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THEORY OF ISOTACHOPHORESIS (DISPLACEMENT ELECTROPHORESIS, TRANSPHORESIS)

T. W. NEE

Department of Physics and Optical Science Center, University of Arizona, Tucson, Ariz. 85721 (U.S.A.)

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SUMMARY

The fundamental formulas of electrophoresis are derived microscopically and applied to the problem of isotachophoresis. A simple physical model of the isotachophoresis front is proposed. The front motion and structure are studied in the simplified case without convection, diffusion and non-electric external forces. This is an essential improvement of Kohlrausch's theory.

INTRODUCTION

Electrophoresis[1] is the separation of charged components of a solution in an electric field due to their different electric mobilities. This technique has been extensively used in bio-medical researches. Many different techniques have been developed. A useful one is the isotachophoresis[2] (displacement electrophoresis[3], transphoresis[4]). Isotachophoresis is the migration in an electric field of different ion species of the same sign, all having common counter-ion species. In the steady state, these different ion species are spatially separated. The ions of higher mobility are moving ahead of those of lower mobility. The boundaries (fronts) between the different ion species all move with the same velocity. In 1897, Kohlrausch[5] developed a theory for the conditions existing in a migrating boundary. He derived a relationship between the two salt concentrations on opposite sides of the boundary and the mobilities of the ions involved. This is the fundamental formula of isotachophoresis experiments.

In this paper, we shall study isotachophoresis from basic microscopic principle. An essential improvement of Kohlrausch's theory will be developed. In the next section, we shall review first the microscopic derivation of fluid dynamics. A physical model of ionic solutions will be introduced and the fundamental equations derived. Further, we shall present a simple model for the front motion in isotachophoresis. In addition to Kohlrausch's formula, expressions for the electric field and ionic densities in the front region will be derived. Discussion and conclusions are given in the final section.

MICROSCOPIC THEORY OF CHARGED FLUID

Classical theory of fluid

We consider a fluid system consisting of N identical particles. Each particle has mass m, position $\vec{r}_j(t)$ and momentum $\vec{p}_j(t)$, $(j = 1,2,\ldots N)$ at a given instant of time t. The particle density $n(\vec{r},t)$ and current density $\vec{J}(r,t)$ at position $\vec{r}$ and time t are defined as

$$n(\vec{r},t) = \langle \sum_{j=1}^{N} \delta\,(\vec{r} - \vec{r}_j(t)) \rangle \tag{1}$$

and

$$\vec{J}(\vec{r},t) = \langle \sum_{j=1}^{N} \delta\,(\vec{r} - \vec{r}_j(t)) \frac{p_j(t)}{m} \rangle \tag{2}$$

where $\langle \quad \rangle$ is the equilibrium ensemble average. From the classical equation of motion

$$\dot{\vec{r}}_j(t) = \frac{1}{m} \vec{p}_j(t) \tag{3a}$$

and

$$\dot{\vec{p}}(t) = \vec{F}_j(t), \tag{3b}$$

we can derive the equation of continuity

$$\frac{\partial}{\partial t} n(r,t) + \nabla \cdot \vec{J}(\vec{r},t) = 0 \tag{4}$$

and the momentum conservation equation

$$m \cdot n \cdot \frac{d}{dt} \vec{v}(\vec{r},t) = \vec{F}_B(\vec{r},t) + \nabla \cdot \overleftrightarrow{T}(\vec{r},t) \tag{5}$$

where ∇, is the gradient operator.

$$\frac{d}{dt} = \frac{\partial}{\partial t} + \vec{v} \cdot \nabla, \tag{6}$$

$$\vec{v}(\vec{r},t) = \frac{1}{n(\vec{r},t)} \cdot \vec{J}(\vec{r},t) \tag{7}$$

is the fluid velocity, and

$$\vec{F}_B(\vec{r},t) = \langle \sum_{j=1}^{N} \delta(\vec{r} - \vec{r}_j(t))\, \vec{F}_j(t) \rangle \tag{8}$$

is the bulk force density. $\vec{F}_j(t)$ is the force acting on the jth particle at time t.

$$\overleftrightarrow{T}(\vec{r},t) = -\,m \cdot \langle \sum_{j=1}^{N} \delta(\vec{r} - \vec{r}_j(t)) \left(\frac{\vec{p}_j(t)}{m} - \vec{v}\right) \left(\frac{\vec{p}_j(t)}{m} - \vec{v}\right) \rangle \tag{9}$$

is the stress force tensor.

Simple model of ionic solution

In order to develop a fundamental theory of ionic motion in solution, we consider a simple model: A system of N ions each with mass m and charge q and moving in a viscous solvent fluid. The force acting on the jth ion is

$$\vec{F}_j(t) = q \cdot \vec{E}(\vec{r}_j(t),t) - \zeta \cdot \dot{\vec{r}}_j(t) + \vec{F}_j^{\text{ext}}(t) \tag{10}$$

where $\vec{E}(\vec{r}_j(t),t)$ is the electric field at the ionic location, ζ the frictional constant due to the viscous surrounding medium —the solvent fluid and other ions. $\vec{F}_j^{\text{ext}}$ is the force due to non-electric external source.

Substituting eqn. 10 into eqn. 8, we obtain the bulk force density

$$\vec{F}_B(\vec{r},t) = q \cdot n(\vec{r},t) \cdot \vec{E}(\vec{r},t) - \zeta \cdot \vec{E}\,\vec{J}(\vec{r},t) + F_B^{\text{ext}}(\vec{r},t) \tag{11}$$

The stress tensor can be written in a standard form[6]

$$\overleftrightarrow{T}(\vec{r},t) = - P_i(\vec{r},t) \cdot \overleftrightarrow{I} + \overleftrightarrow{\sigma}(\vec{r},t) \tag{12}$$

where P_i is the ionic partial pressure, σ the internal frictional force tensor among the ions[6],

$$\nabla \cdot \overleftrightarrow{\sigma} = \eta \cdot \nabla^2 \vec{v} + \left(\nu + \frac{\eta}{3}\right) \nabla (\nabla \cdot \vec{v}) \tag{13}$$

η and ν are the viscosity coefficients. Substituting eqns. 11–13 into eqn. 5, we have the formal equation of motion of the ionic fluid:

$$m \cdot n \cdot \frac{\mathrm{d}}{\mathrm{d}t} \vec{v} = - \zeta\, [\vec{J} \underset{\left(\begin{smallmatrix}\text{positive} \\ \text{negative}\end{smallmatrix}\; q\right)}{\mp} \mu \cdot n \cdot \vec{E}] - \nabla P_i + \eta \cdot \nabla^2 \vec{v} + \left(\nu + \frac{\eta}{3}\right) \nabla (\nabla \cdot \vec{v}) + \vec{F}_B^{\text{ext}} \tag{14}$$

where

$$\mu = |q|/\zeta \tag{15}$$

is the ionic mobility.

In the electrophoresis experiments, the ions are cells or proteins, they are small macroscopic particles or giant molecules. In this situation, the frictional effect is so great that the inertial effect is not important. In the absence of convection,

$$m \cdot n \cdot \frac{\mathrm{d}}{\mathrm{d}t} \vec{v} = 0$$

Then from eqn. 14 we have the current density

$$\vec{J} = \pm \mu \cdot n \cdot \vec{E} + \frac{1}{\zeta} \left\{ -\nabla P_i + \eta \cdot \nabla^2 \vec{v} + \left(\nu + \frac{\eta}{3}\right) \nabla (\nabla \cdot \vec{v}) + \vec{F}_B^{\text{ext}} \right\} \tag{16}$$

Motion of ionic packet in a uniform electric field

As a practical example, we consider the case of dilute solution where the ions can be considered as a system of dilute gas. The pressure P_i is related to the density by the ideal gas law

$$P_i(\vec{r},t) = k_B \cdot T \cdot n(\vec{r},t)$$

where k_B is the Boltzmann constant; and the ionic internal frictional force tensor $\overleftrightarrow{\sigma}$ is negligibly small. Furthermore, we consider the isothermal case (T = constant) where there is no convection. Eqn. 16 reduces to

$$J(\vec{r},\vec{t}) = \pm \mu \cdot n \cdot \vec{E} - D \cdot \nabla \cdot n \tag{17}$$

where

$$D = k_B \cdot T/\zeta \tag{18}$$

is the diffusion constant. If we neglect the electric screening effect, *i.e.* $\vec{E} \simeq$ constant, we can substitute eqn. 17 into eqn. 4 and obtain the equation of motion for $n(r,t)$:

$$\left[\frac{\partial}{\partial t} - D \cdot \nabla^2 \pm \mu \cdot \vec{E} \cdot \nabla\right] n(\vec{r},t) = 0 \tag{19}$$

If we assume initially, $t = 0$, the ionic packet has the Gaussian form of width $1/\sqrt{\alpha}$

$$n(\vec{r},0) = N \left(\frac{\alpha}{\pi}\right)^{3/2} [\exp - \alpha r\, {}^{r^2}] \tag{20}$$

Eqn. 19 can be solved and obtain the solution

$$n(\vec{r},t) = N \left(\frac{\alpha}{\pi}\right)^{3/2} \cdot \frac{1}{(1 + 4D \cdot \alpha \cdot t)^{3/2}} \cdot \exp\,[- \alpha\,(\vec{r} \mp \vec{\mu} \cdot E \cdot t)^2/ (1 + 4\,D \cdot \alpha \cdot t)] \tag{21}$$

The ion packet is moving with the drift velocity $\vec{v}_D = \pm \mu \cdot E$ and being broadened due to diffusion ($D \neq 0$).

SIMPLE MODEL OF ISOTACHOPHORESIS FRONT

As a simple model of isotachophoresis, we consider two salt solutions $A_1^+\,B^-$ and $A_2^+\,B^-$ in a long tube of isotachophoresis experiment. According to Kohlrausch's theory[5], the two salts are separated by a plane boundary (front) that is moving with constant velocity v in the steady state. The densities n_1, n_2 of the two ion species A_1^+ and A_2^+ are related by the relation[2,5]

$$\frac{n_1}{n_2} = \frac{\mu_1}{\mu_1 + \mu_B} \cdot \frac{\mu_2 + \mu_B}{\mu_2} \tag{22}$$

where μ_1, μ_2 and μ_B are the mobilities of A_1^+, A_2^+ and B^-, respectively. The electric fields E_1 and E_2 in the two regions are related by

$$\mu_1 \cdot E_1 = \mu_2 \cdot E_2 = v \tag{23}$$

The physical situations are shown in Fig. 1.

It is shown in Fig. 1 that there is electric field discontinuity across the front plane because of the different mobilities ($\mu_1 < \mu_2$) of the two positive ions, A_1^+ and A_2^+. According to the theory of electromagnetism, there should be surface charges on the front plane. Where do these charges come from? What is the mechanism to build up these surface charges? How much are they?

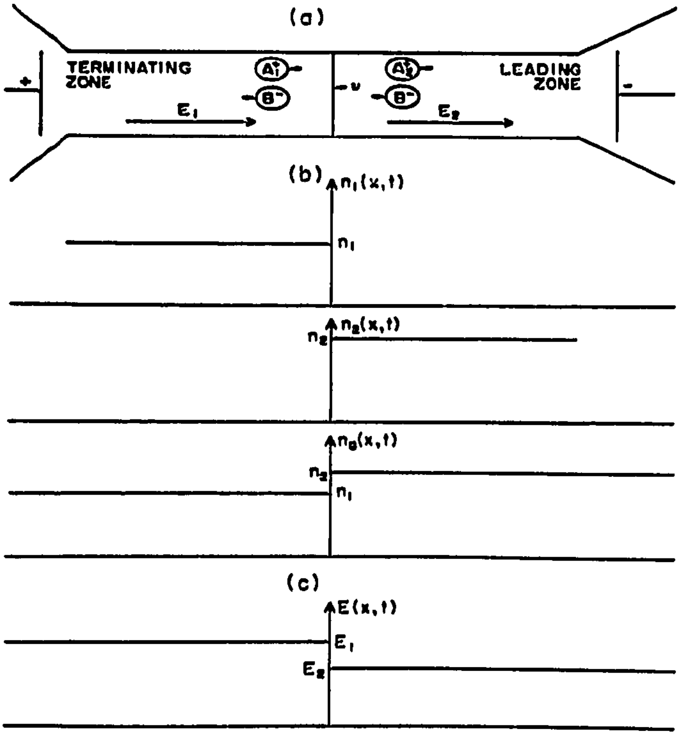

Fig. 1. (a) The ions A_1^+ and A_2^+ are migrating in separated zones. The ions A_2^+ in the leading zone have higher mobilities than A_1^+ in the terminating zone. B^- are the common counter-ions in the two zones. The zone boundary (front) is moving with constant velocity v in the steady state. (b) The ion concentrations in the two zones. (c) The electric fields in the two zones.

In order to answer these questions, we propose a simple model for the structure of the front as follows. The front is not a "plane" but a "region" of finite thickness "δ". Let n_1, n_2 and n_B be the concentrations of the three ions, E the electric field. The fundamental equations are:

$$\frac{\partial}{\partial t} n_i\,(x,t) + \frac{\partial}{\partial x} J_i\,(x,t) = 0,\ (i = 1, 2, \mathrm{B}) \tag{24}$$

where

$$J_i\,(x,t) = \mu_i\, n_i\,(x,t)\, E\,(x,t) - D_i \frac{\partial}{\partial x} n_i\,(x,t)\ (i = 1, 2) \tag{25a}$$

$$J_\mathrm{B}\,(x,t) = -\,\mu_\mathrm{B}\, n_\mathrm{B}\,(x,t)\, E\,(x,t) - D_\mathrm{B} \frac{\partial}{\partial x} n_\mathrm{B}\,(x,t), \tag{25b}$$

and Poisson's equation

$$\frac{\partial}{\partial x} E(x,t) = 4\pi \varrho_e(x,t) \tag{26}$$

where

$$\varrho_e(x,t) = e[n_1(x,t) + n_2(x,t) - n_\mathrm{B}(x,t)] \tag{27}$$

is the electric charge density and e is the positive ion charge. We have chosen the x-axis along the isotachophoresis tube axis, and work the simplest one-dimensional case.

It is understood from eqns. 26 and 27 that inside the front region, there is a non-vanishing negative charge distribution, $\varrho_e < 0$, which comes from the excess of negative ions n_B over the positive ions $n_1 + n_2$. This non-neutral charge distribution is formed because the two kinds of positive ion packets are moving with different velocities:

$$v_1 = \mu_1 \cdot E < \mu_2 \cdot E = v_2$$

at where both kinds of positive ions are present during the electrophoretic separation process. This separation mechanism makes the two positive ion clouds become further apart after separation leaving a region with more negative ions.

With this physical picture, despite the mathematical difficulty in solving these non-equilibrium eqns. 24–27, we may study some characteristic and quantitative properties in a simplified case: the steady state and non-diffusive case. In the absence of diffusion, from eqn. 21, we know the ion packet shape is not changing ($D = 0$). Then we consider the very special situation where the positive ions A_1^+ and A_2^+ have fixed packet with sharp plane boundaries. Therefore, there exist three regions: two neutral regions with $A_1^+B^-$ and $A_2^+B^-$ salt solutions and a front region of finite thickness δ and with ion B^- only. This front region is bounded by the two sharp positive ion boundary planes that are moving with the same velocity v. Inside the front region, there is a non-vanishing charge density $\varrho_e = -e \cdot n_B$, which contributed to the electric field variation $E_2 - E_1 < 0$ over the front region.

For mathematical simplicity, we chose the moving coordinate axis with origin fixed on the left boundary plane of the front, *i.e.*,

$$n_1(x,t) = \begin{cases} n_1 & x < 0 \\ 0 & x > 0 \end{cases} \tag{28a}$$

$$n_2(x,t) \quad \begin{cases} 0 & x < \delta \\ n_2 & x > \delta \end{cases} \tag{28b}$$

and

$$n_B(x,t) = \begin{cases} n_1 & x < 0 \\ n_{Bf}(x) & 0 < x < \delta \\ n_2 & x > \delta \end{cases} \tag{28c}$$

This physical situation is shown in Fig. 2.

The front velocity v is the same as the velocities of the two positive ion packets, *i.e.*,

$$v = \mu_1 \cdot E_1 = \mu_2 \cdot E_2 \tag{29}$$

This is the same as eqn. 23 of Kohlrausch's theory.

On the moving frame, the velocities of ion packets are

$$v_1' = 0 \tag{30a}$$

$$v_2' = 0 \tag{30b}$$

$$v_{B1}' = \mu_B \cdot E_1 - v = -(\mu_B + \mu_1)\, E_1 \,(x < 0) \tag{30c}$$

$$v_{B2}' = \mu_B \cdot E_2 - v = -(\mu_B + \mu_2)\, E_2 \,(x > \delta), \tag{30d}$$

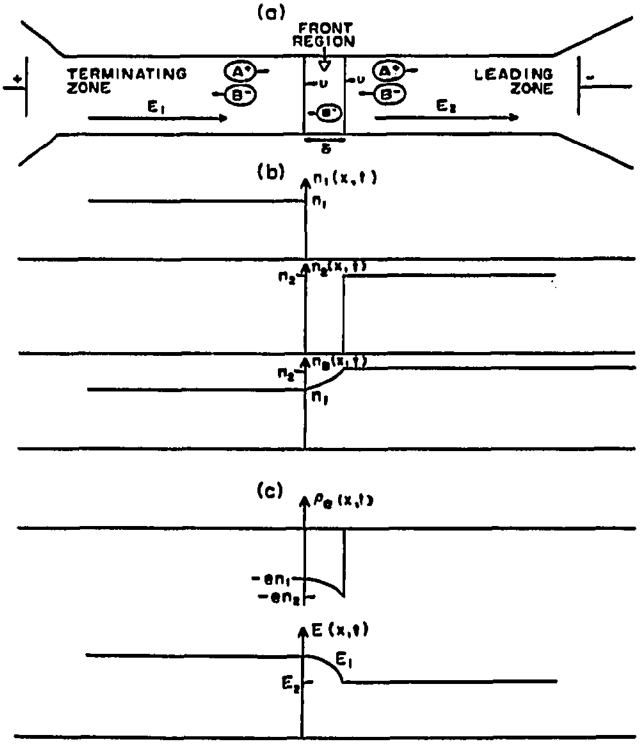

Fig. 2. (a) In addition to the leading and terminating zones, there is a front region with counter-ions B⁻ and moving with constant velocity *v* in the steady state. (b) The ion concentrations in the two zones and the front region. (c) The charge distribution and the electric fields in the two zones and the front region.

and the current densities are

$$J_1' = -e \cdot n_1 \cdot v_{B1}' = e \cdot n_1 (\mu_B + \mu_1) E_1 \quad (x < 0) \tag{31a}$$

and

$$J_2' = -e \cdot n_2 \cdot v_{B2}' = e \cdot n_2 (\mu_B + \mu_2) E_2 \quad (x > \delta) \tag{31b}$$

in the two positive ion regions. In the front region,

$$E = E_f(x) \qquad (0 < x < \delta) \tag{32}$$

and

$$J_f' = e \cdot n_{Bf}(x) \, [\mu_B E_f(x) + v] \quad (0 < x < \delta) \tag{33}$$

From the equation of continuity, we have

$$J_1' = J_f' = J_2' \tag{34}$$

This leads to Kohlrausch's equation

$$\frac{n_1}{n_2} = \frac{\mu_1}{\mu_1 + \mu_B} \cdot \frac{\mu_2 + \mu_B}{\mu_2} \tag{35}$$

and the negative charged particle density in the front region:

$$n_{Bf}(x) = n_j \cdot \frac{E_j + \nu/\mu_B}{E_f(x) + \nu/\mu_B} \quad (j = 1, 2) \tag{36}$$

The electric field $E_f(x)$ in front region is related to $n_{Bf}(x)$ by Poisson's equation:

$$E_f'(x) = -4\pi e \cdot n_{Bf}(x) \tag{37}$$

Substituting eqn. 36 into eqn. 37 and using the boundary condition

$$E_f(0) = E_1, \tag{38}$$

we obtain

$$E_f(x) = -\frac{\nu}{\mu_B} + \sqrt{\left\{\left(E_1 + \frac{\nu}{\mu_B}\right)^2 - 8\pi n_1 \cdot e \left(E_1 + \frac{\nu}{\mu_B}\right) x\right\}} \tag{39}$$

The thickness δ of the front region can be calculated from another boundary condition

$$E_f(\delta) = E_2 = \nu/\mu_2 \tag{40}$$

The ion densities, charge density and electric field can be calculated from eqns. 28, 36 and 39. These are shown in Fig. 2.

DISCUSSION AND CONCLUSION

In this paper, we have presented the derivation of the fundamental formulas for electrophoresis from the microscopic statistical theory and some macroscopic fluid aspect. It is applied to the isotachophoresis system. We have proposed a simple model for the "isotachophoresis front", the ions and electric field distributions have been calculated in the simplified situation without convection, diffusion and non-electric external forces. This is an essential improvement of Kohlrausch's theory.

The fact that the front is a region of charge non-neutrality was also realized by Hinckley and Hall[7]. They call this the "shock region". They discussed some qualitative properties of the shock region, but they did not calculate the charge and electric field distributions.

The general equation of motion, eqn. 14, is very useful. It provides the basic starting point of any macroscopic fluid dynamic calculations. For a simple demonstration of the existence and structure of isotachophoresis front, we have neglected (i) the diffusion effect, (ii) the ionic shearing stress effect, (iii) the convection effect and (iv) the other possible external forces, for instance, the gravity, magnetic force, etc. In addition, we only consider the steady state case and positive ion packets with sharp boundaries for mathematical simplicity. In other words, we have only presented the "minimum theory" for isotachophoresis. Further calculations compatible with real experimental situations will be interesting for further investigations.

The diffusion has two major effects: (1) the ion packets will be broadened, this has been characteristically shown in *Motion of ionic packet in a uniform electric field*; (2) there are no sharp boundaries, this was shown by Konstantinov and Oshurkova[8]. It is expected that the positive ions A_1^+ and A_2^+ will diffuse into the front region and further to the regions of other ions[7]. Furthermore, because the diffusive boundary region is very large[8], it would be meaningless to do any quantitative calculations for real experiment without considering the diffusion. The quantitative solutions can not be obtained without numerically solving the set of exact eqns. 24–27 in computer. The approximate numerical solutions for steady state have been calculated by Coxon and Binder[9]. However, without considering the diffusion, we have clearly seen the qualitative structure of isotachophoresis front from our minimum theoretical calculations.

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