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Nonequilibrium thermodynamic separation model in capillary electrophoresis

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Abstract

This paper describes a separation model in capillary electrophoresis (CE) based on the entropy equation of nonequilibrium thermodynamics. We first related ΔS, the mixed entropy change of the solute system, to plate height (H) and ΔS_s, the contribution of ΔS only due to the net separation process, to resolution (R_s) and resolution product (ΠR_s). In particular, we determined the entropy flow of the solute system, which is composed of both energetic and material exchange terms relating to capillary cooling and relative migrations among solute zones, respectively. It is just the CE separation system, as exterior surroundings, that contributes to the enhanced separation efficiency. The more the CE system (except the solute system) provides the solute system with negative entropy flow, the better the separation efficiency of the CE system. We also determined six thermodynamic forces and their thermodynamic fluxes corresponding to six irreversible processes; heat conduction, four kinds of diffusion (electrical field, axial concentration gradient, electrophoretic dispersion and wall adsorption) and viscous flow, respectively. Entropy production is thus composed of the six terms corresponding to time-dependent CE efficiency loss factors. The bigger the entropy production, the greater the loss of separation efficiency. The objective functions were built based on the entropy equation of solute systems developed between CE separation efficiency (ΔS_s) and the optimizing parameters (electrical strength, coolant temperature; the composition and concentration of buffer; the radius, length and wall adsorption of the capillary; the concentration, charge, molecular weight and conformation of solutes; injection conditions, etc.). The more negative ΔS_s is, the better the separation efficiency. This model was supported by the results of our experiments and data in the literature.

Keywords: Separation optimization; Nonequilibrium thermodynamics

1. Introduction

As capillary electrophoresis (CE) gains more and more importance among biochemists and analysts working in the pharmaceutical industry and for environmental analysis [1], it is increasingly important to relate operating parameters to separation efficiency for optimizing separations in CE.

The current separation theories of CE are based on dynamics and equilibrium thermodynamics [3–5]. Many factors that cause peak broadening have been discussed previously [6,7], such as Joule heat [8], diffusion [9,10] and electrophoretic dispersion in CE [11,12]. Hjerten [5] derived approximate equations for efficiency and resolution as a function of the width of the starting zone and the zone broadening caused by diffusion, Joule heat, adsorption and the differences in conductivity between a solute zone and the background electrolyte. Grushka [2] used the mass balance approach to deal with solute band

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spreading in CE. Some excellent works summarized current separation theories of CE focusing on the factors influencing zone broadening [3,4]. However, there is some weakness in the area of plate theory itself, especially in the case of electrophoresis, as pointed out by Grossman and Colburn [3], who said "It is important to recognize that it is misleading to discuss electrophoretic separation in terms of plates. The concept of a theoretical plate implies that a separation is accomplished as a result of an equilibrium partitioning between two immiscible phases. This is certainly not true in the case of electrophoresis, where the separation -is accomplished through differences in rates of transport. Thus, in the context of electrophoretic separations, theoretical plates is simply a convenient way to describe the shape of a Gaussian curve". Dynamics are absolutely necessary to describe a concrete irreversible process. However, the details of a phenomenon should be fully known, however, this is not always easy to achieve. In addition, dynamics research is generally limited to concrete problems, thus it is difficult to achieve universal conclusions using dynamics as can be done using thermodynamics. Plate theory focuses on factors causing zone broadening using the dynamic methods, however, it considers other factors that impel the separation between two adjoining zones at the same time to a lesser extent. In fact, the factors of the two sides are present simultaneously in the separation processes. Thus, the factors involved on both sides should be taken into account in one theoretical frame. How would one assemble the factors of the two sides in one theoretical frame? What kind of ground theories could deal with this tricky problem? These questions must be answered first.

In the 1950s, nonequilibrium thermodynamics emerged because of the valuable contributions of Prigogine [13] and Nicolis and Prigogine [14] and have been used as a general tool to macroscopically describe the irreversible processes involved in physico-chemical phenomena in biology, meteorology, astrophysics and other subjects. Nonequilibrium thermodynamics extended the fundamental laws of classical thermodynamics (particularly for the second law of thermodynamics) to open systems and presented the entropy change per unit time (dS/dt) of a thermodynamic system to characterize the evolution of irreversible processes [13]. Giddings [15] pointed out that "Displacements toward equilibrium (Separating) are irreversible or, more descriptively, one way only. An elegant discipline describing these displacements is irreversible thermodynamics, sometimes called nonequilibrium thermodynamics." and "Much of the difficulty arises because separation flies in the face of the second law of thermodynamics. Entropy is gained in mixing, not in separation. Therefore it is the process of mixing that occurs spontaneously. To combat this and achieve separation, one must apply and manipulate external work and heat and allow dilution in a thermodynamically consistent way ..., it should be kept in mind that all separation processes must be thermodynamically consistent." So, the separation processes, which are time-dependent physical and irreversible processes, would be described appropriately using nonequilibrium thermodynamics. However, at present, nonequilibrium thermodynamics has not been introduced fully into chromatography and electrophoresis.

The entropy equation occupied an important position in studying the linear phenomena of nonequilibrium thermodynamics. The use of entropy as a general criterion of separation has been proposed by De Clerke and Cloete [16] and Stewart [17]. We have confirmed that the entropy, entropy change and entropy change rate of solute systems make up a group of general criteria for determining separation efficiency [18]. In the electrophoretic separation of solute systems, three main thermodynamic phenomena exist; electrokinetic effects, diffusion and heat transfer, which belong to the category of linear nonequilibrium thermodynamics. Thus, the entropy equation could be used as a basis for establishing an original separation theory of CE, that is based on nonequilibrium thermodynamics.

In this paper, we present a separation model of CE that is based on the entropy equation of nonequilibrium thermodynamics, which can relate most of the operating parameters of separation efficiency for optimizing separation in CE. The prediction of the model developed was supported by the results of our experiments and data from the literature. In this model, entropy production, which is composed of six terms, corresponds to time-dependent CE efficiency loss factors, and entropy flow, which is composed of

two terms (material and energetic exchange), corresponds to the time-dependent CE efficiency increase factors. It is the sense of well-matched in establishing the separation model with nonequilibrium thermodynamics that inspires us to develop this model further.

The significance of this research lies in the fact that it enriched current separation theories and extended the application fields of nonequilibrium thermodynamics.

2. Theory

2.1. Nonequilibrium thermodynamic separation model

2.1.1. Calculating the entropy change (ΔS) from separation results

Here we give a model solute system, which is made up of k species with n_i moles of component i ($i=1, 2,..., k$), the final state is where the solute i is being detected with the final volume (V_{1i}), and the initial state is where the solute i is just injected with the initial volume (V_0). Let ΔS be the mixed entropy change of the solute system between the final state and the initial state. To calculate ΔS, we will relate ΔS to plate height (H), resolution (R_s) and resolution product (ΠR_s), which can be calculated from the separation results. Only equilibrium thermodynamics were required to derive these relationships.

It is well known that the parameter, H, deals with mono-component zone spreading; R_s reflects the relative change in both the separation and spreading of two-component zones; ΠR_s indicates the power of the separation system for the multi-component solute system. To succinctly describe the relationships between ΔS, H, R_s and ΠR_s, we assume that

A. The separation occurs under conditions of defenite temperature and pressure.
B. The solution is ideal.
C. The actual zone width is six times the standard deviation of the Gaussian peak, say, $6\sigma_i$ [15].
D. The solute is evenly distributed in the zone.

Plate height, H, is commonly used to assess column efficiency in chromatographic and electrophoretic systems by measuring the zone spreading of a component. In the monocomponent solute system, with assumptions A and B, in the separation, the entropy change caused by dilution of n_i moles of component i can be expressed as [15]

$$\Delta S = n_i R \ln (V_{1i}/V_0) \quad (1)$$

where R is the gas constant, V_{1i} is the final volume of component i and V_0 is the initial volume. Let A denote the cross-sectional area perpendicular to the separation path, and, with assumption C, we have

$$V_{1i} = 6A\sigma_i \quad (2)$$

where σ_i is the standard deviation of solute zone i. Inserting Eq. (2) and the definition of plate height, $H=\sigma_i^2/X$, into Eq. (1), with $X=l$ (l being the effective length of the capillary), we have

$$\Delta S = n_i R \ln (6A/V_0) + (1/2)n_i R \ln l + (1/2)n_i R \ln H \quad (3)$$

Since the entropy change of the solute system is a function of the state, ΔS is related only to the final and the initial states, and not to the path of the separation process, although the actual separation is involved in nonequilibrium processes [15] . Therefore, it can be calculated by designing a reversible process, as in Fig. 1. In a two-component system, there are n_i and n_j moles of solutes i and j, V_0 is the volume that components i and j commonly occupy in the initial state. V_1, V_{1i} and V_{1j}, respectively, denote the volumes that the two components total, the solute i and j occupy in the final state. We split a whole separation process into two independent processes. One is the process of the sample dilution or concentration without separation, in which there is a change in the volume of the solute only. The other is the net separation process of solutes without any change of the volume that the two components occupy in total. In other words, the imaginary middle state has the same volume as the final state with no separation. This is shown in Fig. 1. In this case, ΔS of the whole separation process can be split into two parts denoting by ΔS_V, the entropy change due to the volume change only, and by ΔS_S, the contribution due to the net separation. This can be expressed as

$$\Delta S = \Delta S_V + \Delta S_S \quad (4)$$

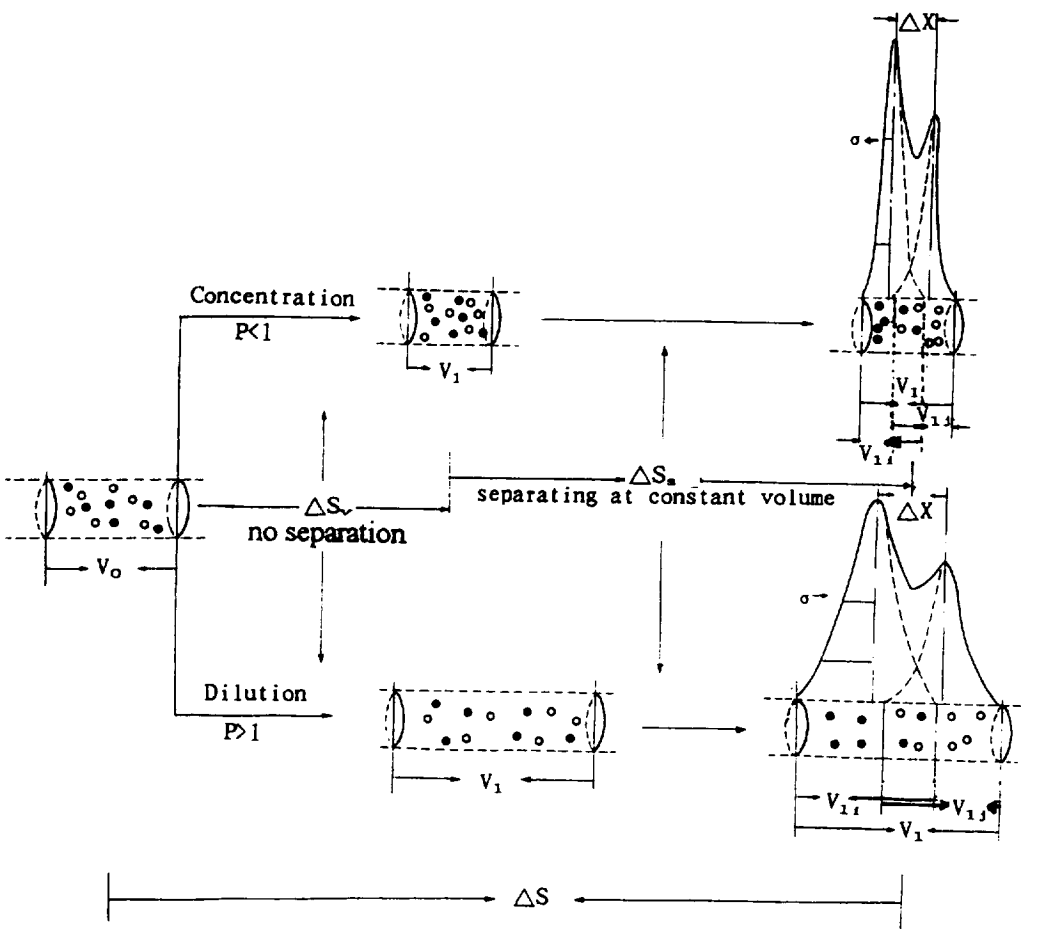

Fig. 1. Zone spreading and separation of the two-component system. The first step is the concentration or dilution of the components with no separation. The second step is the net separation with no change in the total volume.

According to Eq. (1), ΔS_V can be expressed as

$$\Delta S_V = \sum_{i=1}^{2} n_i R \ln P_i \tag{5}$$

where $P_i = V_1/V_0$, and is termed the volume ratio. If $P_i > 1$, it is a dilution process; if $P_i < 1$, it is a concentration process. With Fig. 1 and Eq. (1), for the net separation process, we have,

$$\Delta S_S = \sum_{i=1}^{2} n_i R \ln (V_{1i}/V_1) \tag{6}$$

If $3|\sigma_i - \sigma_{i+1}| \leq \Delta X \leq 3|\sigma_i + \sigma_{i+1}|$, where ΔX is the distance between the centres of gravity of adjoining zones, we can derive the following equation:

$$V_1 = A[3(\sigma_i + \sigma_{i+1}) + \Delta X] \tag{7}$$

Inserting Eqs. (2,7) and the definition of resolution, $R_s = \Delta X/[2(\sigma_i + \sigma_{i+1})]$ into Eq. (6), we have

$$\Delta S_S = \sum_{i=1}^{2} n_i R \ln [6\sigma_i/((\sigma_1 + \sigma_2)(3 + 2R_s))] \tag{8}$$

If $n_i = n_{i+1} = n$ and $\sigma_i = \sigma_{i+1} = \sigma$, Eq. (8) becomes

$$\Delta S_S = 2nR \ln [3/(3 + 2R_s)] \quad (0 < R_s \leq 1.5) \tag{9}$$

In the multi-component system, referring to Eq. (7), we have

$$V_1 = A\left(3\sigma_1 + \sum_{i=1}^{k-1} \Delta X_{i,i+1} + 3\sigma_k\right) \tag{10}$$

where $\Delta X_{i+1,i}$ is the distance between the two centres of gravity of zones $i+1$ and i. Referring to Eq. (5), we get

$$\Delta S_V = \sum_{i=1}^{k} n_i R \ln P_i \tag{11}$$

Considering that entropy is an extensive quantity, referring to Eq. (8), we can get ΔS_S of the multicomponent system due to the net separation, it is a function of $R_{s,i,i+1}$.

$$\Delta S_S = \sum_{i=1}^{k-1} n_i R \ln [6\sigma_i/(\sigma_i + \sigma_{i+1})(3 + 2R_{s,i,i+1})] \tag{12}$$

To relate ΔS to ΠR_s, we can define an equivalent parameter with resolution, name it thermodynamic resolution $R_{s,t}$)

$$R_{s,t,i,i+1} = 3/(3 + R_{s,i,i+1}) \tag{13}$$

If $n_i = n_{i+1} = n$ and $\sigma_i = \sigma_{i+1} = \sigma$, Eq. (12) becomes

$$\Delta S_S = nR \ln \left(\prod_{i=1}^{k-1} R_{s,t,i,i+1} \right) \tag{14}$$

with Eqs. (3,9,14), we can find that the entropy changes have become measurable parameters to indicate separation efficiency. The entropy changes are key physico-chemical parameters which relate to others, which are general optimizing parameters in the separations, especially in the entropy equation. This becomes more important, when we treat separation processes as the irreversible processes of thermodynamics. In truth, we can directly calculate ΔS_S form V_0, V_{1i} and V_{1j}. In the other words, ΔS_S does not depend on H, R_s and ΠR_s to indicate separation efficiency. With those relationships, it is only more convenient to calculate ΔS_S with H, R_s and ΠR_s, if one is used to doing so.

On the other hand, ΔS, as a state variable of the solute system, is controlled by macroscopic physico-chemical parameters, which are just the optimizing parameters in CE separation, inside the solute system and in interactions with its exterior. Therefore ΔS can be described by those parameters on the entropy equation of nonequilibrium thermodynamics; thus Eq. (4) becomes,

$$\Delta S_S = \Delta S_{(\text{Optimizing parameters})} - \Delta S_V \tag{15}$$

after that ΔS is developed with nonequilibrium thermodynamics, Eq. (15) will become objective functions, with which we can optimize CE operational conditions.

2.1.2. Objective optimizing function of CE separation

We choose only the solutes being separated in CE as our study. This system belongs to a non-equilibrium thermodynamic system, in which there are viscosity flows, external electric field and no chemical reaction. The k kinds of different solutes are labelled by i with molecular weight (M_i), charge (Z_i) and conformation parameter (R_{ci}). Let the axial x and the radial r coordinate be the axis and radius of the capillary, respectively. Considering that the capillary is cylindrical symmetry with the total length L (effective length l) and inner radius R_1, therefore the cylinder coordinate can be used to describe thermodynamic variables of the solute system in continuous space- and time-dependent fields.

In nonequilibrium thermodynamics [13,14], a basic entropy equation can be written

$$ds^*/dt = -\nabla \cdot J_s + \sigma_s \tag{16}$$

here ds^*/dt is hydrodynamic derivation of the entropy of solute system per unit volume; $\nabla_{ri} = \partial/\partial r$, a gradient operator, J_s entropy current and σ_s entropy production. For simplification, the following assumptions are adopted:

1. The axial temperature gradient may be neglected.
2. The migration of solutes is uniform velocity along the axial x coordinate.
3. The radius concentration gradient due to thermal diffusion (Soret effect [14]) is neglected.
4. The solute rotation is negligible.
5. The velocity of solutes in radius coordinate is zero.
6. The elasticity of the system is not considered.
7. There are not coupling effects among thermodynamic forces and fluxes.
8. The viscosity of the solute system is not affected by the electrical field.
9. The solute adsorption on capillary wall is not affected by temperature.

With assumption (1) to (9) in CE, for the solute system, we have obtained [18,19]

$$J_s = \sum_{j=1}^{2} J_{s,j} = \Lambda C_b E^2 r/2T_c + \sum_{i=1}^{k} s_i'[\rho_i FE/\alpha]\left(\Theta_i - \rho^{-1}\sum_{i=1}^{k}\rho_i\Theta_i\right) \quad (17)$$

where Λ is the equivalent conductance of buffer, C_b is the concentration of buffer electrolyte solution, E is electrical field strength, T_c is the temperature of cooling, s_i' is the transfer entropy density per unit mass of solute i, F is Faraday constant, ρ and ρ_i are total average density of solute system and solute i, respectively, $\alpha = k' \exp(\epsilon/RT_c)$, it is a constant relating to viscosity of buffer, k' is other constant relating to the viscosity of buffer, ϵ is the activation energy of buffer, and $\Theta_i = Z_i/M_i^{R_{ci}}$, we named it electrophoretic characterization factor of solute i, which is only determined by the solute electrophoretic parameters, Z_i, M_i and R_{ci} [20]. For the entropy production, we got

$$\begin{aligned}\sigma_s &= \sum_{j=1}^{6}\sigma_{s,j}\cdot \\ &= (\Lambda C_b r)^2 E^4/4k_1T_c^2 + \sum_{i=1}^{k}\Lambda_i z_i^2 F^2E^2/T_c^2 \\ &+ \sum_{i=1}^{k}(R^2T_c/C_i\alpha M_i^{R_{ci}})(dC_i/dx)^2 \\ &+ \sum_{i=1}^{k}(\kappa RT_c/C_b)\Delta l_0(FE\Theta_i/\alpha)(dC_i/dx)^2 \\ &+ \sum_{i=1}^{k}(D_i^{surf}R/n_i'^2C_i)(dC_i/dr)^2|_{r=R_1} \\ &+ a'F^2\Theta_i^2\Lambda^2C_b^2\epsilon^2r^2E^6/[4k_1^2\alpha^2T_c^4\exp(\epsilon/RT_c)]\end{aligned} \quad (18)$$

where k_1 is the thermal conductivity of buffer, Λ_i is the equivalent conductance of ith solute, z_i is the electronic charge per unit mass of the solute i, κ is a constant relating to electrophoretic dispersion of solutes, Δl_0 is the initial width of the sample plug, C_i is the concentration of solute i, D_i^{surf} is the diffusion coefficient of solute i on the internal face of capillary wall, n_i' is a constant in Freunidlich adsorption equation, a' is a constant in the viscosity equation, and $|_{r=R_1}$ indicates on the internal face of capillary wall.

In Eq. (17), the entropy flow is composed of both energetic in interaction with the exterior and material exchange terms amoung volume cells, dV, which relate to capillary cooling and solute relative migrations, respectively. They contribute in the CE system to the enhanced separation efficiency. In Eq. (18), the entropy production is composed of the six terms corresponding to time-dependent CE efficiency loss factors, which are heat conduction, four kinds of diffusion (electrical field, axial concentration gradient, electrophoretic dispersion and capillary wall adsorption) and viscous flow, respectively. From Eq. (16), we can obtain the mixed entropy change of the solute system in the whole CE separation process,

$$\Delta S = \Delta S_e + \Delta S_i = -\int_0^t dt \int_\Sigma J_s \cdot d_\Sigma + \int_0^t dt \int_V \sigma_s dV \quad (19)$$

here ΔS_e arises from the exchange of entropy with the surroundings of the solute system and ΔS_i comes from internal production or destruction of entropy in the whole CE separation process, t is the time of solute migration, and d_Σ is the surface element.

With Eq. (19) and the first term in the right-hand side of Eq. (17), the contribution to ΔS_e from heat transportation (energetic exchange) between the solute system and its surroundings, $\Delta S_{e,1}$, can be obtained

$$\Delta S_{e,1} = -\sum_{i=1}^{k}\int_0^{t_i} dt[\Lambda C_b E^2 R_1/(2T_c)](2\pi R_1)\left(3\sigma_1 + \sum_{i=1}^{k-1}\Delta X_{i,i+1} + 3\sigma_k\right) \quad (20)$$

For any solute with a different shape, we have presented an electrophoretic mechanical migration model (EMMM) to describe the solute migration in CE [18,20], so the electrophoretic velocity of ith solute is

$$U_i = [FE/\alpha]\Theta_i \quad (21)$$

thus $\Delta X_{i,i+1}$ in Eq. (20) can be written

$$\Delta X_{i+1,i} = (U_{i+1} - U_i)t_i = [FE/\alpha]\Delta\Theta_{i,i+1}t_i \quad (22)$$

here $\Delta\Theta_{i+1,i} = \Theta_{i+1} - \Theta_i$, it is the difference between solute $i+1$ and i in the electrophoretic characterization factor. As it is known that

$$\sigma_i = \sqrt{2D_i t_i} \quad (23)$$

here D_i is total diffusion coefficient of solute i. Let $D_i = D_k = D$, inserting Eqs. (22,23) into Eq. (20), we have

$$\Delta S_{e,1} = -[\Lambda C_b E^2/T_c]\Psi_k \quad (24)$$

where

$$\Psi_k = \pi R_1^2\left[4\sqrt{2D}(FE\Theta_i/\alpha)^{-3/2}l^{3/2} + (1/2)(FE/\alpha)^{-1}l^2\sum_{i=1}^{k-1}\Theta_i^{-2}\Delta\Theta_{i,i+1}\right] \quad (25)$$

we name Ψ_k the CE time–volume integral unit. If $k=2$, we get

$$\Delta S_{e,1} = -[\Lambda C_b E^2/T_c]\Psi_2 \quad (26)$$

where

$$\Psi_2 = \pi R_1^2[4\sqrt{2D}(FE\Theta_i/\alpha)^{-3/2}l^{3/2} + (1/2)(FE/\alpha)^{-1}l^2\Delta\Theta_{i+1,i}\Theta_i^{-2}] \quad (27)$$

With assumption D, the density of solute i can be obtained

$$\rho_i = n_i/(6\sigma_i A) \quad (28)$$

and the total density of the solute system can be written

$$\rho = n_t \Big/ lA\left(3\sigma_1 + \sum_{i=1}^{k-1}\Delta X_{i,i+1} + 3\sigma_k\right) \quad (29)$$

where n_i and n_t are the number of moles for solute i and the solute system, respectively. Inserting Eqs. (28,29) into Eq. (17), the second term of Eq. (17) can be written

$$J_{s,2} = \sum_{i=1}^{k} s_i'[n_i FE\Theta_i/(6A\alpha\sqrt{2D_i t_i})] \times\left\{1 - \sum_{i=1}^{k}\left(n_i\Big/\sum_{i=1}^{k} n_i\right)\left[1 + (FEt_i/6\alpha\sqrt{32D_i t_i})\sum_{i=1}^{k-1}\Delta\Theta_{i,i+1}\right]\right\} \quad (30)$$

If $k=2$, Eq. (30) becomes

$$J_{s,2} = -[(n_1 + n_2)/(72AD)](FE/\alpha)^2\Theta_1\Delta\Theta_{1,2}s' \quad (31)$$

With the first term on the right-hand side of Eqs. (19,31), the contribution to ΔS_e from mass transportation (material exchange), $\Delta S_{e,2}$ can be obtained

$$\Delta S_{e,2} = -[(n_1 + n_2)/(72D)]s'(FEl/\alpha)\Delta\Theta_{2,1} \quad (32)$$

With the second term of Eq. (19) and the first and second terms on the right-hand side of Eq. (18), the contribution to ΔS_i from heat conduction ($\Delta S_{i,1}$) and electrical field ($\Delta S_{i,2}$) can be written, respectively,

$$\Delta S_{i,1} = [(\Lambda C_b R_1)^2 E^4/8k_1 T_c]\Psi_k \quad (33)$$

$$\Delta S_{i,2} = \left(\sum_{i=1}^{k}\Lambda_i z_i^2 F^2 E^2/T_c^2\right)\Psi_k \quad (34)$$

For the third and fourth terms of Eq. (18), let

$$dC_i/dx = BC_i \quad (35)$$

where B is a constant relating to the axial diffusion of the solutes.

Inserting Eq. (35) into Eq. (18), with the second term of Eq. (19), the contribution to ΔS_i from the axial concentration gradient ($\Delta S_{i,3}$) and electrophoretic dispersion ($\Delta S_{i,4}$) can be written, respectively,

$$\Delta S_{i,3} = \left\{\sum_{i=1}^{k}[R^2 T_c/(A_0 M_i^{R_{ci}})]B^2 C_i\right\}\Psi_k \quad (36)$$

where A_0 is a constant relating to the diffusion of concentration of the solute and

$$\Delta S_{i,4} = \left[\sum_{i=1}^{k}(\kappa RT_c/C_b)\Delta l_0(FE\Theta_i/\alpha)B^2 C_i^2\right]\Psi_k \quad (37)$$

For the fifth term of Eq. (18), let

$$(dC_i/dr)\big|_{r=R_1} = B_1 C_i \quad (38)$$

where B_1 is a constant relating to the radial diffusion of the solutes on the internal face of capillary.

Inserting Eq. (38) into Eq. (18), with the second term of Eq. (19), the contribution to ΔS_i from wall adsorption ($\Delta S_{i,5}$) can be written

$$\Delta S_{i,5} = \left[\sum_{i=1}^{k} (D_i^{\text{surf}} R/n_i'^2) B_1^2 C_i\right] \Psi_k \tag{39}$$

In the same way, the contribution to ΔS_i from viscous flow ($\Delta S_{i,6}$) can be written

$$\Delta S_{i,6} = \{a' F^2 \Theta_i^2 \Lambda^2 C_b^2 \epsilon^2 R_1^2 E^6 / [4k_1^2 \alpha^2 T_c^4 \ln(\epsilon/RT_c)]\} \Psi_k \tag{40}$$

Inserting Eqs. (22,23,10) into Eq. (11), ΔS_V can be expressed

$$\Delta S_V = \sum_{i=1}^{k} n_i R \ln \left\{ (l/\Delta l_0) \left[3\sqrt{\alpha/Fl} (\sqrt{2D_1/\Theta_1} + \sqrt{2D_k/\Theta_k}) + \sum_{i=1}^{k-1} \Delta\Theta_{i,i+1}/\Theta_i \right] \right\} \tag{41}$$

if $k=2$, and let $D_1 = D_k = D$ and $t_i \approx t_{i+1}$, we have

$$\Delta S_V = \sum_{i=1}^{2} n_i R \ln \{ [(l/\Delta l_0)[6\sqrt{2\alpha D/Fl\Theta_1} + \Delta\Theta_{2,1}/\Theta_1]\} \tag{42}$$

Referring to Eqs. (3,41), we can build an objective function to optimize operational parameters for the mono-component system,

$$S_H = (1/2) n_i R \ln H_i = \Delta S_{(\text{Optimizing parameters})} - n_i R \ln (6l^{1/2}/\Delta l_0) \tag{43}$$

In the mono-component system, there is no relative migration of the solute, thus $\Delta S_{e,2} = 0$, the other terms of ΔS in the mono-component system are similar in the two- or multi-component solute system, with Ψ_1 replacing Ψ_k only, here

$$\Psi_1 = \pi R_1^2 [4\sqrt{2D_i} (FE\Theta_i/\alpha)^{-3/2} l^{3/2}] \tag{44}$$

At last, referring to Eqs. (24,33,36,37,39,40,44), then inserting them into Eq. (43), the objective function of the mono-component system can be written

$$\begin{aligned}
\Delta S_H &= \Delta S_{e,1} + \sum_{j=1}^{6} \Delta S_{i,j} - n_i R \ln (6l^{1/2}/\Delta l_0) \\
&= \pi R_1^2 [4\sqrt{2D_i} (FE\Theta_i/\alpha)^{-3/2} l^{3/2}] \{ \\
&\quad - [\Lambda C_b E^2/T_c] + [(\Lambda C_b R_1)^2 E^4/8k_1 T_c] \\
&\quad + (\Lambda_i z_i^2 F^2 E^2/T_c^2) + [R^2 T_c/(A_0 M_i^{R_{ci}})] B^2 C_i \\
&\quad + (\kappa RT_c/C_b) \Delta l_0 (FE\Theta_i/\alpha) B^2 C_i^2 \\
&\quad + (D_i^{\text{surf}} R/n_i'^2) B_1^2 C_i \\
&\quad + a'(F\Theta_i \Lambda C_b \epsilon R_1)^2 E^6 / [4k_1^2 \alpha^2 T_c^4 \ln(\epsilon/RT_c)]\} \\
&\quad - n_i R \ln (6l^{1/2}/\Delta l_0)
\end{aligned} \tag{45}$$

For the two-component system, $k=2$ and $\Psi_k = \Psi_2$, inserting Eqs. (24,32–34,36,37,39,40,42) into Eq. (15), the objective functions of two-component systems can be written

$$\begin{aligned}
\Delta S_S &= \sum_{k=1}^{2} \Delta S_{e,i} + \sum_{j=1}^{6} \Delta S_{i,j} - \Delta S_V \\
&= -[(n_1 + n_2)/(72D)] s'(FEl/\alpha) \Delta\Theta_{1,2} \\
&\quad + \pi R_1^2 [4\sqrt{2D} (FE\Theta_i/\alpha)^{-3/2} l^{3/2} \\
&\quad + (1/2)(FE/\alpha)^{-1} l^2 \Delta\Theta_{i,i+1} \Theta_i^{-2}] \times \Big\{ \\
&\quad - [\Lambda C_b E^2/T_c] + [(\Lambda C_b R_1)^2 E^4/8k_1 T_c] \\
&\quad + \sum_{i=1}^{2} (\Lambda_i z_i^2 F^2 E^2/T_c^2) \\
&\quad + \sum_{i=1}^{2} [R^2 T_c/(A_0 M_i^{R_{ci}})] B^2 C_i \\
&\quad + \sum_{i=1}^{2} (\kappa RT_c/C_b) \Delta l_0 (FE\Theta_i/\alpha) B^2 C_i^2 \\
&\quad + \sum_{i=1}^{2} (D_i^{\text{surf}} R/n_i'^2) B_1^2 C_i \\
&\quad + a'(F\Theta_i \Lambda C_b \epsilon R_1)^2 E^6 / [4k_1^2 \alpha^2 T_c^4 \ln(\epsilon/RT_c)] \Big\} \\
&\quad - \sum_{i=1}^{2} n_i R \ln \{ l/\Delta l_0)[6\sqrt{2\alpha D/Fl\Theta_1} + \Delta\Theta_{1,2}/\Theta_i]\}
\end{aligned} \tag{46}$$

For the multi-component solute system, the objective function can be obtained with the same way.

Up to the present, we have built a nonequilibrium

thermodynamic separation model (NTSM) in CE based on nonequilibrium thermodynamics with assumptions. The outstanding characteristics of the developed model is that the mixed entropy change of the solute system was chosen as a bridge between the separation efficiency of CE found in Eqs. (3,9,14), and optimizing parameters in the entropy equations of CE shown in Eqs. (45,46).

In Eq. (45), ΔS_H indicates the broadening of solute's zone under given CE operation conditions, the value of ΔS_H is determined by the unit of H_i, if $H_i>1$, $\Delta S_H>0$; if $1>H_i>0$, $\Delta S_H<0$; $\Delta S_{e,1}$ in Eq. (45) indicates the mixed entropy reduction of the mono-component solute system due to the heat transferred from the solute system with cooling, it was awarded qualitatively by Grushka [2], he said "cooling down will reduce the solute's diffusion coefficient, which may help to improve the system's efficiency". In NTSM, $\Delta S_{e,1}$ denotes equivalently the level of reduction of the solute's diffusion due to the thermal diffusion of solute zones. $\Sigma_{j=1}^{6}\ \Delta S_{i,j}$ in Eq. (45) are the contributions of ΔS_H due to the entropy production, which corresponds to six irreversible processes in CE separation, heat conduction, four kinds of diffusion (electrical field, axial concentration gradient, electrophoretic dispersion, wall adsorption) and viscous flow, respectively. Under typical CE operating conditions, $\Delta S_{i,j}>0$ and $\Sigma_{j=1}^{6}\ \Delta S_{i,j}>0$, thus the terms of the entropy production correspond to time-dependent efficiency loss factors in CE, respectively. The bigger the entropy production, the greater the loss of separation efficiency. The last term on the right-hand side of Eq. (45) indicates the contributions of ΔS_H due to the solute's migration distance (l) and injecting conditions (Δl_0). Therefore Eq. (45) reveals a quantitative relationship between the separation efficiency of the CE system for given solutes and optimizing parameters.

The fact that $\Delta S_{e,1}$ in Eq. (45) is negative compelled us to introduce the concept of "negative plate height" to indicate that the more negative the term in the contribution of ΔS_H, the better the separation efficiency. It is eminently important in optimizing operational conditions to understand negative plate height.

In Eq. (46), ΔS_S is the mixed entropy change in the net separation process only (see Fig. 1), thus $\Delta S_S<0$ in any effective separations. $\Sigma_{i=1}^{2}\ \Delta S_{e,i}$ in Eq. (46) are the contributions of ΔS_S due to the entropy flow, which relates to the capillary cooling and relative migrations of the solute, respectively, thus $\Delta S_{e,i}<0$ and $\Sigma_{i=1}^{2}\ \Delta S_{e,i}<0$ in any effective separations, it just shows that the CE system, as exterior surroundings, contributes to enhancing the separation efficiency of the solute system. The more the CE system provides the solute system with a negative entropy flow, the better the separation efficiency of the CE system. $\Sigma_{j=1}^{6}\ \Delta S_{i,j}$ in Eq. (46) are the contributions of ΔS_S due to the entropy production. They have the similar meanings in Eq. (45). ΔS_V in Eq. (46) shows the contributions of ΔS_S due to the injecting conditions (Δl_0) and the change of total volume all solutes occupy (V_1). Therefore Eq. (46) reveals a quantitative relationship between CE separation efficiency (ΔS_S) and optimizing parameters (electrical strength, coolant temperature, injecting conditions; the composition and concentration of the buffer; the radius, length and wall adsorption of the capillary; the concentration, charges, molecular weight and conformation of solutes, and so on). The more negative ΔS_S is, the better the separation efficiency is. It is thus obvious that Eq. (46) reflects quantitatively what Giddings said [15], separation is the art and science of maximizing separative transport (making $\Sigma_{i=1}^{2}\ \Delta S_{e,i}$ more negative) relative to dispersive transport (decreasing $\Sigma_{j=1}^{6}\ \Delta S_{i,j}$) in CE.

For the multi-component system, each term of their objective function has a similar meaning in the two-component system.

3. Experimental

All experiments were performed with the BioFocus 3000 capillary electrophoresis system as well as its corresponding capillary columns, samples and reagents (see 96 Life Science Research Products edited by Bio-Rad, Richmond, CA, USA). The complete integration/calibration software package of the BioFocus system allows the quantitation of all data for general separation parameters, such as standard deviation (σ), the number of theoretical plates (N), etc. Free zone capillary electrophoresis (CZE) was used for the determinations of N for the six peptides chosen from a peptide calibration set

(catalog No. 148-2012) with capillary cartridge (catalog No. 148-3031), coated capillary, 24 cm (effective length 17.5 cm)×25-μm I.D.; each peptide 50 ng/μl; buffer: 0.1 *M* phosphate, pH 2.5; load pressure, 20 p.s.i. s (1 p.s.i. = 6894.76 Pa); detection, UV, 200 nm, 0.02 AUFS; Cooling temperature, 25°C. Non-gel sieving of capillary electrophoresis (NGS) run with a coated capillary, 23.5 cm (effective length 17 cm)×50-μm I.D.; sample: CE–sodium dodecyl sulphate (SDS) protein sample buffer (catalog No. 148-5033); buffer: TBE (0.267 *M* Tris–boric acid, 2 m*M* EDTA) and polymer solution 0.8%; load pressure 40 p.s.i. s; detection, UV, 220 nm; cooling temperature, 25°C. The run voltage of two experiments is shown in Figs. 4 and 6 as field strength. As for the reproducibility, the relative standard deviations (R.S.D.) of the CZE were 0.5% for migration times and 1.2% for peak areas. For the NGS, R.S.D. is 0.4% for migration times and 1.0% for peak areas, the times taken for each of the two experiments was six, respectively. In CZE, the parameter N given by the software package automatically was used to directly calculate the relative value of ΔS_H of peptides with the equation: $\Delta S_H = \ln(l/N) = \ln(17.5/N)$, which was similar to Eq. (43), so the value of ΔS_H was negative in Fig. 4, the qualitative shape of $\Delta S_H \sim E$ was not affected by this simplified method. In NGS, the parameters σ given by the software package and R_s calculated by us with electrophoresis results of two adjoining peaks of SDS–proteins were used to calculate the relative value of ΔS_S with the equation:

$$\Delta S_S = \sum_{i=1}^{2} \ln[6\sigma_i/((\sigma_1 + \sigma_2)(3 + 2R_s))],$$

which was similar to Eq. (8) in Fig. 6.

4. Results and discussion

It is necessary to classify functionally optimizing parameters. Factors influencing separation efficiency include instrument operating conditions, voltage (V), cooling temperature (T_c) and injection width (Δl_0); capillary conditions, capillary dimensions [length to detector (l), total length (L), inside radius (R_1)] and wall adsorption (D_i^{surf}, n_i'); buffer electrolyte concentration (C_b), thermal conductivity (k_1), equivalent conductance (Λ), viscosity (η); electrophoretic parameters of solutes, charges (Z_i), molecular weight (M_i) and conformation (R_{ci}). The four kinds of factors influencing performance are reflected in the terms on the right-side of Eqs. (45,46) to influence CE separation efficiency (ΔS_H or ΔS_S). Fig. 2 shows the complete picture of how the factors affect on the separation efficiency in NTSM.

There are two kinds of variables in optimizing CE parameters. One is the independent variables, which are independent and adjustable in CE practice, and the other is the derived variables, whose changes are caused by other independent variables. Obviously, if a lot of parameters could be optimized with one objective function, it is required that the derived variables should be as few as possible in the function. At least, when a parameter is optimized, the other parameters are constant for this parameter in the objective function. For example, we have, $E = V/L$, $t_i = l/U_i$, $U_i = [FE/\alpha]\Theta_i$ and $\alpha = k' \exp(\epsilon/RT_c)$, etc., here V, L, l, Θ_i, T_c and ϵ are independent variables, but E, t, U_i and α are derived variables, thus we have to replace derived variables with independent variables in the building of objective optimizing functions.

The pH of the buffer is a special variable, although pH does not appear in the objective functions, in fact, the pH influences CE performance by the other variables in NTSM. For example, the change of pH will cause the change of solute's migration due to the changes of the solute electrophoretic parameters (Z_i, M_i, R_{ci}), wall adsorption (D_i^{surf}, n_i') and electrophoretic dispersion (C_b, Θ_i). To optimize pH with NTSM, we must know the relationships between pH and solute's migration, wall adsorption as well as electrophoretic dispersion.

In building NTSM, we assume that electroosmotic mobility (U_{eo}) is zero, thus NTSM is only valid in the types of CE with $U_{eo} = 0$. For example, capillary zone electrophoresis (CZE) in the lower pH range (<2.5) or NGS and capillary gel electrophoresis (CGE) with a very small U_{eo}. Electroosmotic flow (EOF) itself does not play a significant role in influencing separation, since it influences directly the centre of mass velocity of zones, but it does not relate directly to the separation among solute zones. When EOF, the adsorption of solutes on separation

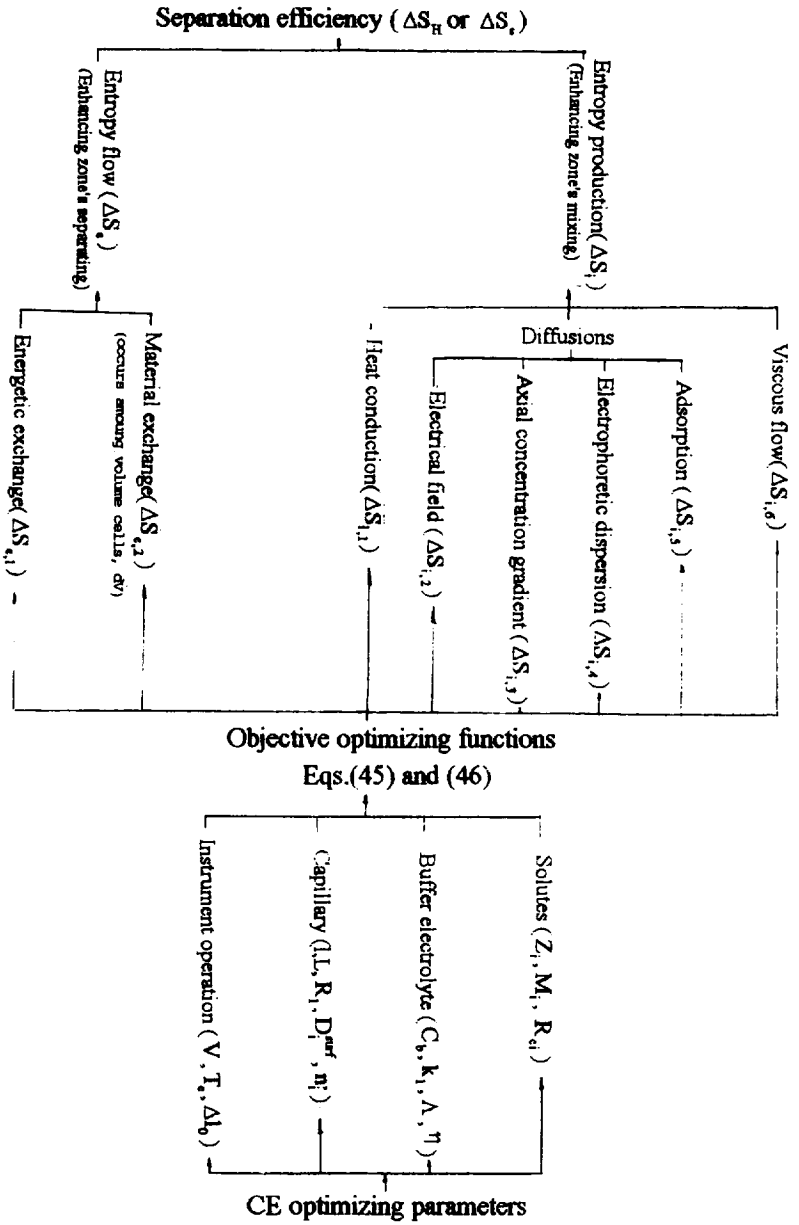

Fig. 2. Influence of operational parameters on the CE separation efficiency in NTSM.

surfaces (e.g. SDS micelle) and other factors are considered in NTSM, this model will be used in other separation systems, such as micellar electrochromatography, electrokinetic chromatography, etc.

In NTSM, we emphasized that the CE system should be matched to the solute system, which is reflected in the CE time–volume integral unit (Ψ_k), other terms relating to solute's migration (Θ_i) and the adsorption of solute on to the capillary wall (D_i^{surf}, n_i'). In other words, only when the solutes are determined, can the function (ΔS_S) be used to evaluated the efficiency of the CE system.

In principle, we can optimize any operation parameter in the objective functions with NTSM. In

this paper, only injection sample length (Δl_0) and field strength (E) are discussed with experimental data.

4.1. Injection conditions

The contribution of injection conditions to band broadening is usually described by Eq. (47),

$$\sigma_1^2 = \Delta l_0^2/12 \tag{47}$$

where σ_1^2 is the variance caused by finite injection volume. Grossman and Colburn [3] pointed out that Eq. (47) is not valid if the sample is injected directly into the capillary, some quantitative expression was derived to estimate the contribution of the injection plug to the overall variance. Huang [10] found that there was some obvious deviation between the experiment data and the model based on Eq. (47) for injection lengths greater than 0.6 cm. In NTSM, inserting the definition of plate height into the left-side of Eq. (45), we can obtain

$$\ln w = A_2 + B_2 \ln \Delta l_0 \tag{48}$$

where w is the spatial width of peaks [10], and

$$A_2 = \Delta S_{e,1} + \sum_{j=1}^{6} \Delta S_{i,j} - n_i R \ln (6l^{1/2}) + \ln l,\ B_2 = n_i R,$$

we dealt with Huang's experimental data using Eq. (48) with linear correlation, the results are shown in Table 1.

Under typical experimental conditions, A_2 and B_2 must be constant for the given solutes, we will find a linear relationship between ln w and ln Δl_0. We must point out that none of the 11 experimental points were cut off for the three types of solutes, when we dealt with this experimental data using Eq. (48). From Table 1, we can find Eq. (48) has a better correlation coefficient (R), the smallest value (0.9399) of R is obviously bigger than 0.735. On statistics, the linear relationship is true, if $R>0.735$ with $n-2=9$ degrees of freedom and significance level is 1%. In Eq. (48), A_2 is a constant relating to the character of the solute, Table 1 shows obvious differences between the three types of solute; B_2 is a constant relating to the mole number of the solutes, the concentration of each solute is $1\cdot10^{-5}$ M in the experiment. Just as expected, the correlation results shows that the average B_2 is 0.1441 ± 0.0046 for the solutes. Thus NTSM agreed with experimental facts on the estimate for injection conditions influencing separation efficiency.

Table 1
The correlation parameters by Eq. (48) with Huang's experimental data [10]

Solutes	A_2	B_2	R	$n-2$[a]
Adenosine	−1.3402	0.1431	0.9711	9
Adenosine monophosphate	−1.0037	0.1510	0.9399	9
Pyroximine	−1.7076	0.1383	0.9497	9
Average		0.1441 ± 0.0046		

[a] n is the number of experimental points.

4.2. Field strength

Many authors [3–5] studied deeply about optimizing field strength in CE, they have noted that as E increases, the variance caused by diffusion decreases, and the variance caused by temperature profile increases, thus there must exist an optimal field strength (E_{opt}) that minimizes the combined variance. In NTSM, it was schematically shown in Fig. 3 that the quantitative relationship between field strength and separation efficiency (ΔS_H) in Eq. (45) for mono-component system.

In Fig. 3, as E increases, the terms of the viscous flow ($\Delta S_{i,6}$), heat conduction ($\Delta S_{i,1}$) and electrical field ($\Delta S_{i,2}$) increase with difference power of E, thus these three kinds of factors lose separation efficiency (making ΔS_H increase) more greatly as E increases. On the other hand, the terms of axial concentration gradient ($\Delta S_{i,3}$), electrophoretic dispersion ($\Delta S_{i,4}$) and wall adsorption ($\Delta S_{i,5}$) decrease as E increases, which is due mainly to the decrease of solute migration time. Heat transportation ($\Delta S_{e,1}$) is negative across the whole scope of field strength, thus it is a factor that enhances separation efficiency (making ΔS_H decrease) due to the fact that more Joule heat is taken off from the solute system as E increases. The objective function (ΔS_H) is the sum of those seven effects ($\Delta S_{e,1}$, $\Sigma_{j=1}^{6}$ $\Delta S_{i,j}$), the optimal field strength (E_{opt}), which is shown in Fig. 3, and can be derived by using Eq. (45).

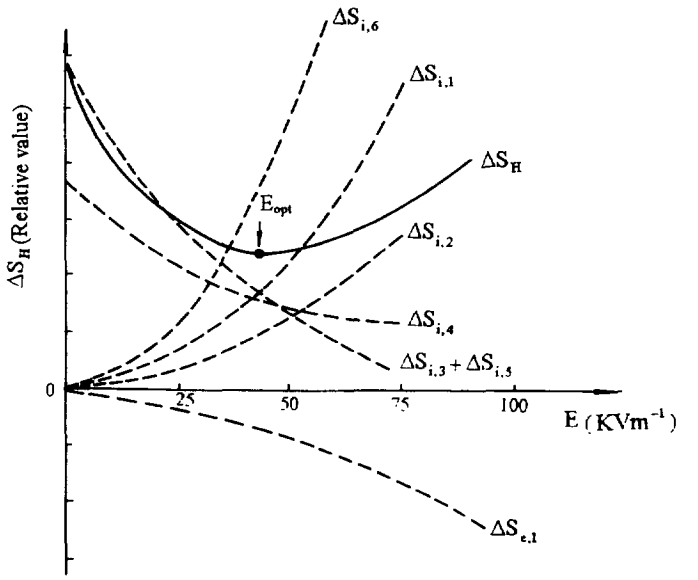

Fig. 3. Schematic drawing of the influence of field strength on separation efficiency for the monocomponent system under typical experimental conditions of CE. These curves were generated using Eq. (45). E_{opt} is also shown.

Fig. 4 shows the relationship between ΔS_H and E from our experimental results for five peptides in CZE [18]. It was found that the experimental results supported the theoretical prediction of Eq. (45) by comparing Fig. 4 and Fig. 3. There are other experimental data in the literature [21] that support the estimate of Eq. (45) also.

Fig. 5 is based on Eq. (46) for the two-component

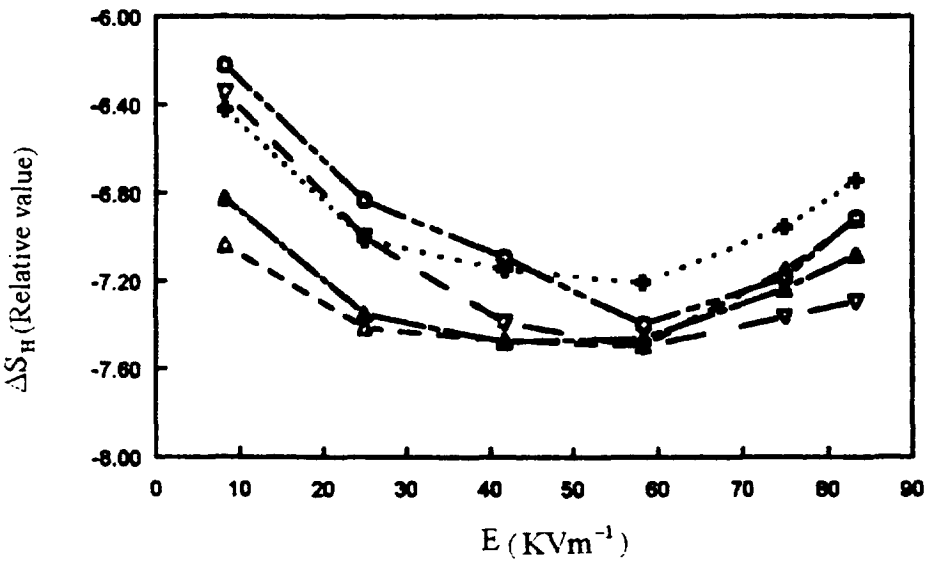

Fig. 4. Dependence of separation efficiency (ΔS_H) on field strength for the experimental data for peptides in CZE. ○, Bombesin; ▽, Leucine enkephalin; +, TRH; ▲, LHRH; △, Angiotensin II. For operating conditions, refer to Section 3.

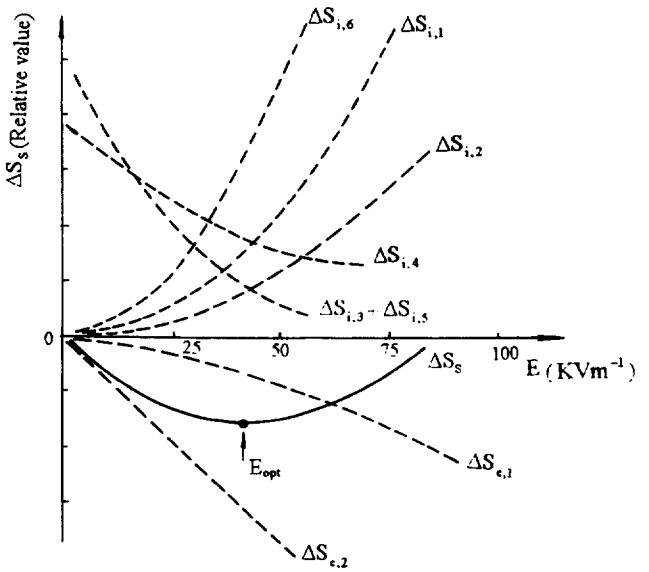

Fig. 5. Schematic drawing of influence of field strength on the separation efficiency (ΔS_S) for the two-component system, under typical experimental conditions of CE. These curves were generated using Eq. (46). E_{opt} is also shown.

system, it shows how eight kinds of factors influence separation efficiency (ΔS_S) as E increases. Here $\Delta S_{e,1}$ and $\Sigma_{j=1}^{6} \Delta S_{i,j}$ have the similar meanings to those in Fig. 3, and $\Delta S_{e,2}$ corresponds to the relative migration between two adjoining solute zones. The terms of energy transportation ($\Delta S_{e,1}$) and mass transportation ($\Delta S_{e,2}$) are negative across the whole scope of field strength. $\Delta S_{e,1}$ and $\Delta S_{e,2}$ are more negative, which correspond to the enhanced separation system as E increases. This is why the high

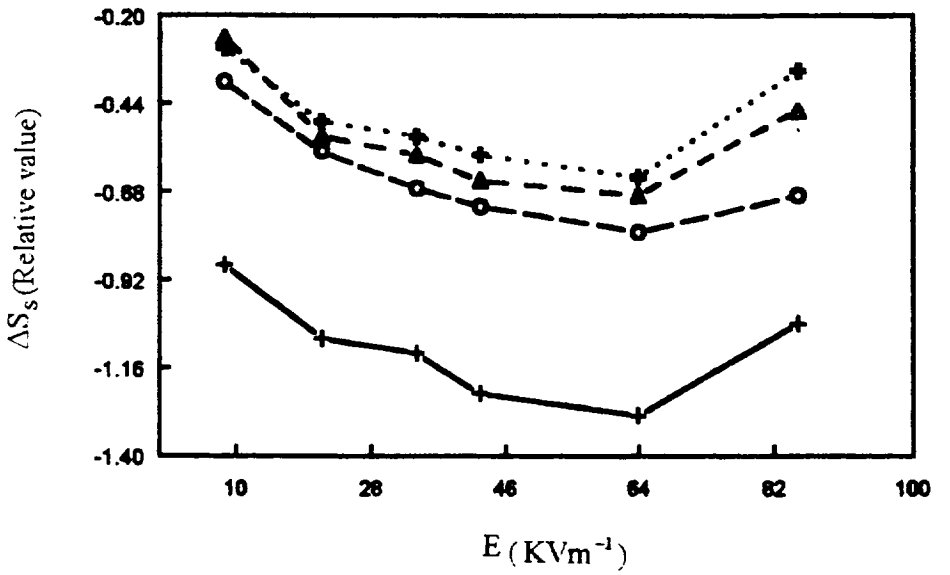

Fig. 6. Dependence of separation efficiency (ΔS_S) on field strength for the experimental data between SDS–proteins in NGS. ···+···, phosphorylase B and β-galactosidase; △, ovalbumin and serumalbumin; ○, serum albumin and phosphorylase B; – + –, trypsin inhibitor and carbonic anhydrase. For operating conditions, refer to Section 3.

field strength is adopted as the driving force for the CE separation with fine cooling (making $\Delta S_{e,1}$ more negative). The objective function (ΔS_S) is the sum of those eight effects ($\Sigma_{i=1}^{2}\ \Delta S_{e,i}$, $\Sigma_{j=1}^{6}\ \Delta S_{i,j}$). The optimal field strength (E_{opt}) is shown in Fig. 5, and corresponds to the minimum of ΔS_S for the best separation efficiency, and it can be derived by using Eq. (46).

Fig. 6 shows the relationship between ΔS_S and E from our experimental results for the separation between two adjoining SDS-proteins in NGS [18]. The similar shape between Fig. 5 and Fig. 6 reflects that the experimental data support our theoretical prediction of Eq. (46), experimental data that support Eq. (46) can be found in the literature [22].

Analogously, the cooling temperature, the concentration of buffer, the radius and length of capillaries, etc. could be optimized with the objective functions in NTSM.

5. Conclusions

(1)The nonequilibrium thermodynamic separation model (NTSM) was presented based on nonequilibrium thermodynamics. The mixed entropy change of the solute system was chosen as a bridge between the separation efficiency and operational parameters.

(2) The objective functions in NTSM can be used to evaluate the separation efficiency of CE systems and to optimize operational parameters for the given solutes.

(3) This method or procedure could be extended in other separation technology.

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