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Moving chemical reaction boundary and isoelectric focusing I. Conditional equations for Svensson–Tiselius' differential equation of solute concentration distribution in idealized isoelectric focusing at steady state

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Abstract

In this paper, the differential equation, which is formulated by Svensson–Tiselius for the concentration distribution of protolytes in idealized isoelectric focusing (IEF) at steady state, is discussed. The general condition equations are formulated for Svensson–Tiselius' differential equation, and from the general conditional equations, the conditional equations for the acidic anolyte and alkaline catholyte used in Svensson's IEF are derived. It is theoretically shown that the condition equations for the anolyte and catholyte are the same as the stationary neutralization reaction boundary equations (SNRBEs) that can be deduced from the moving chemical reaction boundary equations (MCRBEs) in special cases. The conditional equations for the anolyte and catholyte reveal that an idealized IEF, viz., a stable pH gradient in IEF, can be achieved, if the fluxes of protons and hydroxyl ions migrating in opposite directions are equal to each other. According to the conditional equations for the anolyte and catholyte and the fact that the pH gradient in Svensson's IEF is quasi-stable, but not completely stable (due to the drifts and plateau of the pH gradient in IEF), one can infer that the quasi/equal fluxes of hydrogen and hydroxyl ions must be present in Svensson's IEF. As shown in the accompanying paper, this inference coincides with important findings, i.e. the existence of quasi/equal fluxes of hydrogen and hydroxyl ions in stationary electrolysis, on which Svensson's IEF is based. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Isoelectric focusing; Moving chemical reaction boundary; Concentration distribution

1. Introduction

The famous theory of isoelectric focusing (IEF) was advanced by Svensson (now, Rilbe) [1–3] in 1961–1962. The theory is successful and important in the field of IEF. Since, according to this theory, the important invention of carrier ampholyte (CA) was achieved by Vesterberg [4,6] and Carlstrom and Vesterberg [5] in 1963–1969, and with Vesterberg's

CA, the actual utilization of IEF was soon achieved. Due to the combination between IEF and high-performance capillary electrophoresis (as first described by Hjertén in 1967, see p. 215 in Ref. [7]), IEF has gradually become a rapid and automatic method of separating proteins and other substances [7–36].

However, there still exist some instabilities of pH gradients, such as the cathodic and/or anodic drift of

pH gradient [37–56], the plateau phenomenon [37–39,55–57], which cannot be elucidated by Svensson's theory [1,2].

The instabilities of pH gradients in IEF may be caused by “outer” and “inner” factors. Those caused by electroosmotic flow (EOF) [39,55,56] and diffusion of Ampholine out of and/or electrolytes into the gel [39] are obviously “outer” instabilities of pH gradients. But what are the “inner” factors which cause the “inner” instabilities of pH gradients in Svensson's IEF?

In order to explain the “inner” instability of pH gradients, several hypotheses were proposed, and a few of these are listed here: (1) isotachophoretic migration of isoelectric carrier ampholytes advanced by Chrambach's group [37–45,47–56]; (2) CO₂ adsorption at the cathodic gel end [39,50,55,56]; (3) formation of a zone of pure water in the neutral region of the pH gradient [39,56]; (4) isoelectric focusing of water (considered to be an ampholyte), which results in its accumulation in the neutral region and back-flow towards the periphery of the gel [39,55,56]; (5) selective deficiency of carrier ampholyte [38,39]; (6) progressive gain or loss of charged ligand by Ampholine [39]; (7) interactions between the carrier ampholytes themselves and the presence of poor amphoteric species [39,55,56,58–60], (8) non-balance between mobilities and concentrations of protons and hydroxyl ions migrating in opposite directions, proposed by Murel et al. [61].

These hypotheses may explain some phenomena of “inner” instabilities of pH gradients in Svensson's IEF. It is difficult to use them to explain the dynamic mechanism of Hjertén's mobilization of pH gradient [10–23] (will be discussed below) and the movement of ampholyte dyes in electrically controlled electrofocusing observed by Pospichal et al. [32,34] (also discussed below).

In the 1980s, Hjertén showed the “inherent” natural drift of pH gradient in Svensson's IEF [12,16]. By using electroneutrality condition $\sum c_{H^+} + \sum c_{NH_3^+} = \sum c_{OH^-} + \sum c_{COO^-}$ (where c_{H^+} , $c_{NH_3^+}$, c_{OH^-} and c_{COO^-} are the concentrations in equivalents per litre of protons, hydroxyl ions and positive and negative groups in the carrier ampholytes, respectively), Hjertén revealed that the ampholytes located in the region of the tube where the pH is above 7

must carry positive charge(s) and will therefore move toward the cathode by the force of electric field, and analogously that the ampholytes positioned in the region of the tube where the pH is below 7 must carry negative charge(s) and drift toward the cathode under the electric field. This process of ampholytes moving toward anode and cathode causes the plateau phenomena of pH gradient and the cathodic and/or anodic drift of pH gradient in Svensson's IEF. When a sufficiently large cathodic flow phenomenon (for instance by EOF) is superimposed upon the plateau only a “cathodic drift” of pH gradient is observed.

Also by using electroneutrality condition, Hjertén firstly invented Hjertén's mobilization of pH gradient in IEF [10–16]. Now Hjertén's mobilization has gradually been used for the detection of *pI* of protein [10–23]. Hjertén's mobilization, which is caused by adding salt like NaCl into the anolyte of acid or catholyte of base after the steady-state of IEF, may, *controllably*, move toward the anode or cathode [12–23]. For this reason, Hjertén's mobilization is, in nature, different from the EOF mobilization invented by Thormann et al. [18,19,24–26] and hydrodynamic mobilization of pH gradient [10,18,19,27–30], and for this reason, Hjertén's mobilization caused by the addition of a salt like NaCl is also different from the “inner” stability of pH gradient that yields naturally in Svensson's IEF.

In 1989–1994, Pospichal et al. [32–34] invented the apparatus of electrically controlled electrofocusing used for the study of IEF. With the apparatus, Pospichal et al. [33] can control the fluxes (or transference numbers) of hydrogen ion in the anolyte and hydroxyl ion in the catholyte, and observe the movement of ampholyte dyes focused in the neutralization boundary. In their experiments, they find that if the flux of hydrogen ion in the anolyte is over that of hydroxyl ion in the catholyte, the ampholyte dyes move toward the cathode, conversely, they observe the dyes migrate the anode, and if the fluxes of hydrogen and hydroxyl ions are equal to each other, they discover the dyes electrofocused in the neutralization boundary is stationary.

More and more clues show that there is always an electromigration reaction between the hydrogen and hydroxyl ions in stationary electrolysis and in Svensson's IEF (also see Sections 8 and 9), since in

Svensson's IEF, the anolyte is always a pure acid (namely, hydrogen ion exists) and the catholyte is a pure base (namely, hydroxyl ion is present). Clearly, under the electric field, the hydrogen ion in the anolyte and the hydroxyl ion in the catholyte must migrate in opposite directions and react with each other when they meet. This kind of electromigration reaction has been recognized by Murel et al. [61] and Hagedorn and Fuhr [57] and has been, successfully, proved by the experiments of "precipitate reaction front", carried out by Deman and Rigole [62,63], by those of electrically controlled electrofocusing by Pospichal et al. [32–34] (see above and Section 8.2), and by those of moving precipitate boundary by us [64] (see also Section 8.1). The electromigration reaction has been used to elucidate the mechanisms of the cathodic drift of pH gradient [61] and of the plateau phenomena of pH gradient (see Figs. 6 and 7 in Ref. [57]), which occur in Svensson's IEF.

However, the electromigration reaction between proton and hydroxyl ion, usually, is not present in a moving boundary system (MBS) or in isotachopheresis (ITP), since, in an ITP, the pH values of both leading and terminating electrolytes are usually less than or greater than pH 7 simultaneously. Obviously, with such an electrolyte system of ITP, there is not an electromigration reaction between hydrogen and hydroxyl ions in an ITP.

Based upon the clues mentioned above and the ideas of the "precipitate reaction front" of Deman and Rigole [62,63], of the electromigration reaction between hydrogen and hydroxyl ions migrating in opposite directions of Hagedorn and Fuhr [57] and of the stationary neutralization reaction boundary (SNRB) of Pospichal et al. [32–34], the author developed the concepts of moving and stationary chemical reaction boundaries (MCRB, SCRb), formed by strong or weak reaction electrolytes [65–67], such as $\text{H}_2\text{SO}_4\text{--NaOH}$ and $\text{CH}_3\text{COOH--CH}_3\text{CH}_2\text{NH}_2$, and deduced a series of moving or stationary chemical reaction boundary equations (MCRBEs, SCRBEs; see Eqs. (69)–(80)). The theoretical results [65–67] show that (1) the MCRBEs are very similar to, but in nature different from, the moving boundary equations (MBEs) formulated about 50 years ago [68–72], (2) the MCRB theory is the counterpart of the theory of moving boundary

system (MBS) developed by Longworth [68], Dole [69], Svensson [70], Alberty [71] and Nichol [72].

Mainly according to the experimental method developed by Deman and Rigole [62,63], we performed some experiments of MCRB formed by the strong reaction electrolytes, CoCl_2 and NaOH , together with 0.1 M background electrolyte, KCl . The predictions with the MCRBEs (see Eqs. (69)–(71)) are, quantitatively, in agreement with the experiments of MCRB [64].

It is well known that ITP is based on the mechanism of MBS [68–75]. Because of this, ITP is sometimes called moving boundary electrophoresis. Is Svensson's IEF based upon the mechanism of chemical reaction boundary, exactly, neutralization reaction boundary formed by hydrogen and hydroxyl ions, just like ITP being based on the mechanism of MBS [68–75]. Those studies by Murel et al. [61], Deman and Rigole [62,63], Hagedorn and Fuhr [57], Pospichal et al. [32–34] and us [64–67] have, in a preliminary manner, shown the idea of IEF being based on a neutralization reaction boundary. Thus, in the series of papers entitled "Moving chemical reaction boundary and isoelectric focusing:...", we will show the relationships between MCRB and IEF and explain the mechanisms of the instability of pH gradients in IEF and of Hjertén's mobilization of pH gradients after IEF in a step-by-step manner.

In this paper, the main purposes are to derive the condition equations (including the general condition equations (see Eqs. (38), (39), (44)–(49)) and the condition equations for the acidic anolyte and alkaline catholyte (see Eqs. (61)–(68)) for Svensson–Tiselius' differential equation (see Eq. (3)) that describes an idealized IEF at steady state, to show the relationships between the condition equations and SNRBEs, and to give the tests briefly.

Before the launch of the formulation of the condition equations, it ought to be emphasized that Svensson's pH gradient, based upon stationary electrolysis, is very different from Chrambach's pH gradient, which is based on the mechanism of MBS, viz., ITP [40–45,49–54], from Rilbe's pH gradient of the steady state rheoelectrolysis [76–78], from Troitsky's pH gradient formed by the concentration gradient of a non-electrolyte [79,80], from Shukun's

pH gradient formed with borate–mannitol in a free-flow electrophoretic apparatus [81–83] and from the thermal pH gradient [84–87]. The IEF discussed here is Svensson's IEF, but not those based on "non-stationary electrolysis" [40–45,49–54,76–87].

2. Notations

C ,	the molar concentration (mol/l).
c ,	the equivalent concentration (see Eqs. (17) and (20b), equiv./l). The bar of "–" over the c means the constituent concentration [49,71,72,74,75,88]. The sign is positive if the ion carries net positive charge(s) and negative if it has net negative charge(s), as defined by Longworth [68] and others [69–72].
m ,	the electrophoretic mobility of ion ($\text{m}^2 \text{V}^{-1} \text{s}^{-1}$). The bar of "–" over the m indicates the mobility of the constituent [49,71,72,74,75]. The sign is positive if the ion carries net positive charge(s) and negative if it has net negative charge(s), as has been defined by Longworth [68] and others [69–72].
I ,	the electric current (A).
q ,	the cross-sectional area of electrolytic medium (m^2).
κ ,	the specific conductivity (S/m).
E ,	the electric field strength ($=I/q\kappa$ (1))
D ,	the diffusion coefficient of the component corresponding to the ion constituent.
x ,	coordinate along the direction of current.
F ,	Faraday's constant ($=96\,500$ coulombs per equivalent).
z ,	the valence of an ion constituent.
T ,	the transference number of an ion constituent $=FCzm/\kappa$ (2)
$(Tib)/(FDzq)$,	the integration constant (see Eq. (4)).
v ,	the displacement of boundary, $\alpha\beta$,

V ,	in $\text{m}^3/\text{coulomb}$. The superscript, $\alpha\beta$, indicates the boundary $\alpha\beta$.
J ,	the displacement of boundary $\alpha\beta$ in $\text{m}^3/\text{Faraday}$. The superscript, $\alpha\beta$, indicates the boundary $\alpha\beta$.
J ,	the mass flux of ion or ion constituent (in equiv./s m^2).
pH :	the pH value, the superscripts, α (or acid) and β (or base), indicate the acidic anolyte and the alkaline catholyte, respectively.
κ_w :	the product of water.

For the significances of the superscripts and subscripts before or after a variant, such as J and C , see the following examples

$${}_{i}^{i-1}J_{i,\text{H}^+}; {}_{i}^{i-1}C_{i,(1-k)}$$

the superscript $i-1$ and the subscript i before the variants J or C mean boundary $i-1-i$; the subscript, i , after the variants J or C indicates medium i , or carrier ampholyte $i(\text{CA}_i)$; the subscript, H^+ , after the J implies the hydrogen ion, and the subscript, $(1-k)/$ or $(k-1)$, implies the number of charge(s) carried by the CA, which diffuses from its own medium into the adjacent medium ($k=0, 1-k=+$; $k=1, 1-k=0$ and $k=2, 1-k=-$ (see Fig. 1 and Eqs. (16)–(18)).

3. Short reviews of Svensson's theory of IEF

To formulate the conditional equations for Svensson–Tiselius' differential equation [1,88], which describes an idealized IEF, it is necessary to review Svensson's theory briefly.

3.1. Svensson–Tiselius' differential equations and its analytical solutions

Svensson–Tiselius' differential equation, which depicts the concentration course of an electrolytic component, regardless of whether it is an ampholyte or a non-ampholyte or whether it is an end or an intermediate component, is

$$\frac{CmI}{q\kappa} = D \frac{dC}{dx} \quad (3)$$

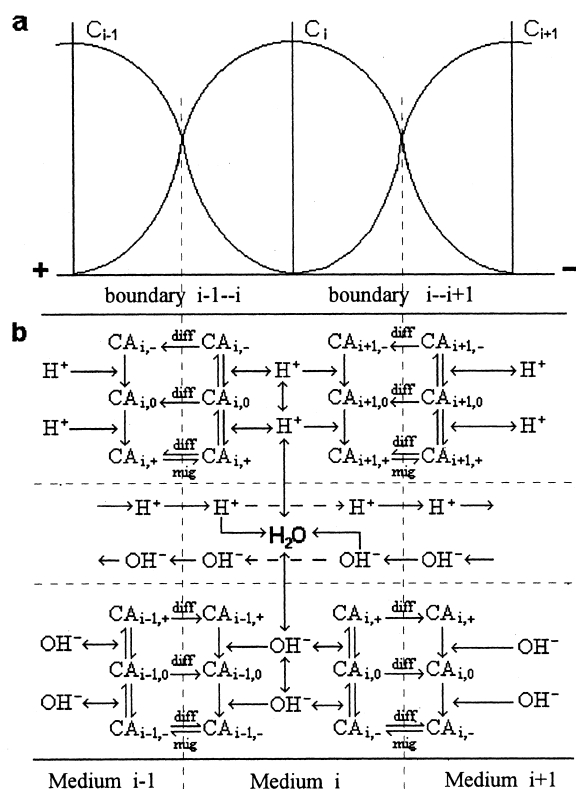


Fig. 1. The distribution, diffusion and electromigration of CA_{i-1} , CA_i and CA_{i+1} focused at their pI values in an idealized IEF at steady state. (a) The Gaussian distributions of CA_{i-1} , CA_i and CA_{i+1} , and (b) shows (1) the diffusion and electromigration of CA_{i-1} , CA_i and CA_{i+1} , (2) the electromigration of free hydrogen and hydroxyl ions, (3) the formation of water and (4) the balance between different subspecies of a CA. "Diff" and "mig" indicate the diffusion and electromigration of CA, respectively, and the symbols "+" and "-" in (a) indicate the anode and cathode, respectively. The subscript "+" and "-" in (b) indicate a proton/or hydroxyl ion carried by CA, and "0" is the zwitterionic and undissociated CA. For other symbols, see text.

As stated by Svensson [1], each term in Eq. (3) expresses the mass flow per second and the cross-sectional area, with that on the left being the electric- and that on the right being the diffusional mass flow. The differential equation describes the stationary state of an electrolytic component, i.e. the electric mass flow of an ion constituent is equal to the diffusional mass flow. Like most differential equations in physics and chemistry, Svensson–Tiselius' differential equation is rather useless without solutions concerning specific experimental conditions.

If the cross-section of medium, the transference number of an ion constituent and the diffusional coefficient are all constant, then the solution of the differential equation for a pure end component, viz., acid or base, is given by

$$C = \frac{TI(x-b)}{FDzq} \quad (4)$$

Of course, the validity of the equation is restricted to the space between the electrodes, viz., the interval between $x=0$ and $x=a$) and to positive concentration, as has been pointed out by Svensson [1]. The coordinate b may assume any positive and negative values. Negative b may be obtained for Tz values and means that the concentration is finite and steadily increasing from the cathode to the anode. Positive b values smaller than a indicate that the concentration drops to zero somewhere in the apparatus, and Eq. (4) is then restricted to the interval between b and that electrode where the component exists. For the interval between b and the other electrode, one has

$$C = 0 \quad (5)$$

which also satisfies Svensson–Tiselius' differential equation.

Clearly, Eq. (4) describes the linear concentration distribution of anolyte or catholyte along an electrophoretic apparatus and indicates that the concentration is not uniform. Of course, the anolyte and catholyte are uniform, due to the convection caused by joule's heat in IEF. Thus, in this paper, the conductance and concentration of the anolyte (viz., acid) and the catholyte (viz., base) are assumed to be constant and uniform (see assumption 3 in Section 5). This treatment is for the convenience of the following derivations.

If the diffusional coefficient, conductance and the derivative

$$p = -\frac{dm}{dx} = -\frac{dm}{d(pH)} \frac{d(pH)}{dx} \quad (6)$$

are all regarded as constant, then the analytical solution of Eq. (3) for an intermediate component, viz., carrier ampholyte (CA), can be expressed as

$$C = C_0 \exp\left(\frac{I}{q\kappa} \frac{px^2}{2D}\right) = C_0 \exp\left(E \frac{px^2}{2D}\right) \quad (7)$$

The solution of Eq. (7) describes the stationary state, i.e. a Gaussian concentration distribution (see Fig. 1a) of an intermediate component, viz., CA, focused in an idealized IEF at steady state, with the inflection point at

$$x_i = \left[\frac{D}{p} \frac{q\kappa}{I} \right]^{1/2} = \left[\frac{D}{p} \frac{1}{E} \right]^{1/2} \quad (8)$$

If the conductance of medium is not constant but is a linear function of x , it is still possible to integrate Svensson–Tiselius' differential equation analytically. For the linear function

$$\kappa = \kappa_0 + rx \quad (9)$$

(see Eq. (7) and Fig. 1a) the analytic solution is

$$C = C_0 \exp \left[\frac{pI\kappa_0}{Dqr^2} \ln \left(1 + \frac{rx}{\kappa_0} \right) - \frac{pIx}{Dqr} \right] \quad (10)$$

and the solution indicates a skew concentration distribution.

The consequence of the diffusion–electromigration theory is that a CA necessarily extends to or beyond the concentration maximum of the adjacent one. Every CA is therefore more or less contaminated at the center of its zone. This concentration distribution of CA has been described using computer simulations by Almgren [89], Murel et al. [61], Bier and co-workers [47,73,90–94], Shimao [74,75] and Šlais [95]. Of course, the distribution of CA in some IEF systems is not Gaussian, but a skew concentration distribution and some wide concentration distribution (see references [96–98] and Fig. 1a in Ref. [95]).

Even if such contamination and skew or a wide concentration distribution exist, a boundary is assumed to be present between two adjacent CAs focused in an idealized IEF at steady state (see Figs. 1 and 2). The treatment that has been used in the studies on MBS [49,50,68–72,74,75], ITP [49,50,75]

and IEF [33,49,50,57,61,73,74,89–95] is for the sake of convenience.

3.2. Ionization of ampholyte in its isoelectric state

As stated by Svensson [2], for the great majority of low-molecular-mass ampholytes, only two pK values exert a measurable influence on the ionization about the pI value. Thus, one has a simple relationship between pI and the two pK values

$$pI = \frac{1}{2}(pK_1 + pK_2) \quad (11)$$

if the cation and anion concentrations of ampholyte, i , are denoted by $C_{i,+}$ and $C_{i,-}$, respectively, and the added concentrations of zwitterionic and undissociated ampholyte is denoted by $C_{i,0}$, then the total concentration, C_i , is given as

$$C_i = C_{i,+} + C_{i,-} + C_{i,0} \quad (12)$$

and the degree of ionization is defined as

$$a_i = (C_{i,+} + C_{i,-})/C_i \quad (13)$$

If the ampholyte, i , is focused at its pI , one gets the degree of ionization for the isoelectric state

$$a_i = 2/(2 + 10^{pI - pK_1}) \quad (14)$$

With Eq. (14), we can calculate the ionization degrees of the ampholytes in Table 1.

It can be seen from Table 1 that, in the isoelectric state, the degree of ionization of a good ampholyte is, in general, over 5% and, in some cases, over 20%. Therefore, when treating the diffusion of CA in an isoelectric state, as depicted by the Svensson–Tiselius' differential equation, we ought to consider not only the zwitterionic and undissociated forms, but also the dissociated forms, viz., positive and negative forms, as shown in Fig. 1b.

+	anolyte acid	CA _{min} pI _{min}	CA _{m+1} pI _{m+1}	CA _{m+2} pI _{m+2}	...	CA _i pI _i	...	CA _{m-2} pI _{m-2}	CA _{m-1} pI _{m-1}	CA _{max} pI _{max}	catholyte base	-
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Fig. 2. Arrangement of CAs, the anolyte and catholyte in an idealized IEF at steady state. The symbols “+” and “-” indicate the anode and cathode, respectively, the subscripts “min” and “max” imply the CA with minimum and maximum pI values, respectively. For other symbols, see text.

Table 1
Calculated degrees of ionization for different ampholytes with known concentrations^a

Ampholyte	Concentration (<i>M</i>)	$pI - pK_1$	α	Good or poor ampholyte?
<i>o</i> -Aminophenylarsonic acid	0.0092	0.77	0.2532	Good
Lysine	0.358	0.79	0.2448	Good
<i>m</i> -Aminobenzoic acid	0.0546	0.81	0.2367	Good
Aspartic acid	0.563	0.89	0.2049	Good
<i>p</i> -Aminophenylarsonic acid	0.0130	0.92	0.1938	Good
Glutamic acid	0.100	1.03	0.1574	Good
<i>p</i> -Aminobenzoic acid	0.036	1.30	0.0911	Good
Nicotinic acid	0.154	1.37	0.0786	Good
<i>o</i> -Aminobenzoic acid	0.0547	1.47	0.0635	Good
Histidine	0.0886	1.50	0.0595	Good
Arginine	0.100	1.72	0.0367	Poor
Picolinic acid	0.100	2.15	0.0140	Poor

^a Data are cited from Ref. [2].

4. Concentration distributions of CA for high and low electric field strengths

It is obvious from Eqs. (7) and (10) and computer simulations [61,73,74,89,91,95] that there is a thin concentration distribution of CA in IEF, if a high electric field strength is used. Thus, under this condition, only two neighboring CAs contaminate each other.

However, if low electric field strength exists, the concentration distribution of CA in IEF is wide. This will lead to the existence of numerous CAs with different pI values in a boundary, as has been simulated by Murel [61], Almgren [89], Bier et al. [73,91], Shimao [74] and Šlais [95].

Thus, for high electric field, we ought to consider the transference of hydrogen or hydroxyl ion by the two adjacent CAs in IEF (see Fig. 2 and Section 7), however, for low electric field, we should consider the transport of the hydrogen or hydroxyl ion not only by the two adjacent CAs, such as CA_{i-1} and CA_i , but also by other CAs, such as CA_{i-3} , CA_{i-2} , CA_{i+1} and CA_{i+2} (see Fig. 2).

5. Assumptions

For the sake of convenience, the following assumptions are given simply: (1) uniform mobility of an ion or a subspecies; (2) zero mobility for uncharged or zwitterionic ampholyte; (3) uniform and constant conductance of the acidic anolyte and

alkaline catholyte; (4) uniform and constant diffusional coefficient amongst different subspecies; (5) no EOF; (6) no convection caused by Joule's heat in the pH gradient; (7) the achievement of a steady state of IEF; (8) the omission of the formation of water due to the electromigration reaction between hydrogen and hydroxyl ions in IEF (this will be discussed elsewhere); (9) CA has two pK values, viz., pK_1 and pK_2 , or only two adjacent pK s of CA exert a measurable influence on the ionization of CA in an isoelectric state, as used by Svensson [2], Almgren [89], and Bier et al. [73,90,91]. These assumptions have been used by numerous scientists [1,2,68–74,76–78,89–95].

6. Conditional equations

6.1. General conditional equations

From the above discussions, it is evident that Svensson–Tiselius' differential equation describes a complete stationary state of a CA, in which the flow of diffusion of a CA is exactly equal to that of electromigration of the CA, regardless of whether it is a Gaussian or a skew concentration distribution or whether there is a wide or thin distribution of CA [96–98]. For better perception of Eq. (3) and the derivation of the following general conditional equation, Fig. 1 is given.

Fig. 1a shows the Gaussian concentration distributions of CA_{i-1} , CA_i and CA_{i+1} in an idealized

IEF at steady state. For CA_{i-1} and CA_{i+1} , only two halves are shown. Note that CA_i may be the anolyte, or CA_{\min} with pI_{\min} , or CA_{m+1} with pI_{m+1} , or..., or CA_{m-1} with pI_{m-1} , or CA_{\max} with pI_{\max} , or the catholyte (see Fig. 2).

Fig. 1b shows (1) the equilibrium state in which the diffusion and electromigration fluxes of CA, for example CA_i , are equal to each other, (2) the electromigration of hydrogen and hydroxyl ions carried by the charged CAs, (3) the (de)protonizations of (un)charged CAs during their diffusion and electromigration, (4) the electromigration of free hydrogen and hydroxyl ions and (5) the formation of water due to the electromigration reaction between hydrogen and hydroxyl ions (note, this will be discussed elsewhere). The acid anolyte is on the left and the base catholyte is on the right.

Due to assumption 9 in Section 5, we only consider two adjacent pK values, viz., pK_1 and pK_2 , which have a measurable influence on the ionization of CA in an isoelectric state. This treatment is necessary, since, without the treatment, the general condition equations are too complex to be expressed conveniently. This treatment has been used by Svensson [2], Almgren [89], and Bier et al. [73,90,91].

It can be seen from Table 1 that, even in an isoelectric state of CA, equivalently, at the steady-state of CA in IEF, the degree of ionization of CA is over 5% and, in some cases, is over 20%. Thus, at the steady state of IEF, CA_i , focused at its pI , may exist as $CA_{i,-}$, $CA_{i,+}$ and $CA_{i,0}$. Of course, the zwitterionic and undissociated $CA_{i,0}$ is of the highest ratio.

Consider the diffusion–electromigration of CA_i through boundary $i-1-i$ in an idealized IEF at steady state, clearly, using Eq. (3) and assumptions 4 and 9 in Section 5, one has

$$\frac{{}^{i-1}C_i {}^{i-1}m_i I}{q_i^{i-1} \kappa} = D_i \left(\frac{d({}^{i-1}C_{i,-})}{d(x_i)} + \frac{d({}^{i-1}C_{i,0})}{d(x_i)} + \frac{d({}^{i-1}C_{i,+})}{d(x_i)} \right) = D_i \frac{d({}^{i-1}C_i)}{d(x_i)} (x_i < 0) \quad (15)$$

The right side and the middle of Eq. (15) indicate the diffusion flow of CA_i from medium i into medium

$i-1$. Since the pH value of medium $i-1$ is lower than pI_i , thus (see Fig. 1b):

(1) After diffusing into medium $i-1$, the $CA_{i,0}$ (the subscript of “0” indicates that no net charge is carried by zwitterionic or undissociated CA) must be charged by a free proton, and becomes the charged $CA_{i,+}$ (subscript “+” means a hydrogen ion carried by CA_i). The charged $CA_{i,+}$ will electrically migrate into its own medium, where the $CA_{i,+}$ loses the charge, viz., the proton. Because the pH value of medium i is equal to pI_i . Hence, from the diffusion of $CA_{i,0}$ to the electromigration of $CA_{i,+}$, a proton is carried from medium $i-1$ to medium i .

(2) After diffusing into medium $i-1$, the $CA_{i,-}$ (the subscript of “-” indicates a hydroxyl ion carried by CA_i) must be charged by two free protons, and also becomes $CA_{i,+}$, which will move into medium i electrically. Obviously, from the diffusion of $CA_{i,-}$ to the electromigration of $CA_{i,+}$, two hydrogen ions are transferred from medium $i-1$ to medium i .

(3) Diffusing into medium $i-1$, the $CA_{i,+}$ must move into medium i under the electric field strength, since the $CA_{i,+}$ has been charged by a proton. Clearly, from the diffusion of $CA_{i,+}$ to the electromigration of $CA_{i,+}$, no proton or hydroxyl ion is transported.

As discussed above, three protons are carried by the charged $CA_{i,+}$, from medium $i-1$ into medium i during the diffusion of $CA_{i,0}$ to the electromigration of $CA_{i,+}$ and the diffusion of $CA_{i,-}$ to the electromigration of $CA_{i,+}$. Thus, the flux of electromigration of the hydrogen ion carried by $CA_{i,+}$ from medium $i-1$ into medium i in unit time and unit cross-sectional area can be expressed as

$$\begin{aligned} {}^{i-1}J_{i,H^+} &= 2 {}^{i-1}C_{i,-} {}^{i-1}m_{i,-} \frac{I}{q_i^{i-1} \kappa} \\ &+ 1 {}^{i-1}C_{i,0} {}^{i-1}m_{i,0} \frac{I}{q_i^{i-1} \kappa} \\ &+ 0 {}^{i-1}C_{i,+} {}^{i-1}m_{i,+} \frac{I}{q_i^{i-1} \kappa} \\ &= \sum_{k=0}^2 k {}^{i-1}C_{i,(1-k)} {}^{i-1}m_{i,(1-k)} \frac{I}{q_i^{i-1} \kappa} \quad (16) \end{aligned}$$

with the equivalent concentration given as

$${}^{i-1}c_{i,(1-k)} = k {}^{i-1}C_{i,(1-k)} \quad (17)$$

Thus, Eq. (16) can be re-expressed as follows

$${}^{i-1}J_{i,H^+} = \sum_{k=0}^2 {}^{i-1}c_{i,(1-k)} {}^{i-1}m_{i,(1-k)} \frac{I}{q_i^{i-1}\kappa} \quad (18)$$

obviously, $k=0$ means that no proton is carried by $CA_{i,+}$ from the diffusion of $CA_{i,+}$ ($=CA_{i,(1-k)}$, $k=0$) to the electromigration of $CA_{i,+}$ (see point 3 above); $k=1$ indicates a proton is carried by $CA_{i,+}$ from the diffusion of $CA_{i,0}$ ($=CA_{i,(1-k)}$, $k=1$) to the electromigration of $CA_{i,+}$ (see point 1 above), and $k=2$ implies that two protons are transferred from the diffusion of $CA_{i,-}$ ($=CA_{i,(1-k)}$, $k=2$) to the electromigration of $CA_{i,+}$ (see point 2).

Consider the diffusion–electromigration of CA_{i-1} through boundary $i-1-i$ in an idealized IEF at steady state, one may get, like the formation of Eq. (15) with Eq. (3)

$$\begin{aligned} \frac{{}^{i-1}C_{i-1} {}^{i-1}m_{i-1} I}{q_i^{i-1}\kappa} &= D_{i-1} \left(\frac{d({}^{i-1}C_{i-1,+})}{d(x_{i-1})} \right. \\ &\quad + \frac{d({}^{i-1}C_{i-1,0})}{d(x_{i-1})} \\ &\quad \left. + \frac{d({}^{i-1}C_{i-1,-})}{d(x_{i-1})} \right) \\ &= D_{i-1} \frac{d({}^{i-1}C_{i-1})}{d(x_{i-1})} (x_{i-1} > 0) \quad (19) \end{aligned}$$

Clearly, the right side and the middle of Eq. (19) indicate the diffusion of CA_{i-1} into medium i . Because the pH value of medium i is over pI_{i-1} , therefore (see Fig. 1b):

(1) After diffusing into medium i , the $CA_{i-1,0}$ must be charged by an hydroxyl ion, and becomes the charged $CA_{i-1,-}$ (subscript “-” indicates an hydroxyl ion carried by CA_{i-1}), which will return to its own medium, where it loses the hydroxyl ion. Apparently, an hydroxyl ion is carried from the diffusion of $CA_{i-1,0}$ to the electromigration of $CA_{i-1,-}$.

(2) It is obvious from Fig. 1b that two hydroxyl ions are transported from the diffusion of $CA_{i-1,+}$ to the electromigration of $CA_{i-1,-}$.

(3) Fig. 1b shows clearly that no hydrogen or

hydroxyl ion is carried from the diffusion of $CA_{i-1,-}$ to the electromigration of $CA_{i-1,-}$.

Similar to the formation of Eq. (18), the flux of electromigration of hydroxyl ion carried by the $CA_{i-1,-}$ from medium i into $i-1$ may be written as

$$\begin{aligned} {}^{i-1}J_{i-1,OH^-} &= 2 {}^{i-1}C_{i-1,+} {}^{i-1}m_{i-1,+} \frac{I}{q_i^{i-1}\kappa} \\ &\quad + 1 {}^{i-1}C_{i-1,0} {}^{i-1}m_{i-1,0} \frac{I}{q_i^{i-1}\kappa} \\ &\quad + 0 {}^{i-1}C_{i-1,-} {}^{i-1}m_{i-1,-} \frac{I}{q_i^{i-1}\kappa} \\ &= \sum_{k=0}^2 k {}^{i-1}C_{i-1,(k-1)} {}^{i-1}m_{i-1,(k-1)} \frac{I}{q_i^{i-1}\kappa} \\ &= \sum_{k=0}^2 {}^{i-1}c_{i-1,(k-1)} {}^{i-1}m_{i-1,(k-1)} \frac{I}{q_i^{i-1}\kappa} \quad (20a) \end{aligned}$$

$${}^{i-1}c_{i-1,(k-1)} = k {}^{i-1}C_{i-1,(k-1)} \quad (20b)$$

where, $k=0$ indicates no hydroxyl ion is carried by $CA_{i-1,-}$ from the diffusion of $CA_{i-1,-}$ ($=CA_{i-1,(k-1)}$, $k=0$) to the electromigration of $CA_{i-1,-}$ (see point 3 above), $k=1$ implies that an hydroxyl ion is carried by $CA_{i-1,-}$ from the diffusion of $CA_{i-1,0,-}$ ($=CA_{i-1,(k-1)}$, $k=1$) to the electromigration of $CA_{i-1,-}$ (see point 1), and $k=2$ indicates that two hydroxyl ions are transferred from the diffusion of $CA_{i-1,+}$ ($=CA_{i-1,(k-1)}$, $k=2$) to the electromigration of $CA_{i-1,-}$ (see point 2).

As both free hydrogen and hydroxyl ions exist in boundary $i-1-i$, they must move through boundary $i-1-i$ electrically (see Fig. 1b). The fluxes of electromigration of free hydrogen and hydroxyl ions are, respectively,

$${}^{i-1}J_{H^+} = {}^{i-1}c_{H^+} {}^{i-1}m_{H^+} \frac{I}{q_i^{i-1}\kappa} \quad (21)$$

$${}^{i-1}J_{OH^-} = {}^{i-1}c_{OH^-} {}^{i-1}m_{OH^-} \frac{I}{q_i^{i-1}\kappa} \quad (22)$$

Treatment 1: The electromigration of free hydroxyl ion can be regarded as the converse electromigration of free hydrogen ion [99,100], namely, the electromigration of free hydroxyl ion from the cathode to

the anode is equivalent to that of the hydrogen ion constituent with the mobility of an hydroxyl ion, i.e. $-2.05 \cdot 10^{-7} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ at 25°C from the anode to the cathode. Thus, the flux of electromigration of free hydroxyl ion (viz., Eq. (22)), from medium i into medium $i-1$ can be expressed as that of the hydrogen ion constituent with a mobility $-2.05 \cdot 10^{-7} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ from medium $i-1$ into medium i and vice versa.

Treatment 2: Similarly, the flux of electromigration of hydroxyl ion (viz., Eqs. (20a) and (20b)) carried by $\text{CA}_{i-1,-}$ (see Fig. 1b) from medium i into medium $i-1$ may also be expressed as that of the hydrogen ion constituent from medium $i-1$ into medium i . The flux of electromigration of hydrogen ion (viz., Eq. (18)) carried by $\text{CA}_{i,+}$ (see Fig. 1b) from medium $i-1$ into medium i is also equivalent to that of the hydroxyl ion constituent from medium i into $i-1$.

With the aid of the above treatments, it is evident that additivity exists between Eqs. (18), (20a), (21), (22). Thus, one has the total flux of the hydrogen or hydroxyl ions constituents through boundary $i-1-i$ as

$$\begin{aligned} {}^{i-1}J_{\text{all,H}^+} &= \sum_{k=0}^2 {}^{i-1}c_{i-1,(k-1)} {}^{i-1}m_{i-1,(k-1)} \frac{I}{q_i^{i-1} \kappa} \\ &+ \sum_{k=0}^2 {}^{i-1}c_{i,(1-k)} {}^{i-1}m_{i,(1-k)} \frac{I}{q_i^{i-1} \kappa} \\ &+ \frac{{}^{i-1}c_{\text{H}^+} {}^{i-1}m_{\text{H}^+}}{q_i^{i-1} \kappa} \\ &+ \frac{{}^{i-1}c_{\text{OH}^-} {}^{i-1}m_{\text{OH}^-}}{q_i^{i-1} \kappa} \end{aligned} \quad (23)$$

$$\begin{aligned} {}^{i-1}J_{\text{all,H}^+} &= \sum_{k=0}^2 {}^{i-1}c_{i-1,(k-1)} {}^{i-1}m_{i-1,(k-1)} \frac{I}{q_i^{i-1} \kappa} \\ &+ \sum_{k=0}^2 {}^{i-1}c_{i,(1-k)} {}^{i-1}m_{i,(1-k)} \frac{I}{q_i^{i-1} \kappa} \\ &+ \frac{{}^{i-1}c_{\text{H}^+} {}^{i-1}m_{\text{H}^+}}{q_i^{i-1} \kappa} \\ &+ \frac{{}^{i-1}c_{\text{OH}^-} {}^{i-1}m_{\text{OH}^-}}{q_i^{i-1} \kappa} + {}^{i-1}J_{\text{oth,H}^+} \end{aligned} \quad (24)$$

$${}^{i-1}J_{\text{oth,H}^+} = \sum_{\text{oth}} \sum_{k=0}^2 {}^{i-1}\bar{c}_{\text{oth},(1-k)} {}^{i-1}\bar{m}_{\text{oth},(1-k)} \frac{I}{q_i^{i-1} \kappa} \quad (25)$$

where, the “oth” in Eqs. (24) and (25) means other CAs, such as..., $\text{CA}_{i-2}, \text{CA}_{i+1}, \dots$ (see Fig. 2), but not $\text{CA}_{i-1}, {}^{i-1}\text{H}^+, {}^{i-1}\text{OH}^-$ and CA_i . Eqs. (23) and (24) are for high and low electric loads, respectively. Eq. (23) is different from Eq. (24), as shown by the last term on the right-hand-side of Eq. (24), viz., Eq. (25). This difference is due to the following causes (see Section 4): Under high electric load, the distribution of CA is thin, no other CAs, such as $\text{CA}_{i-3}, \text{CA}_{i-2}, \text{CA}_{i+1}$ and CA_{i+2} (see Figs. 1 and 2), take part in the transference of hydrogen and hydroxyl ions through boundary $i-1-i$; whereas, under low electric load, other CAs will also take part in the transference of hydrogen and hydroxyl ions, due to the wide distribution of CAs in IEF [95–98].

Both Eqs. (23) and (24) may be written as follows, with the hydrogen ion constituent

$${}^{i-1}J_{\text{all,H}^+} = \sum_{\text{all}} \sum_{k=0}^2 {}^{i-1}\bar{c}_{\text{all},(1-k)} {}^{i-1}\bar{m}_{\text{all},(1-k)} \frac{I}{q_i^{i-1} \kappa} \quad (26)$$

Eqs. (23) and (24) can also be expressed in terms of the hydroxyl ion constituent

$${}^{i-1}J_{\text{all,OH}^-} = \sum_{\text{all}} \sum_{k=0}^2 {}^{i-1}\bar{c}_{\text{all},(k-1)} {}^{i-1}\bar{m}_{\text{all},(k-1)} \frac{I}{q_i^{i-1} \kappa} \quad (27)$$

where, the “all” must be $\text{CA}_{i-1}, {}^{i-1}\text{H}^+, {}^{i-1}\text{OH}^-$ and CA_i , and may be other CAs like..., $\text{CA}_{i-2}, \text{CA}_{i+1}, \dots$ (see Fig. 2). Evidently, Eq. (26) is equivalent to Eq. (27) and vice versa.

Consider the diffusion and electromigration of CA_i through boundary $i-i+1$ in an idealized IEF, the flux of electromigration of the hydroxyl ion carried by the charged $\text{CA}_{i,-}$ from medium $i+1$ into i may be written as follows, being analogous to Eqs. (18), (20a), (20b):

$${}^i J_{i,\text{OH}^-} = \sum_{k=0}^2 {}^i c_{i,(k-1)} {}^i m_{i,(k-1)} \frac{I}{q_{i+1}^i \kappa} \quad (28)$$

That of the hydrogen ion carried by the charged

CA_{*i+1,+*} from medium *i* into medium *i+1* may be given as

$${}^i J_{i+1,H+} = \sum_{k=0}^2 {}^i c_{i+1,(1-k)} {}^i m_{i+1,(1-k)} \frac{I}{q_{i+1}^i \kappa} \quad (29)$$

Similarly (see Eqs. 21 and 22), those of free proton and hydroxyl ion through boundary *i-i+1* are, respectively

$${}^i J_{H+} = {}^i c_{H+} {}^i m_{H+} \frac{I}{q_{i+1}^i \kappa} \quad (30)$$

$${}^i J_{OH-} = {}^i c_{OH-} {}^i m_{OH-} \frac{I}{q_{i+1}^i \kappa} \quad (31)$$

With the same treatments used to obtain Eqs. (25) and (26), clearly, there is also additivity between Eqs. (28)–(31). Thus, by using hydrogen or hydroxyl ion constituents to express the total flux, one obtains the following expressions for both high and low electric loads, viz., thin and wide distributions of CAs

$${}^i J_{all,H+} = \sum_{all} \sum_{k=0}^2 {}^i \bar{c}_{all,(1-k)} {}^i \bar{m}_{all,(1-k)} \frac{I}{q_{i+1}^i \kappa} \quad (32)$$

$${}^i J_{all,OH-} = \sum_{all} \sum_{k=0}^2 {}^i \bar{c}_{all,(k-1)} {}^i \bar{m}_{all,(k-1)} \frac{I}{q_{i+1}^i \kappa} \quad (33)$$

where, the “all” in Eqs. (32) and (33) must be CA_{*i*}, ^{*i*}H⁺, ^{*i+1*}OH⁻ and CA_{*i+1*}, and may be other CAs like..., CA_{*i-1*}, CA_{*i+2*},... (see Fig. 2). Evidently, Eq. (33) is equivalent to Eq. (32), and vice versa.

Up to now, we have considered the net increase of the hydrogen (or hydroxyl ion) constituent of medium *i*.

Obviously, if the hydrogen ion constituent is used to express the net increase in the hydrogen ion constituent of medium *i*, by using Eqs. (26) and (32), one obtains

$$J_{i,netH+} = \sum_{all} \sum_{k=0}^2 {}^i \bar{c}_{all,(1-k)} {}^i \bar{m}_{all,(1-k)} \frac{I}{q_{i+1}^i \kappa} - \sum_{all} \sum_{k=0}^2 {}^{i-1} \bar{c}_{all,(1-k)} {}^{i-1} \bar{m}_{all,(1-k)} \frac{I}{q_i^{i-1} \kappa} \quad (34)$$

which can be reduced to simply

$$J_{i,netH+} = {}^i J_{all,H+} - {}^{i-1} J_{all,H+} \quad (35)$$

Using the hydroxyl ion constituent to express the net increase in the hydroxyl ion constituent of medium *i*, one gets, using Eqs. (27) and (33)

$$J_{i,netOH-} = \sum_{all} \sum_{k=0}^2 {}^{i-1} \bar{c}_{all,(k-1)} {}^{i-1} \bar{m}_{all,(k-1)} \frac{I}{q_{i+1}^i \kappa} - \sum_{all} \sum_{k=0}^2 {}^i \bar{c}_{all,(k-1)} {}^i \bar{m}_{all,(k-1)} \frac{I}{q_{i+1}^i \kappa} \quad (36)$$

$$J_{i,netOH-} = {}^{i-1} J_{all,OH-} - {}^i J_{all,OH-} \quad (37)$$

It is clear that Eqs. (34) and (35) are equivalent to Eqs. (36) and (37), respectively, and vice versa.

Now, we can formulate the conditional equations for Svensson–Tiselius’ differential equation. As will be discussed below, the following conditions must be satisfied for Svensson–Tiselius’ differential equation, viz., an idealized IEF at steady state.

Condition: The net increase in hydrogen or hydroxyl ion constituent of medium *i* must be equal to zero during the continuance of steady-state IEF, namely, the following two equalities must be satisfied for the idealized IEF described by Svensson–Tiselius’ differential equation

$$J_{i,netH+} = {}^i J_{all,H+} - {}^{i-1} J_{all,H+} = 0; \quad (38)$$

or

$$J_{i,netOH-} = {}^{i-1} J_{all,OH-} - {}^i J_{all,OH-} = 0. \quad (39)$$

This is due to the reasons discussed in the following.

From Eqs. (34) and (35) or Eqs. (36) and (37), three possibilities exist. The first is Eq. (38) or Eq. (39), viz., the condition of Eqs. (38) and (39), the second and third are inequalities in Eqs. (40a), (40b), (41a), (41b), respectively.

$$J_{i,netH+} > 0 \quad (40a)$$

or

$$J_{i,netOH-} < 0 \quad (40b)$$

$$J_{i,netOH-} > 0 \quad (41a)$$

or

$$J_{i,\text{netH}^+} < 0 \quad (41b)$$

By multiplying the two sides of Eqs. (35) and (37) by the electrophoretic time, t , we get Eqs. (42) and (43), respectively:

$$J_{i,\text{netH}^+}t = ({}^i_{i+1}J_{\text{all,H}^+} - {}^{i-1}_iJ_{\text{all,H}^+})t \quad (42)$$

$$J_{i,\text{netOH}^-}t = ({}^{i-1}_iJ_{\text{all,OH}^-} - {}^i_{i+1}J_{\text{all,OH}^-})t \quad (43)$$

It is obvious from the inequality in Eq. (40a) and Eq. (42) that, if enough electrophoretic time t is given, a sufficient net quantity of hydrogen ion constituents will accumulate in medium i , where the hydrogen ion constituents may partly be present as positively charged CA_i and partly be transferred as free proton into medium i . The former consequence of accumulation of hydrogen ion constituent is that CA_i carries net positive charge(s) directly, and the latter is the same, since the accumulation of free proton relieved from the hydrogen ion constituent causes the pH value of medium i to be lower than pI_i and, consequently, CA_i carries net positive charge(s). The CA_i , which hauls net positive charge(s), must move towards the cathode electrically. Therefore, under the condition of inequality given in Eq. (40a) or Eq. (40b), the complete stationary concentration distribution of CA_i described by Svensson–Tiselius’ differential equation, viz., an idealized IEF at steady state (see assumption 7 in Section 5), is broken, and the pH gradient must drift towards the cathode.

The inequalities in Eqs. (41a) and (43) indicate that, if enough time is given, a sufficient net quantity of hydroxyl ion constituents will collect in medium i where the hydroxyl ion constituents may, to some extent, exist as negatively charged CA_i and partially be transferred as free hydroxyl ion into medium i . The former consequence of the accumulation of the hydroxyl ion constituent in medium i is that CA_i carries net negative charge(s); the latter is the same, since the free hydroxyl ion relieved from the hydroxyl ion constituent causes the pH of medium i to be greater than the pI_i value and, consequently, CA_i carries net negative charge(s). The CA_i , which carries net negative charge(s), must migrate towards the anode electrically. Thus, under the condition of inequality given in Eq. (41a) or Eq. (41b), the

complete stationary concentration distribution of CA_i , depicted by Svensson–Tiselius’ differential equation, viz., assumption 7 in Section 5, is also broken, and the pH gradient must drift towards the anode.

Under the conditions of Eq. (38) or Eq. (39), it is obvious that no net quantity of hydrogen or hydroxyl ion accumulates in medium i . Since the steady state of an idealized IEF has been achieved (see assumption 7 in Section 5), and no predominant (de)protonization of CA_i causes itself carries net positive or negative charge(s); thus, no drift of the pH gradient occurs. This is complete stationary concentration distribution as described by Svensson–Tiselius’ differential equation. (Note, the formation of water [57], caused by the electromigration reaction of hydrogen and hydroxyl ions has not been discussed here). Under the conditions of Eq. (38) or Eq. (39) (and the other conditions given in Section 5), it is clear that an idealized complete stationary pH gradient can last forever.

From the above discussions, it is evident that, under the conditions of Eq. (38) or Eq. (39), in association with those given in Section 5, one has a stable pH gradient continuously, whereas, if one doesn’t have the conditions of Eq. (38) or Eq. (39), one cannot get the stable pH gradient in IEF, as described by Svensson–Tiselius’ differential equation. Thus, Eqs. (38) and (39), coupled with Eqs. (44)–(49), (61), (62), (65)–(68), which will be derived from Eqs. (38) and (39), are the conditional equations for Svensson–Tiselius’ differential equation. Obviously, under the conditions of Eq. (38) or Eq. (39), the following general conditional equations can be derived from Eq. (38):

$$\begin{aligned} & \sum_{\text{all}} \sum_{k=0}^2 {}^{i-1}_i \bar{c}_{\text{all},(1-k)} {}^{i-1}_i \bar{m}_{\text{all},(1-k)} \frac{I}{q_i^{i-1} \kappa} \\ &= \sum_{\text{all}} \sum_{k=0}^2 {}^i_{i+1} \bar{c}_{\text{all},(1-k)} {}^i_{i+1} \bar{m}_{\text{all},(1-k)} \frac{I}{q_{i+1}^i \kappa} \end{aligned} \quad (44)$$

Elimination of I and q yields

$$\begin{aligned} & \sum_{\text{all}} \sum_{k=0}^2 {}^{i-1}_i \bar{c}_{\text{all},(1-k)} {}^{i-1}_i \bar{m}_{\text{all},(1-k)} {}^i_{i+1} \kappa \\ &= \sum_{\text{all}} \sum_{k=0}^2 {}^i_{i+1} \bar{c}_{\text{all},(1-k)} {}^i_{i+1} \bar{m}_{\text{all},(1-k)} {}^{i-1}_i \kappa \end{aligned} \quad (45)$$

or simply

$${}_{i+1}^i J_{\text{all,H}^+} = {}_i^{i-1} J_{\text{all,H}^+} \quad (46)$$

If the hydroxyl ion constituent is used to express Eqs. (44)–(46), we have

$$\sum_{\text{all}} \sum_{k=0}^2 {}_i^{i-1} \bar{c}_{\text{all},(k-1)} {}_i^{i-1} \bar{m}_{\text{all},(k-1)} \frac{I}{q_i^{i-1} \kappa} = \sum_{\text{all}} \sum_{k=0}^2 {}_i^{i-1} \bar{c}_{\text{all},(k-1)} {}_i^{i-1} \bar{m}_{\text{all},(k-1)} \frac{I}{q_{i+1}^i \kappa} \quad (47)$$

$$\sum_{\text{all}} \sum_{k=0}^2 {}_i^{i-1} \bar{c}_{\text{all},(k-1)} {}_i^{i-1} \bar{m}_{\text{all},(k-1)} {}_i^{i-1} \kappa = \sum_{\text{all}} \sum_{k=0}^2 {}_i^{i-1} \bar{c}_{\text{all},(k-1)} {}_i^{i-1} \bar{m}_{\text{all},(k-1)} {}_i^{i-1} \kappa \quad (48)$$

$${}_{i-1}^i J_{\text{all,OH}^-} = {}_{i+1}^i J_{\text{all,OH}^-} \quad (49)$$

Eqs. (38), (39), (44)–(49) ought to be called general condition equations, since the conditional equations for the anolyte and catholyte can be derived from them, as will be shown below.

6.2. Condition equations for the anolyte and catholyte

Now, we can use the general condition equations to derive the condition equation for the anolyte, viz., an acid, and the catholyte, viz., a base, as used in Svensson's IEF.

If CA_i = the anolyte (i.e., acid), obviously, CA_{i-1} is also the anolyte, CA_{i+1} is the CA_{min} . Using assumption 3 in Section 5 and assuming the existence of a boundary between CA_{i-1} and CA_i (see the broken line in the anolyte in Fig. 2), we can express Eq. (44) as follows

$$\bar{c}_{\text{H}^+}^{\text{acid}} \bar{m}_{\text{H}^+} = \frac{I}{q \kappa^{\text{acid}}} = \sum_{\text{all}} \sum_{k=0}^2 \bar{c}_{\text{all},(1-k)}^{\text{acid}} \bar{m}_{\text{all},(1-k)}^{\text{acid}} \frac{I}{q_{\text{min}}^{\text{acid}} \kappa} \quad (50)$$

If $CA_i = CA_{\text{min}}$, clearly, CA_{i-1} = the anolyte, and $CA_{i+1} = CA_{m+1}$, hence, Eq. (44) can be rewritten as

$$\sum_{\text{all}} \sum_{k=0}^2 \bar{c}_{\text{all},(1-k)}^{\text{acid}} \bar{m}_{\text{all},(1-k)}^{\text{acid}} \frac{I}{q_{\text{min}}^{\text{acid}} \kappa} = \sum_{\text{all}} \sum_{k=0}^2 \bar{c}_{\text{all},(1-k)}^{\text{min}} \bar{m}_{\text{all},(1-k)}^{\text{min}} \frac{I}{q_{m+1}^{\text{min}} \kappa} \quad (51)$$

Evidently, if $CA_i = CA_{m+1}$, then $CA_{i-1} = CA_{\text{min}}$, $CA_{i+1} = CA_{m+2}$, thus Eq. (44) can be expressed as

$$\sum_{\text{all}} \sum_{k=0}^2 \bar{c}_{\text{all},(1-k)}^{\text{min}} \bar{m}_{\text{all},(1-k)}^{\text{min}} \frac{I}{q_{m+1}^{\text{min}} \kappa} = \sum_{\text{all}} \sum_{k=0}^2 \bar{c}_{\text{all},(1-k)}^{m+1} \bar{m}_{\text{all},(1-k)}^{m+1} \frac{I}{q_{m+2}^{m+1} \kappa} \quad (52)$$

If $CA_i = CA_{i-1}$, then $CA_{i-1} = CA_{i-2}$, and $CA_{i+1} = CA_i$, thus, Eq. (44) becomes

$$\sum_{\text{all}} \sum_{k=0}^2 \bar{c}_{\text{all},(1-k)}^{i-2} \bar{m}_{\text{all},(1-k)}^{i-2} \frac{I}{q_{i-1}^{i-2} \kappa} = \sum_{\text{all}} \sum_{k=0}^2 \bar{c}_{\text{all},(1-k)}^{i-1} \bar{m}_{\text{all},(1-k)}^{i-1} \frac{I}{q_i^{i-1} \kappa} \quad (53)$$

If $CA_i = CA_{i-1}$, then $CA_{i-1} = CA_{i-1}$, and $CA_{i+1} = CA_{i+1}$, one gets Eq. (44).

Evidently, the right-hand side of Eq. (50) is the same as the left-hand side of Eq. (51), and the right-hand side of Eq. (51) is the same as the left-hand side of Eq. (52),..., the right-hand side of Eq. (53) is the same as the left-hand side of Eq. (44). Thus, adding Eq. (50) with Eq. (51), with Eq. (52), with..., with Eq. (53), and finally with Eq. (44), we have

$$\bar{c}_{\text{H}^+}^{\text{acid}} \bar{m}_{\text{H}^+} + \frac{I}{q \kappa^{\text{acid}}} = \sum_{\text{all}} \sum_{k=0}^2 \bar{c}_{\text{all},(1-k)}^i \bar{m}_{\text{all},(1-k)}^i \frac{I}{q_{i+1}^i \kappa} \quad (54)$$

Similarly (see Eqs. 50–53), let CA_i = the catholyte (viz., base), and $CA_{i-1} = CA_{\text{max}}$, and $CA_{i+1} = CA_{m-1}$, and..., and CA_{i+2} , CA_{i+1} (see Fig. 2) and use Eq. (47) repeatedly, we obtain a series of equations

$$\sum_{\text{all}} \sum_{k=0}^2 \bar{c}_{\text{all},(k-1)}^{\text{max}} \bar{m}_{\text{all},(k-1)}^{\text{max}} \frac{I}{q_{\text{base}}^{\text{max}} \kappa} = \bar{c}_{\text{OH}^-}^{\text{base}} \bar{m}_{\text{OH}^-} - \frac{I}{q \kappa^{\text{base}}} \quad (55)$$

$$\sum_{\text{all}} \sum_{k=0}^2 \bar{c}_{\text{all},(k-1)}^{m-1} \bar{m}_{\text{all},(k-1)}^{m-1} \frac{I}{q_{\text{max}}^{m-1} \kappa} = \sum_{\text{all}} \sum_{k=0}^2 \bar{c}_{\text{all},(k-1)}^{\text{max}} \bar{m}_{\text{all},(k-1)}^{\text{max}} \frac{I}{q_{\text{base}}^{\text{max}} \kappa} \quad (56)$$

$$\begin{aligned} & \sum_{\text{all}} \sum_{k=0}^2 \frac{m-2}{m-1} \bar{c}_{\text{all},(k-1)} \frac{m-2}{m-1} \bar{m}_{\text{all},(k-1)} \frac{I}{q \frac{m-2}{m-1} \kappa} \\ &= \sum_{\text{all}} \sum_{k=0}^2 \frac{m-1}{\max} \bar{c}_{\text{all},(k-1)} \frac{m-1}{\max} \bar{m}_{\text{all},(k-1)} \frac{I}{q \frac{m-1}{\max} \kappa} \end{aligned} \quad (57)$$

$$\begin{aligned} & \sum_{\text{all}} \sum_{k=0}^2 \frac{i+1}{i+2} \bar{c}_{\text{all},(k-1)} \frac{i+1}{i+2} \bar{m}_{\text{all},(k-1)} \frac{I}{q \frac{i+1}{i+2} \kappa} \\ &= \sum_{\text{all}} \sum_{k=0}^2 \frac{i+2}{i+3} \bar{c}_{\text{all},(k-1)} \frac{i+2}{i+3} \bar{m}_{\text{all},(k-1)} \frac{I}{q \frac{i+2}{i+3} \kappa} \end{aligned} \quad (58)$$

$$\begin{aligned} & \sum_{\text{all}} \sum_{k=0}^2 \frac{i}{i+1} \bar{c}_{\text{all},(k-1)} \frac{i}{i+1} \bar{m}_{\text{all},(k-1)} \frac{I}{q \frac{i}{i+1} \kappa} \\ &= \sum_{\text{all}} \sum_{k=0}^2 \frac{i+1}{i+2} \bar{c}_{\text{all},(k-1)} \frac{i+1}{i+2} \bar{m}_{\text{all},(k-1)} \frac{I}{q \frac{i+1}{i+2} \kappa} \end{aligned} \quad (59)$$

On combining Eqs. (55)–(59), we obtain

$$\begin{aligned} & \sum_{\text{all}} \sum_{k=0}^2 \frac{i}{i+1} \bar{c}_{\text{all},(k-1)} \frac{i}{i+1} \bar{m}_{\text{all},(k-1)} \frac{I}{q \frac{i}{i+1} \kappa} \\ &= \bar{c}_{\text{OH}^-}^{\text{base}} \bar{m}_{\text{OH}^-} \frac{I}{q \kappa^{\text{base}}} \end{aligned} \quad (60)$$

Since, the right-hand side of Eq. (54) is equivalent to the left-hand side of Eq. (60) (see Treatments 1 and 2 in Section 6.1 and compare Eqs. (44)–(46) with Eqs. (47)–(49)), the combination of Eqs. (54) and (60) yields

$$\bar{c}_{\text{H}^+}^{\text{acid}} \bar{m}_{\text{H}^+} \frac{I}{q \kappa^{\text{acid}}} = \bar{c}_{\text{OH}^-}^{\text{base}} \bar{m}_{\text{OH}^-} \frac{I}{q \kappa^{\text{base}}} \quad (61)$$

The elimination of I and q yields

$$\bar{c}_{\text{H}^+}^{\text{acid}} \bar{m}_{\text{H}^+} \kappa^{\text{base}} = \bar{c}_{\text{OH}^-}^{\text{base}} \bar{m}_{\text{OH}^-} \kappa^{\text{acid}} \quad (62)$$

Due to (see Refs. [49,71–75])

$$\bar{c}_{\text{H}^+} \bar{m}_{\text{H}^+} = c_{\text{H}^+} m_{\text{H}^+} \quad (63)$$

$$\bar{c}_{\text{OH}^-} \bar{m}_{\text{OH}^-} = c_{\text{OH}^-} m_{\text{OH}^-} \quad (64)$$

Thus, the insertion of Eqs. (63) and (64) into Eq. (62) yields

$$c_{\text{H}^+}^{\text{acid}} m_{\text{H}^+} \kappa^{\text{base}} = c_{\text{OH}^-}^{\text{base}} m_{\text{OH}^-} \kappa^{\text{acid}} \quad (65)$$

by using Eq. (2), one gets from Eq. (65)

$$T_{\text{H}^+}^{\text{acid}} = T_{\text{OH}^-}^{\text{base}} \quad (66)$$

$$J_{\text{H}^+}^{\text{acid}} = J_{\text{OH}^-}^{\text{base}} \quad (67)$$

and if we use the pH values of the anolyte and catholyte to express Eq. (65), we get

$$p\text{H}^{\text{acid}} = \lg\left(\frac{m_{\text{H}^+}^{\text{acid}}}{m_{\text{OH}^-}^{\text{base}}}\right) - \lg\left(\frac{\kappa^{\text{acid}}}{\kappa^{\text{base}}}\right) + p\kappa_w - p\text{H}^{\text{base}} \quad (68)$$

It is evident from Eqs. (66) and (67) that a stationary concentration distribution of CA, as depicted by Eq. (3), viz., idealized IEF, can be achieved, if the fluxes or transference numbers of hydrogen and hydroxyl ions migrating in opposite directions are equal to each other.

7. Relationships with MCRBEs

As stated in Section 1, the MCRB theory was developed [65–67] and a series of MCRBEs were formulated. Here we list some of them

$$\frac{\bar{c}_+^{\alpha} \bar{m}_+^{\alpha}}{\kappa^{\alpha}} - \frac{\bar{c}_-^{\beta} \bar{m}_-^{\beta}}{\kappa^{\beta}} = v^{\alpha\beta} (\bar{c}_+^{\alpha} - \bar{c}_-^{\beta}) \quad (69)$$

$$\frac{c_+^{\alpha} m_+^{\alpha}}{\kappa^{\alpha}} - \frac{c_-^{\beta} m_-^{\beta}}{\kappa^{\beta}} = v^{\alpha\beta} (c_+^{\alpha} - c_-^{\beta}) \quad (70)$$

$$T_+^{\alpha} - T_-^{\beta} = V^{\alpha\beta} (c_+^{\alpha} - c_-^{\beta}) \quad (71)$$

If the boundary displacement is set to be stationary, viz.,

$$v^{\alpha\beta} = 0; \text{ or } V^{\alpha\beta} = 0 \quad (72)$$

then Eqs. (69)–(71) become Eqs. (73)–(75), respectively.

$$\bar{m}_+^{\alpha} \bar{c}_+^{\alpha} \kappa^{\beta} = \bar{m}_-^{\beta} \bar{c}_-^{\beta} \kappa^{\alpha} \quad (73)$$

$$m_+^{\alpha} c_+^{\alpha} \kappa^{\beta} = m_-^{\beta} c_-^{\beta} \kappa^{\alpha} \quad (74)$$

$$T_+^{\alpha} = T_-^{\beta} \quad (75)$$

If the boundary is formed by weak or strong acid and base, then Eqs. (73)–(75) can be expressed as follows:

$$\bar{m}_{\text{H}^+}^{\alpha} \bar{c}_{\text{H}^+}^{\alpha} \kappa^{\beta} = \bar{m}_{\text{OH}^-}^{\beta} \bar{c}_{\text{OH}^-}^{\beta} \kappa^{\alpha} \quad (76)$$

$$m_{\text{H}^+}^{\alpha} c_{\text{H}^+}^{\alpha} \kappa^{\beta} = m_{\text{OH}^-}^{\beta} c_{\text{OH}^-}^{\beta} \kappa^{\alpha} \quad (77)$$

$$T_{\text{H}^+}^\alpha = T_{\text{OH}^-}^\beta \quad (78)$$

$$J_{\text{H}^+}^\alpha = J_{\text{OH}^-}^\beta \quad (79)$$

$$\text{pH}^\alpha = \lg\left(\frac{m_{\text{H}^+}^\alpha}{m_{\text{OH}^-}^\beta}\right) - \lg\left(\frac{\kappa^\alpha}{\kappa^\beta}\right) - \text{p}\kappa_w - \text{pH}^\beta \quad (80)$$

Eqs. (72)–(75), coupled with Eqs. (76)–(80), should be called stationary chemical reaction boundary equations (SCRBEs) and Eqs. (76)–(80) stationary neutralization reaction boundary equations (SNRBEs).

Obviously, if a chemical reaction boundary was formed with acid and base, then phases α and β become acid and base, respectively. Thus, Eqs. (76)–(80) are the same as Eq. (62) and Eqs. (65)–(68), respectively. This indicates the conditional equations for the acidic anolyte and alkaline catholyte used in Svensson's IEF can also be deduced from the MCRBEs, when the boundary, which is formed with acid and base as anolyte and catholyte, respectively, is stationary.

Thus, it is shown theoretically that an idealized IEF is based upon a stationary neutralization reaction boundary (SNRB). The theoretical conclusion will be verified in Sections 8 and 9 and the accompanying report [101].

8. Tests

8.1. Experiments of electromigration reaction and chemical reaction boundary

A chemical reaction boundary can be created and an electromigration reaction may occur if the anolyte and catholyte are chosen appropriately. This has been proven directly by the experiments of “precipitate reaction front” of Deman and Rigole [62,63] and by us [64], and those of stationary neutralization boundary have been proven by Pospichal et al. [33] and will also be verified by the following experiments of precipitate reaction boundary.

The experiments of precipitate reaction boundary, using CoCl_2 and NaOH , were performed according

to the procedure of Deman and Rigole [62,63], but with some modifications (for details, see Figs. 3 and 4).

Initially, phase α contains 0.01 M CoCl_2 and 0.1 M KCl , phase β contains 0.005, 0.01 or 0.015 M NaOH and 0.1 M KCl (see Figs. 3 and 4). It was observed that, on the passage of electric current, the reaction boundary was created and moved towards the anode, and the blue precipitate zone of $\text{Co}(\text{OH})_2$ developed just after the boundary. In Fig. 4, the arrows indicate the boundaries formed just after the ends of experiments of electromigration reaction; the weak blue zones or disks near the anode can be observed clearly after the end of the experiment, at about 12 h, and are probably formed by the diffusion reactions of the cobalt and hydroxyl ions. Fig. 4 shows that the boundaries are sharp and clear, even after the experiment has been carried out for about two weeks. It was calculated that the velocities of boundaries in Fig. 4 quantitatively coincide with the predictions with MCRBEs, viz., Eqs. (70) and (71) (not shown).

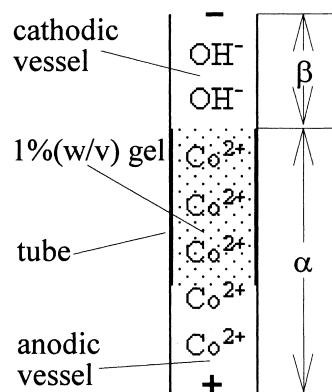


Fig. 3. The experimental procedure of precipitate reaction boundary formed with CoCl_2 and NaOH , together with 0.1 M KCl . The symbols, “ α ” and “ β ”, imply phases α and β , respectively, and “+” and “-” indicate the anode and cathode, respectively. For other symbols, see text. The initial phase α , contains 0.01 M CoCl_2 and 0.1 M KCl and phase β contains 0.005, 0.01 or 0.015 M NaOH and 0.1 M KCl . The gel used was a 1% (w/v) agarose gel (very low EOF). Experiments were performed with a power supply (DDYIII8A; constant voltage, 0–300 or 0–600 V; constant current, 0–25 or 0–100 mA; timer equipment, Beijing Luyi Instrument Factory, Beijing, China) and a disk electrophoretic trough (DDYIII, Beijing Luyi Instrument Factory).

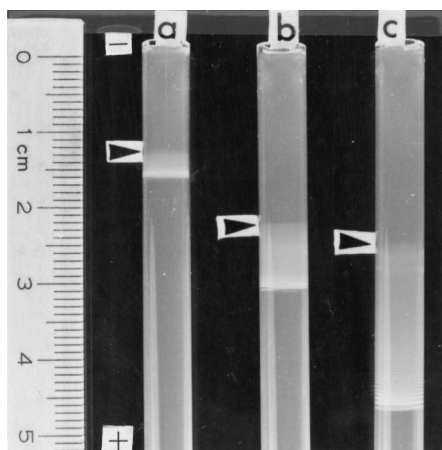


Fig. 4. Precipitate reaction boundary formed by CoCl_2 and NaOH . The arrows indicate the boundaries formed just after the ends of runs, the symbols “-” and “+” indicate the cathode and anode, respectively. The weak yellow zones from the mark of “-” to the arrows are the precipitate zones, the weak blue zones or disks near the anode of “+” are developed completely after the end of runs about 12 h. The concentration, $c_{\text{Co}^{2+}}^{\alpha}$, is fixed at 0.01 M; a: $c_{\text{OH}^-}^{\beta} = 0.005$ M, b: $c_{\text{OH}^-}^{\beta} = 0.01$ M, c: $c_{\text{OH}^-}^{\beta} = 0.015$ M. Conditions: Background electrolyte, 0.1 M KCl; current intensity, 0.61 mA/mm²; I.D of tube=4.6 mm and length of tube=90 mm; run time, 10 min.

8.2. Computer simulations and experiments of electrically controlled electrofocusing

There are two facts that show the validity of the condition equation for the acidic anolyte and the alkaline catholyte used in Svensson’s IEF.

Firstly, the predictions of Eq. (80), viz., Eq. (68), are in exact agreement with the computer simulation based on Kohlrausch’s beharrliche function [66]. Fig. 5 (cited from Ref. [66]) was obtained from Eq. (80) or Eq. (68) under the condition of uniform conductivity due to the existence of a large amount of background electrolyte (KCl) [33]. From Fig. 5, it can be seen that SNRBs, viz., stationary ampholyte dyes, can be observed, if the pH values of the anolyte and catholyte are symmetrical to each other at pH 7.124. This prediction is in almost complete agreement with the computer simulations based upon Kohlrausch’s beharrliche function (compare Fig. 5 here with Figs. 2a and 2b in Ref. [33]).

Secondly, the two SNRBs [21], one at pH 4.26–10

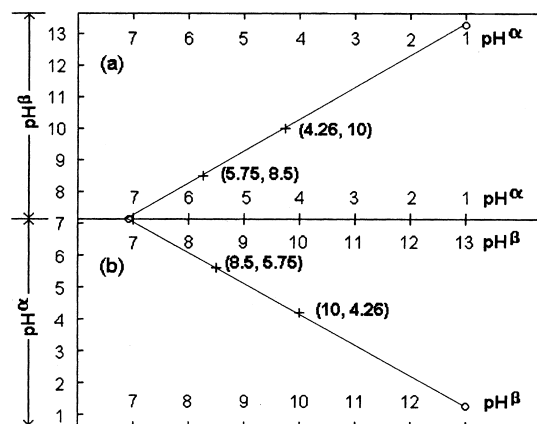


Fig. 5. Relationship between the pH values of phase α (viz., acid) and β (viz., base), pH^{α} and pH^{β} . (a) Dependence of pH^{α} on pH^{β} ; (b) dependence of pH^{β} on pH^{α} ; the symbols “+” marked with (4.26, 10) and (5.75, 8.5) in (a) and by (8.5, 5.75) and (10, 4.26) in (b) are the pH intervals for two SNRBs created by Pospichal et al. [33], with electrically controlled electrofocusing (cited from Ref. [66]).

and another at pH 5.75–8.5, which are observed with stationary ampholyte dyes focused in the capillary electrophoretic apparatus, fit the predictions of Eq. (80) or Eq. (68) completely (see Fig. 5).

8.3. Existence of quasi/equal fluxes of hydrogen and hydroxyl ions in IEF

The conditional equations for the anolyte and catholyte, viz., Eqs. (66) and (67), show that the stationary concentration distribution of CA, depicted by Eq. (3), viz., an idealized IEF, can be achieved, if the fluxes or transference numbers of hydrogen and hydroxyl ions migrating in opposite directions are equal to each other.

Thus, according to the condition equations for the anolyte and catholyte used in Svensson’s IEF and the fact that the quasi-stable, but not completely stable, pH gradients exist in Svensson’s IEF, one can infer that quasi/equal fluxes of hydrogen and hydroxyl ions should be present in Svensson’s IEF. This inference coincides with the important findings, i.e. the existence of quasi/equal fluxes of hydrogen and hydroxyl ions in stationary electrolysis and in Sven-

sson's IEF, as will be shown in the accompanying report [101].

9. Discussion

Classic IEF is based on stationary electrolysis of salt(s). Tiselius [88] and Svensson [1], in their articles, described classic IEF as follows: "In the case of electrolysis of sodium sulfate, one gets evolution of hydrogen at the cathode and of oxygen at the anode (which should be of platinum or carbon), while sodium hydroxide collects in the catholyte and sulfuric acid in the anolyte... If an ampholyte is added to the electrolyzer, it must be repelled both from the anode and the cathode as acid solutions will give it a positive charge, alkaline solutions a negative charge. Consequently, the ampholyte should move until it finds the pH value equal to its pI and remain there as long as electrolysis is continued". Evidently, the classic IEF depicted by Tiselius [88] and Svensson [2] is based on stationary electrolysis.

Classic IEF is based on a neutralization reaction boundary. In stationary electrolysis, acid and base are accumulated in the anolyte and catholyte, respectively. Evidently, the proton in the acidic anolyte and the hydroxyl ion in the alkaline catholyte will migrate in opposite directions under the electric field and will react with each other when they meet. Thus, during stationary electrolysis, on which IEF is based, an electromigration reaction exists between hydrogen and hydroxyl ions and a neutralization reaction boundary is created. The electromigration reaction and neutralization boundary have been recognized by Murel et al. [61], Pospichal et al. [33] and Hayedorn and Fuhr [57] and have been used to explain the mechanism of the instability of the pH gradient in IEF [57,61]. The conclusion is verified directly by the theoretical results, which show that the condition equations (see Eqs. (62), (65)–(68)) for the anolyte and catholyte used in IEF are the same as the SNRBEs (see Eqs. 76–80) that can be derived from the MCRBEs (see Eqs. (69)–(71)) in special cases, and also proved by the experiments and computer simulations described in Sections 8.1 and 8.2. As shown in Section 8.2, (1) the predictions of Eq. (68) or Eq. (80) are in agreement with the computer

simulations based on Kohlrausch's beharrliche functions, (2) the prediction with Eqs. (66)–(68) coincide exactly with the experiments performed on SNRBEs with electrically controlled electrofocusing of ampholyte dyes.

Quasi/equal fluxes of hydrogen and hydroxyl ions are present in Svensson's IEF. It is evident from the conditional equations for the anolyte and catholyte, e.g. Eqs. (66) and (67), that a stationary concentration distribution of CA, depicted by Svensson–Tiselius' differential equation, viz., idealized IEF, can be achieved, if the fluxes or transference numbers of hydrogen and hydroxyl ions migrating in opposite directions are equal to each other. Therefore, according to the condition equations for the anolyte and catholyte (see Eqs. (66) and (67)) and the fact that the quasi-stable, but not the completely stable (due to weak cathodic or anodic drift and plateau of pH gradient in IEF) pH gradient, exists in Svensson's IEF, we can conclude that the flux of hydrogen ion in the acidic anolyte should be equal or very close to that of hydroxyl ion in the alkaline catholyte. This conclusion is in agreement with the finding of the existence of quasi/equal fluxes of hydrogen and hydroxyl ions in stationary electrolysis and Svensson's IEF, as will be shown in the accompanying paper [101].

10. Conclusions

From above discussions, one can conclude that Svensson's IEF is based on stationary electrolysis, in which the electromigration reaction occurs between the hydrogen and hydroxyl ions migrating in opposite directions, in which a neutralization reaction boundary is created by the hydrogen and hydroxyl ions, and in which quasi/equal fluxes (or transference numbers) of hydrogen and hydroxyl ions are present.

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