel is chosen as the medium of anti-convection.

Even if there is the influence on the boundary velocity, the coincidence between the theoretical and experimental results is still quite good.

3.6. Statistical analyses of experimental data

In total, 38 experiments of MNRBs created with HCl and NaOH were completed. Among those runs, 20 runs were carried out at 7–9°C and the remaining 18 runs were performed at 18–20°C.

The statistical analyses of the 18 runs show that (1) the correlation coefficient between the observed and theoretical velocities is equal to 0.9988 ($n = 18$) and (2) the regression equation is $y = 0.9889x - 0.0364$ ($y =$ the theoretical velocity of the MNRB and $x =$ the observed velocity). Those, as a whole, imply good agreement between the experiments of MNRB and the predications with the MCRBEs.

Thus, those statistical results further manifest the validity of the MCRB theory for a MNRB created with the strong reactive electrolytes HCl and NaOH.

4. Conclusions

The MCRB theory possesses strong validity for the MNRB created with the strong reactive electrolytes of acid and alkali, e.g., HCl and NaOH. From our above experiments in Figs. 4A, 5, 6A and 7, it is clear that, if the conditions are chosen appropriately (such as 18–20°C temperature, 1% agarose gel), the observed velocities are in high coincidence with the theoretical computations. Fig. 8, including the statistic analyses in Section 3.6, closely manifests the outstanding conformity which is, as a whole, present between the observed and theoretical velocities. However, poor agreements are
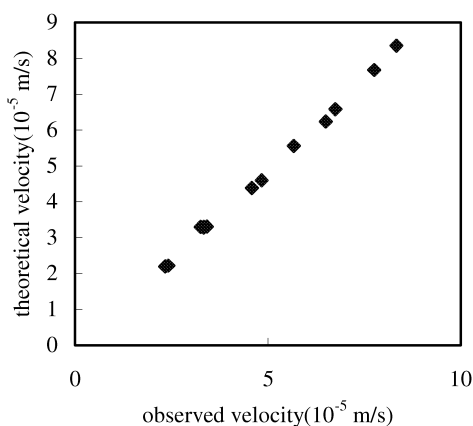
Fig. 8. The linear regression analyses between the theoretical and observed velocities of MNRB created with strong acid of HCl and alkali of NaOH ($n = 18$). All of the runs are performed at 18–20°C.

observed as given in Figs. 4B and 6B as compared those in Figs. 4A and 6A. Even so, the results of Figs. 4B and 6B can at least show the validity of the theory semi-quantitatively. The poor agreements are, as will be discussed, caused by the low temperature of 7–9°C.

The MCRB theory is also of valid for IEF, including electrofocusing [19]. In our early publications, it was theoretically demonstrated that IEF is based upon the MNRB, the stationary neutralization reaction boundary (SNRB) to be exact [12]. The conclusion has been proved by the analyses of experimental data that prove the existence of (or quasi-) equal transference numbers of hydrogen and hydroxyl ions in the classic IEF [12,20,21]. The paper displays that the theory is valid for a MNRB created with strong acid and alkali, coupled with the SNRB, on which IEF and electrofocusing is based. Thus, the results in this paper also verified the validity of the MCRB theory to IEF, including electrofocusing, indirectly and experimentally.

The method described in this paper is stable, efficient and convenient, but inexpensive and uncomplicated, and is very useful for the study on MNRBs and electrofocusing. Fig. 2 shows the boundaries created with HCl and NaOH are clear and sharp and we can conveniently determine the length of displacement from the photograph. Fig. 8 shows that the high agreements exist between the observed

and theoretical data if appreciate experimental conditions are chosen, this implies the efficiency of the method.

The temperature has a strong influence on the movement of the MNRB created with HCl and NaOH. The influence is achieved through two ways. The first is that temperature has a strong influence on the equivalent conductance (Λ) of electrolyte as clearly shown in Fig. 9, which means the linear increase of conductance of HCl solution as a function of temperature.

The second is that temperature greatly affects ionic mobility. Owing to the co-existence of the quite constant of ionic transference number (see Fig. 12 in Ref. [41]) and the equation $m = T\Lambda/F$ (where $m =$ ionic actual mobility, $T =$ ionic transference number, $\lambda =$ equivalent of solution, and $F =$ Faraday's constant), thus from the fact that the equivalent conductance of electrolyte is proportional to the temperature as given in Fig. 9, one can conclude the temperature has a strong influence on ionic mobility.

However, in the theoretical computation from Figs. 4–7, the important parameters, such as the mobilities of hydrogen and hydroxyl ions, can only be obtained at the standard temperature of 25°C. Up to n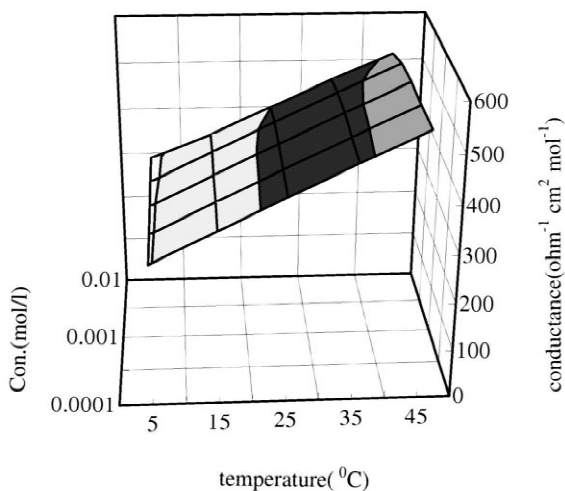ow, we cannot get those ionic mobilities at different temperatures. Therefore, the great differences between Fig. 4A and B and between Fig. 6A and B can be well understood now.

Thus, it is imperative to develop a method of obtaining ionic mobilities at different temperatures and different ionic strengths, in order to compute the movement of the MCRB more exactly. The study on this subject has also significance on zone electrophoresis and ionic mobility itself [34–38].

Acknowledgements

Fig. 9. The equivalent conductances (Ω^{-1} cm^2 mol^{-1}) of HCl at different temperature and concentration (data cited from Ref. [40]).

References

[1] H. Svensson, Acta Chem. Scand. 15 (1961) 325.

[2] H. Svensson, Acta Chem. Scand. 16 (1962) 456.

[3] O. Vesterberg, Acta Chem. Scand. 23 (1969) 2653.

[4] P.G. Righetti, J.W. Drysdale, J. Chromatogr. 98 (1974) 271.

[5] P.G. Righetti, E. Gianazza, K. Ek, J. Chromatogr. 184 (1980) 415.

[6] P.G. Righetti, in: T.S. Work, R.H. Burden (Eds.), 1st ed., Isoelectric Focusing – Theory, Methodology and Applications, Vol. II, Elsevier Biomedical Press, Amsterdam, New York, Oxford, 1983, pp. 153, 299.

[7] L.E.M. Miles, J.E. Simmons, A. Chrambach, Anal. Biochem. 49 (1972) 109.

[8] G. Baumann, A. Chrambach, in: P.G. Righetti (Ed.), Mechanisms of pH Gradient Instability in Isoelectric Focusing, 1st ed., North-Holland, Amsterdam, New York, Oxford, 1975, p. 13.

[9] H. Rilbe, in: P.G. Righetti (Ed.), Stable pH Gradients – A Key Problem in Isoelectric Focusing, 1st ed., Walter de Gruyter, Berlin, New York, 1977, p. 35.

[10] A. Murel, I. Kirjanen, O. Kirret, J. Chromatogr. 174 (1979) 1.

[11] W. Thormann, R.A. Mosher, M. Bier, J. Chromatogr. 351 (1986) 17.

[12] C.-X. Cao, J. Chromatogr. A 813 (1998) 153.

[13] C.-X. Cao, Acta Phys.-Chim. Sin. 13 (1997) 827.

[14] C.-X. Cao, Acta Phys.-Chim. Sin. 13 (1997) 843.

[15] C.-X. Cao, Acta Chem. Scand. 52 (1998) 709.
[16] C.-X. Cao, J.-H. Zhu, H. Liu, W.-H. Fang, W.-Z. Tang, L.-H. Song, W.-K. Chen, Acta Chem. Scand. 53 (1999) 955.
[17] J. Deman, W. Rigole, J. Phys. Chem. 74 (1970) 1122.
[18] J. Deman, Anal. Chem. 42 (1970) 321.
[19] J. Pospichol, M. Deml, P. Bocek, J. Chromatogr. 638 (1993) 179.
[20] C.-X. Cao, J. Chromatogr. A 813 (1998) 172.
[21] C.-X. Cao, Prog. Nat. Sci. 9 (1999) 602.
[22] C.-X. Cao, W.-K. Chen, Chem. J. Chin. Univ. 20 (Suppl.) (1999) 296.
[23] L.G. Longsworth, J. Am. Chem. Soc. 67 (1945) 1109.
[24] V.P. Dole, J. Am. Chem. Soc. 67 (1945) 1119.
[25] H. Svensson, Acta Chem. Scand. 2 (1948) 841.
[26] R.A. Alberty, J. Am. Chem. Soc. 72 (1950) 2361.
[27] J.C. Nichol, J. Am. Chem. Soc. 72 (1950) 2367.
[28] C.-X. Cao, W.-K. Chen, Acta Chem. Scand. 52 (1998) 714.
[29] W.-K. Chen, R.-Z. Li, C.-X. Cao, Acta Phys.-Chim. Sin. 14 (1998) 852.
[30] W.-K. Chen, J.-M. Bai, R.-Z. Li, C.-X. Cao, Acta Phys.-Chim. Sin. 14 (1998) 913.
[31] B.L. Williams, K. Wilson, in: A Biologist's Guide to Principles and Techniques of Practical Biochemistry, 2nd ed., Edward Arnold, London, 1981, p. 106.
[32] B.D. Hames, D. Rickwood, in: Gel Electrophoresis of Proteins. A Practical Approach, IRL Press Ltd., 1981, p. 112.
[33] D.N. Heiger, High Performance Capillary Electrophoresis – An Introduction, Hewlett-Packard, 1992.
[34] J.C. Reijenga, E. Kenndler, J. Chromatogr. A 659 (1994) 403.
[35] J.C. Reijenga, E. Kenndler, J. Chromatogr. A 659 (1994) 416.
[36] W. Friedl, J.C. Reijenga, E. Kenndler, J. Chromatogr. A 709 (1995) 163.
[37] C.-X. Cao, J. High Resol. Chromatogr. 20 (1997) 701.
[38] C.-X. Cao, J. Chromatogr. A 771 (1997) 374.
[39] W.T. Moore, in: Physical Chemistry, 6th ed., Longman, London, 1976, p. 435.
[40] R.L. David, in: CRC Handbook of Chemistry and Physics, 73rd ed., CRC Press, Boca Raton, FL, 1992–1993, p. D164.
[41] D.A. MacInnes, L.G. Longsworth, Chem. Rev. 11 (1932) 172.