Journal of Chromatography, 95 (1974) 133–145

CHROM. 7434

ISOTACHOPHORESIS (DISPLACEMENT ELECTROPHORESIS, TRANSPHORESIS) THEORY

STRUCTURE OF THE IONIC SPECIES INTERFACE

M. COXON and M. J. BINDER

Aerospace and Mechanical Engineering Department, University of Arizona, Tucson, Ariz. 85721 (U.S.A.)

(Received January 2nd, 1974)

SUMMARY

An approximate solution is given for the equations governing the structure of the inter-species ionic interface in discrete sample isotachophoresis (displacement electrophoresis, transphoresis) in an ideal one-dimensional system, in the presence of a common counterion and in the absence of continuous mobility spectrum spacer ampholytes. It is shown that the approximation is reasonably good for values of the Kohlrausch-regulated terminator leader concentration ratio greater than 0.5. The dependence of interface thickness on mobility and concentration ratios is discussed and results are compared to those in previous literature. Interface thickness is found to be inversely proportional to leader voltage gradient.

INTRODUCTION

Isotachophoresis[1] (displacement electrophoresis[2], transphoresis[3]) is an electrophoretic separation method characterized by equal velocities for all ions migrating in the system once equilibrium has been attained. The sample to be separated is placed in a tube between leader and terminator electrolyte solutions whose ions are of the same sign as the sample ions, and whose mobilities are respectively higher and lower than the mobilities of any of the sample ions. In the absence of co-moving continuous mobility spectrum spacer ampholytes[1], the sample ions separate into a number of contiguous compartments arranged in the order of their mobilities, each compartment being of uniform characteristic concentration governed by the Kohlrausch regulating function[4] (see eqn. 33) except in the vicinity of the interfaces.

The system was first described by Kendall[5], and subsequent work, including direct method transport number determinations, has been partly reviewed elsewhere[1,3,6–10].

Because of diffusion effects, the interfaces between the compartments are not sharp (although in most cases they are much sharper than ionic discontinuities in other forms of electrophoresis), but rather a continuous distribution of all ions exists throughout the entire tube. However, in discrete sample (as opposed to continuous frontal) separations, the bulk of the concentration change of the individual ion species occurs in a very short length of tube if the mobility differences are not excessively small. We refer to the distance over which the concentration changes from 99% to 1% as the interface thickness, Δ. This paper presents an approximate method for determining the structure of the concentration and potential gradient profiles and the interface thicknesses based on a one-dimensional analysis of the interface which would exist between leader and terminator in a sample-free model.

Such a one-dimensional system has no physical existence, but may approximate isotachophoretic behaviour under ideal conditions. It is, therefore, a first step in the theory from which the three-dimensional case may follow if the radial and longitudinal non-uniformities of real systems can be estimated. Such non-uniformities include the effects of radial and longitudinal temperature gradients and the various property variations which result from them, electro-osmosis, electro-convection, and gravity-dependent phenomena such as sedimentation and thermal convection. Further corrections are also required for partly ionized compounds and macromolecules.

A theoretical prediction of the thickness of isotachophoretic interfaces is of importance in evaluating the maximum resolution of the method for preparative applications, and in determining in what ways experimental parameters may be varied to achieve optimal performance.

A list of symbols is given at the end of the paper.

PREVIOUS WORK

Previous work concerning the determination of the interface thickness has been restricted for the most part to one-dimensional interfaces (*i.e.*, no variations in a radial direction, steady state conditions) between two essentially 100% dissociated electrolyte solutions having a common counterion. The effects of electro-osmosis and temperature gradients have been dealt with only in a qualitative manner. The basic differences between the various analyses to date lie in the additional assumptions made in each case to render the problem tractable.

Longsworth[11] obtained solutions for the concentration profiles of leader, terminator, and counterion for the case in which the mobilities of leader and counterion were equal and twice the mobility of the terminator. In his analysis he imposed the condition of electrical neutrality throughout the entire system. Although this is certainly true for points removed from the interface region, Gauss's law requires some net charge to exist (however small) in the interface region in order that an electric field gradient exists there.

Martin and Everaerts[2] consider the case where leader and terminator mobilities are approximately equal and assume that diffusion effects can be characterized by a single diffusion coefficient for all three ion species. Their results do not show a dependence of front thickness on counterion mobility. They and Routs[12] rewrite the Kohlrausch regulating function to account for partial ionization and compartmental pH differences, with attendant mobility and concentration differences.

Westhaver[13] considers the case of a very thick interface where there is a small mobility difference, the interface representing a form of frontal enrichment.

Konstantinov and Oshurkova[14] consider only the species continuity equations for leader and terminator and obtain solutions for the ratio of either leader or terminator concentration to the sum of the leader and terminator concentrations. A similar solution is obtained by Routs[15] for the ratio of leader concentration to terminator concentration.

Hall and Hinckley[16] obtain solutions valid at points removed from the vicinity of the interface and then estimate interface thicknesses for all three ion species from this. Hinckley[17] has predicted that the interface thickness should be inversely proportional to the leader voltage gradient on the basis that difussion depends on both mobility and concentration gradient, and the latter increases with reduction of interface thickness (*cf.* Routs[15]).

Brouwer and Postema[18] consider the unsteady problem but neglect diffusion, thus arriving at a steady state in which all components are separated into distinct compartments, each compartment containing only one component, and hence do not obtain estimates of interface thickness.

GOVERNING EQUATIONS FOR THE ONE-DIMENSIONAL INTERFACE

The governing equations for the one-dimensional interface in isotachophoresis are the species continuity equations for the leader, terminator, and common counterion, and Gauss's law. These may be written as

$$\frac{\partial n_1^*}{\partial t} + \nabla\cdot(\mu_1 n_1^* \vec{E}^*) = D_1 \nabla^2 n_1^* \tag{1}$$

$$\frac{\partial n_2^*}{\partial t} + \nabla\cdot(\mu_2 n_2^* \vec{E}^*) = D_2 \nabla^2 n_2^* \tag{2}$$

$$\frac{\partial n_3^*}{\partial t} - \nabla\cdot(\mu_3 n_3^* \vec{E}^*) = D_3 \nabla^2 n_3^* \tag{3}$$

$$\nabla\cdot\vec{E}^* = \frac{q}{\varepsilon^*}(n_1^* + n_2^* - n_3^*) \tag{4}$$

If variations in properties over the cross-section of the tube are neglected, then the above equations may be simplified to give

$$\frac{\partial n_1^*}{\partial t} + \mu_1 \frac{\partial (n_1^* E^*)}{\partial x^*} = D_1 \frac{\partial^2 n_1^*}{\partial x^{*2}} \tag{5}$$

$$\frac{\partial n_2^*}{\partial t} + \mu_2 \frac{\partial (n_2^* E^*)}{\partial x^*} = D_2 \frac{\partial^2 n_2^*}{\partial x^{*2}} \tag{6}$$

$$\frac{\partial n_3^*}{\partial t} - \mu_3 \frac{\partial (n_3^* E^*)}{\partial x^*} = D_3 \frac{\partial^2 n_3^*}{\partial x^{*2}} \tag{7}$$

$$\frac{\partial E^*}{\partial x^*} = \frac{q}{\varepsilon^*}(n_1^* + n_2^* - n_3^*) \tag{8}$$

Transforming the problem to a coordinate system moving with the interface by putting $\bar{x}=x^*-Ut$, the following equations are obtained:

$$D_1 \frac{d^2 n_1^*}{d\bar{x}^2} = \mu_1 \frac{d}{d\bar{x}}(n_1^* E^*) - U \frac{dn_1^*}{d\bar{x}} \tag{9}$$

$$D_2 \frac{d^2 n_2^*}{d\bar{x}^2} = \mu_2 \frac{d}{d\bar{x}}(n_2^* E^*) - U \frac{dn_2^*}{d\bar{x}} \tag{10}$$

$$D_3 \frac{d^2 n_3^*}{d\bar{x}^2} = -\mu_3 \frac{d}{d\bar{x}}(n_3^* E^*) - U \frac{dn_3^*}{d\bar{x}} \tag{11}$$

$$\frac{dE^*}{d\bar{x}} = \frac{q}{\varepsilon^*}(n_1^* + n_2^* - n_3^*) \tag{12}$$

Eqns. 9, 10 and 11 may be integrated once to obtain

$$D_1 \frac{dn_1^*}{d\bar{x}} = \mu_1 n_1^* E^* - U n_1^* + C_1 \tag{13}$$

$$D_2 \frac{dn_2^*}{d\bar{x}} = \mu_2 n_2^* E^* - U n_2^* + C_2 \tag{14}$$

$$D_3 \frac{dn_3^*}{d\bar{x}} = -\mu_3 n_3^* E^* - U n_3^* + C_3 \tag{15}$$

The boundary conditions are:

$$\bar{x} \to -\infty \qquad \begin{aligned} n_1^* &\to N \\ n_2^* &\to 0 \\ n_3^* &\to N \\ E^* &\to E_A \end{aligned} \tag{16}$$

$$\bar{x} \to +\infty \qquad \begin{aligned} n_1^* &\to 0 \\ n_2^* &\to M \\ n_3^* &\to M \\ E^* &\to E_B \end{aligned} \tag{17}$$

Applying boundary conditions 16, and noting that $\mu_1 E_A = \mu_2 E_B = U$, eqns. 13, 14 and 15 become

$$D_1 \frac{dn_1^*}{d\bar{x}} = \mu_1 n_1^* E^* - U n_1^* \tag{18}$$

$$D_2 \frac{dn_2^*}{d\tilde{x}} = \mu_2 n_2^* E^* - U n_2^* \tag{19}$$

$$D_3 \frac{dn_3^*}{d\tilde{x}} = -\mu_3 n_3^* E^* - U n_3^* + E_A N(\mu_1 + \mu_3) \tag{20}$$

According to the Einstein relation $D = \mu kT/e$. For monovalent ions, this is equivalent to $D = \mu kT/q$. Using this, and introducing the dimensionless variables n, E and x, eqns. 18, 19, 20 and 12 become

$$\frac{dn_1}{dx} = n_1 E - n_1 \tag{21}$$

$$\frac{dn_2}{dx} = n_2 E - \frac{n_2}{b_2} \tag{22}$$

$$\frac{dn_3}{dx} = -n_3 E - \frac{n_3}{b_3} + \theta_2(1 + 1/b_3) \tag{23}$$

$$\varepsilon \frac{dE}{dx} = n_1 + n_2 - n_3 \tag{24}$$

In non-dimensional terms, the boundary conditions become

$$x \rightarrow -\infty \qquad n_1 \rightarrow \theta_2 \tag{25a}$$

$$n_2 \rightarrow 0 \tag{25b}$$

$$n_3 \rightarrow \theta_2 \tag{25c}$$

$$E \rightarrow 1 \tag{25d}$$

$$x \rightarrow +\infty \qquad n_1 \rightarrow 0 \tag{26a}$$

$$n_2 \rightarrow 1 \tag{26b}$$

$$n_3 \rightarrow 1 \tag{26c}$$

$$E \rightarrow \theta_1 \tag{26d}$$

Eliminating E from eqns. 21 and 22 gives

$$n_2 = n_1 C e^{\alpha x}$$

Choosing the origin at the point where $n_1 = n_2$ gives $C = 1$, thus

$$n_2 = n_1 e^{\alpha x} \tag{27}$$

From eqn. 21

$$E = 1 + 1/n_1 \frac{dn_1}{dx} \tag{28}$$

Thus

$$\frac{\mathrm{d}E}{\mathrm{d}x}=\frac{1}{n_1^2}\left[n_1\frac{\mathrm{d}^2n_1}{\mathrm{d}x^2}-\left(\frac{\mathrm{d}n_1}{\mathrm{d}x}\right)^2\right] \tag{29}$$

Substitution of eqns. 27 and 29 in eqn. 24 yields

$$n_3=n_1(1+\mathrm{e}^{\alpha x})-\varepsilon\frac{n_1''}{n_1}+\varepsilon\left(\frac{n_1'}{n_1}\right)^2 \tag{30}$$

where

$$n_1'=\frac{\mathrm{d}n_1}{\mathrm{d}x}\text{, etc.}$$

Thus

$$n_3'=n_1\alpha\mathrm{e}^{\alpha x}+n_1'(1+\mathrm{e}^{\alpha x})-\varepsilon\left(\frac{n_1n_1'''-n_1'n_1''}{n_1^2}\right)+\varepsilon\left(\frac{2n_1^2n_1'n_1''-2n_1n_1'^3}{n_1^4}\right) \tag{31}$$

Substitution of eqns. 28, 30 and 31 in eqn. 23 gives

$$2n_1^3n_1'(1+\mathrm{e}^{\alpha x})+n_1^4\alpha\mathrm{e}^{\alpha x}-\varepsilon n_1^2n_1'''+\varepsilon n_1^2n_1''(3-\beta)-\varepsilon n_1'^3-$$
$$\varepsilon n_1n_1'n_1''+\beta\varepsilon n_1n_1'^2+\beta n_1^4(1+\mathrm{e}^{\alpha x})-\frac{\alpha\theta_2}{1-\theta_2}n_1^3=0 \tag{32}$$

The last term in eqn. 32 is obtained by using the relationship

$$\beta=(1+1/b_3)=\alpha/(1-\theta_2) \tag{33}$$

This may be obtained by applying boundary conditions 25 and 26 to eqn. 15 after it has been put in dimensionless form. Boundary condition 25 yields as before

$$\bar{C}_3=\theta_2(1+1/b_3)$$

Boundary condition 26 gives

$$\bar{C}_3=\theta_1+1/b_3$$

Equating the two expressions for $\bar{C}_3$ gives

$$\theta_2(1+1/b_3)=\theta_1+1/b_3$$

Noting that $b_2\theta_1=1$, eqn. 33 is obtained by algebraic manipulation. Eqn. 33 has been described by Kohlrausch[4] and Weber[19] and is known as the Kohlrausch regulating function.

Eqn. 32 may be rearranged to give

$$2n_1'(1+\mathrm{e}^{\alpha x})+n_1\alpha\mathrm{e}^{\alpha x}-\varepsilon\frac{n_1'''}{n_1}+\varepsilon\frac{n_1''}{n_1}(3-\beta)-\varepsilon\left(\frac{n_1'}{n_1}\right)^3-\varepsilon\frac{n_1'n_1''}{n_1^2}+$$
$$+\beta\varepsilon\left(\frac{n_1'}{n_1}\right)^2+\beta n_1(1+\mathrm{e}^{\alpha x})=\frac{\alpha\theta_2}{(1-\theta_2)} \tag{34}$$

SOLUTION OF THE PROBLEM

The problem consists of finding a solution for eqn. 34 that satisfies the boundary conditions 25 and 26. The nature of the problem and the boundary conditions indicate the general shape of the solution curve (it might be referred to as a distorted sigmoid). The results of the aforementioned references tend to confirm this statement. Hence we assume a solution of the form

$$n_1 = \frac{\theta_2}{(1+\psi(x)e^{\phi(x)})^{N_1}} \tag{35}$$

Hence

$$n_2 = \frac{\theta_2 e^{\alpha x}}{(1+\psi(x)e^{\phi(x)})^{N_2}}$$

It is seen that boundary condition 25b is satisfied if

$$\phi(x) = (\alpha/N_1)x$$

Boundary condition 26b is satisfied only if the following conditions are met:

(1) $\phi(x) = (\alpha/N_1)x$

(2) $\psi(x) = K$

(3) $\frac{K^{N_1}}{\theta_2} = 1$

Hence the assumed solution for n_1 becomes

$$n_1 = \frac{\theta_2}{(1+Ke^{\alpha x/N_1})^{N_1}} \tag{36}$$

where

$$K = \theta_2^{1/N_1} \tag{37}$$

It is easily seen that eqn. 35 also satisfies the other boundary conditions.

Substitution of eqn. 36 into eqn. 34 yields an equation of the form

$$f(x) = \beta\theta_2 \tag{38}$$

As an indication of how well our assumed solution satisfies eqn. 38, we define the error in the system to be

$$\text{Error (\%)} = \left| \frac{100[f(x) - \beta\theta_2]}{\beta\theta_2} \right|$$

In general, the error will be a function of x, and the exponent N_1 must be chosen in such a way as to minimize the maximum error for each set of experimental conditions considered.

RESULTS

A numerical search routine was employed to determine the optimum value of N_1 for each set of parameters considered, the calculations being performed on the CDC-6400 computer at the University of Arizona Computer Center. The optimum value of N_1 and the resulting maximum error were found to be highly dependent on θ_2 but essentially independent of α and ε. The variations of N_1 and the maximum error with θ_2 are shown in Figs. 1 and 2, respectively.

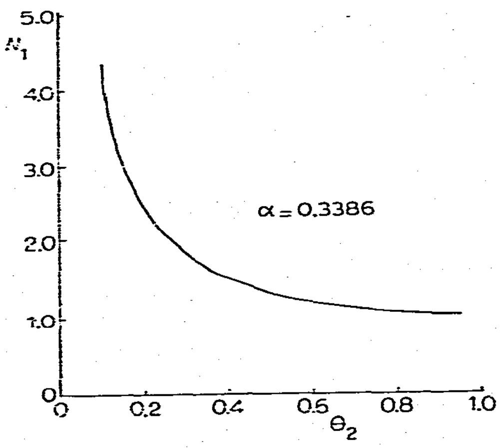


Fig. 1. Variation of N_1 with θ_2.

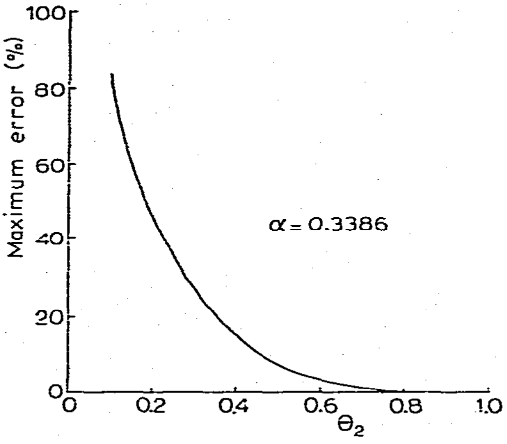


Fig. 2. Variation of maximum error with θ_2.

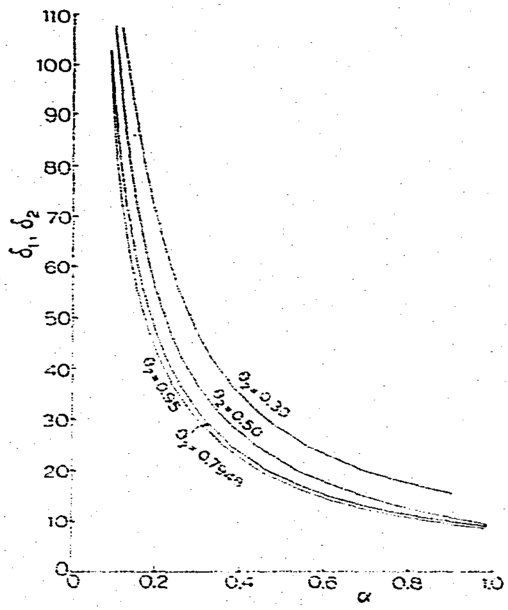
The values of interface thickness for leader and terminator ions were found to be equal and highly dependent on α and θ_2, but independent of ε. This same parameter dependency was found to be true for the counterion and electric field interface thicknesses. The variations of interface thickness with α and θ_2 are shown in Figs. 3–5.

DISCUSSION

As can be seen from Fig. 2, the assumed solution satisfies the governing equations with negligible error for $\theta_2 > 0.75$. The error increases rapidly for decreasing θ_2. At $\theta_2 = 0.5$ the maximum error is about 7.5%.

A comparison of Figs. 3 and 4 shows that δ_3 is less than δ_1 and δ_2. This result was also predicted by Hall and Hinckley[16].

A comparison of Figs. 3 and 5 shows that for large values of θ_2, δ_E is approximately equal to δ_1, but that for small values of θ_2, δ_E is considerably greater than δ_1. Since a common means of determining δ_1 experimentally is to measure δ_E, it would appear that at low values of θ_2 this method would give values of δ_1 which are larger than they should be. However, since it is for low values of θ_2 that the error in our calculations is very large, this may not actually be the case.



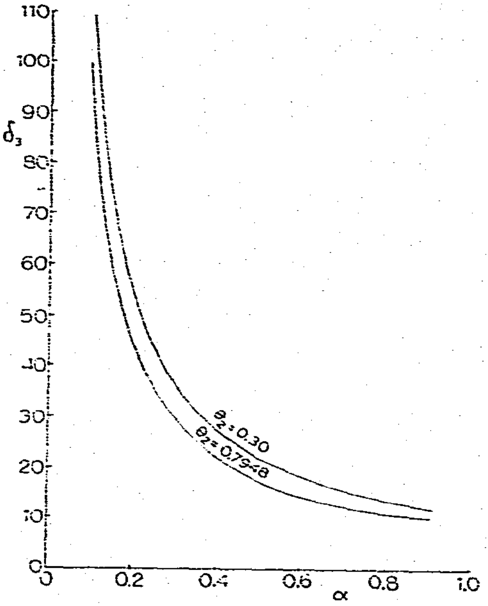


Fig. 3. Leader and terminator interface thickness.

Fig. 4. Counterion interface thickness.

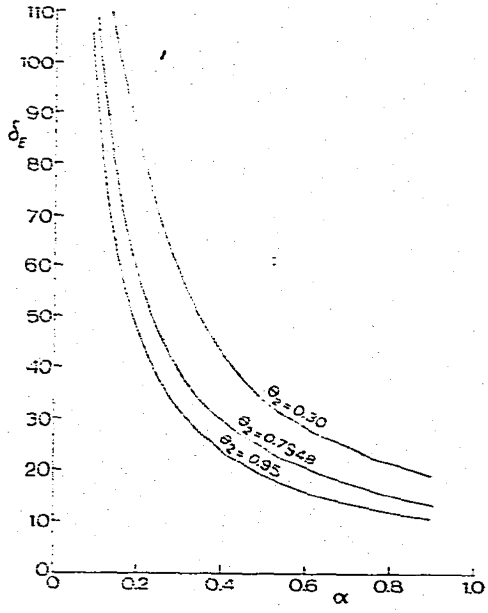


Fig. 5. Electric field interface thickness.

TABLE I

EXPERIMENTAL PARAMETERS

Parameter	*Experimental value*
$n_1^{\pm}$	Sodium
$n_2^{\pm}$	Potassium
$n_3^{\pm}$	Chloride
T	283 K
μ_1	$35.98 \cdot 10^{-5}$ cm$^2 \cdot$V$^{-1} \cdot$sec^{-1}
μ_2	$54.4 \cdot 10^{-5}$ cm$^2 \cdot$V$^{-1} \cdot$sec^{-1}
μ_3	$55.25 \cdot 10^{-5}$ cm$^2 \cdot$V$^{-1} \cdot$sec^{-1}
E_A	250 V/cm
M	1 millimolar
ε^*	$7.4471 \cdot 10^{-10}$ F/m
α	0.3386
θ_2	0.79494
ε	$1.98 \cdot 10^{-4}$

As a numerical example for use in comparison with the results of previous analyses we consider the conditions given in Table I. For the parameters given, Fig. 3 gives $\delta_1 = 27.68$. This is a dimensionless interface thickness, and it may be put back into dimensional form by employing the definition of the dimensionless distance x given in the symbol table. Thus

$$\Delta = \frac{\delta_1 kT}{qE_A}.$$

For the conditions given this yields an interface thickness Δ of 0.027 mm.

Martin and Everaerts[2] predict an interface thickness of 1.2 mm from the results of their analysis. Applying the data of Table I to the formulations of Hall and Hinckley[16] and Konstantinov and Oshurkova[14] gives values of 0.0038 mm and 0.0115 mm, respectively, although the latter is based on a definition of interface thickness different to that used here.

Arlinger and Routs[20], using a UV detector, detected interface thicknesses of less than 1 mm, and Hinckley[17] has detected alkali metal interfaces of 0.2 mm or less with a d.c. electrometric detector although exact measurement is difficult because of the short distances involved.

The concentration and electric field profiles for the conditions of Table I are shown in Fig. 6.

Since the non-dimensional interface thickness is seen to be independent of ε, the variation of dimensional interface thickness with terminator voltage gradient (and hence leader voltage gradient because they are proportional) may be determined directly from the definition of x. Thus

$$\Delta = \frac{\delta_1 kT}{qE_A} = \frac{\delta_1 (1-\alpha) kT}{qE_B}$$

Interface thickness is seen to be inversely proportional to the terminator or leader voltage gradients with the temperature of the system as a parameter for a

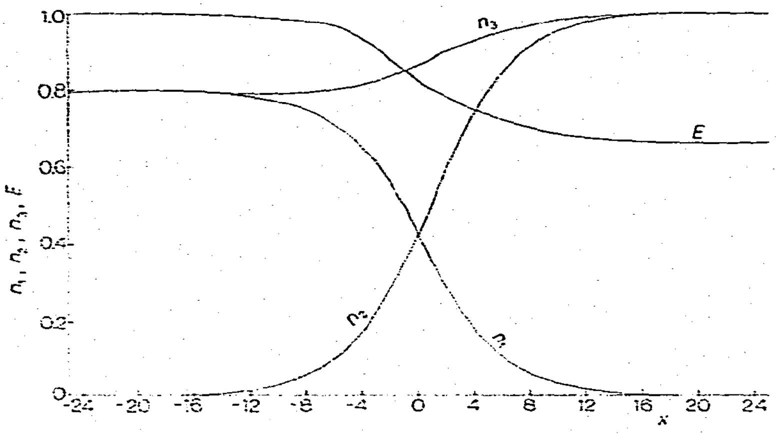


Fig. 6. Concentration and electric field profiles.

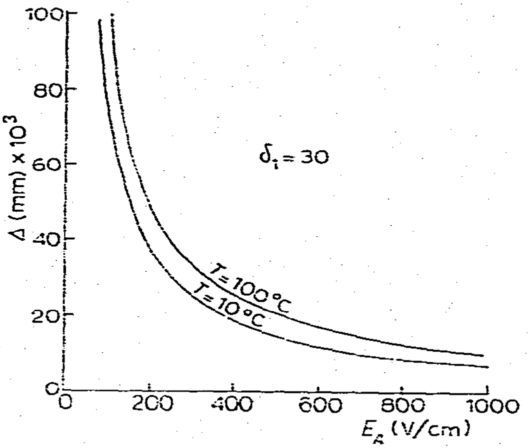


Fig. 7. Variation of interface thickness with terminator voltage gradient.

given δ_1. This variation is depicted in Fig. 7 for a value of δ_1 equal to 30, and is of the form predicted by Hinckley[17] on the grounds previously mentioned.

CONCLUSIONS

The approximate solution given is seen to satisfy the governing equations for the one-dimensional interface in isotachophoresis with a maximum error of 7.5% for values of terminator to leader concentration ratio greater than 0.5. It yields values of interface thickness that are less than those of previous analyses, and this prediction seems to be in line with experimental observations. It is seen that the sum of the concentrations of leader, terminator, and counterion in the region of the interface is very small but not zero, in accordance with Gauss's law. The interface thickness is found to be inversely proportional to the leader voltage gradient as predicted by Hinckley[17].

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Dr. Milan Bier for his interest and comments in discussion. Thanks are also due to J. O. N. Hinckley for suggesting this study and for providing us with previous publications and unpublished material related to the problem.

This study was supported by the National Aeronautics and Space Administration under contract NAS8-29566.

LIST OF SYMBOLS

b_2 = Dimensionless mobility $= \mu_2/\mu_1$
b_3 = Dimensionless mobility $= \mu_3/\mu_1$
C = Integration constant
C_1 = Integration constant (ions·cm^{-2}·sec^{-1})
C_2 = Integration constant (ions·cm^{-2}·sec^{-1})
C_3 = Integration constant (ions·cm^{-2}·sec^{-1})
$\bar{C}_3$ = Integration constant
D_1 = Terminator diffusion coefficient (cm^2/sec)
D_2 = Leader diffusion coefficient (cm^2/sec)
D_3 = Counterion diffusion coefficient (cm^2/sec)
E^* = Electric field (V/cm)
E = Dimensionless electric field $= E^*/E_A$
E_A = Electric field in terminator zone (V/cm)
E_B = Electric field in leader zone (V/cm)
e = Electronic charge (C/electron)
K = Constant
k = Boltzmann's constant (erg/°K)
M = Ionic number density in leader zone (ions/cm^3)
N = Ionic number density in terminator zone (ions/cm^3)
N_1 = Constant, exponent in assumed n_1 solution
n_1^* = Terminator ionic number density (ions/cm^3)
n_2^* = Leader ionic number density (ions/cm^3)
n_3^* = Counterion ionic number density (ions/cm^3)
n_1 = Dimensionless terminator ionic number density
n_2 = Dimensionless leader ionic number density
n_3 = Dimensionless counterion ionic number density
q = Ionic charge (C/ion)
T = Temperature (°K)
t = Time (sec)
U = Speed of migrating ions (cm/sec)
x^* = Distance along axis of tube referred to a fixed coordinate system (cm)
$\tilde{x}$ = Distance along axis of tube referred to a moving coordinate system (cm)
x = Dimensionless distance along axis of tube referred to a moving coordinate system $= \tilde{x}qE_A/kT$
α = Dimensionless parameter $= (b_2-1)/b_2 = 1-\theta_1$
β = Dimensionless parameter $= (b_3+1)/b_3$

δ_1 = Dimensionless terminator interface thickness
δ_2 = Dimensionless leader interface thickness
δ_3 = Dimensionless counterion interface thickness
δ_E = Dimensionless electric field interface thickness
Δ = Dimensional leader or terminator interface thickness (mm)
ε^* = Dielectric constant of the medium (F/m)
ε = Dimensionless dielectric constant $= E_A^2 \varepsilon^* / kTM$
θ_1 = Dimensionless electric field in leader zone $= E_B/E_A = 1 - \alpha$
θ_2 = Dimensionless number density in terminator zone $= N/M = (b_3\theta_1 + 1)/(b_3 + 1)$
μ_1 = Mobility of terminator ion ($\mathrm{cm^2 \cdot V^{-1} \cdot sec^{-1}}$)
μ_2 = Mobility of leader ion ($\mathrm{cm^2 \cdot V^{-1} \cdot sec^{-1}}$)
μ_3 = Mobility of counterion ($\mathrm{cm^2 \cdot V^{-1} \cdot sec^{-1}}$)
ϕ = Function of x in assumed n_1 solution
ψ = Function of x in assumed n_1 solution

REFERENCES

1 H. Haglund, *Sci. Tools*, 17 (1970) 2.
2 A. J. P. Martin and F. M. Everaerts, *Proc. Roy. Soc., Sect. A.*, 316 (1970) 493.
3 J. O. N. Hinckley, in E. Reid (Editor), *Methodological Developments in Biochemistry*, Vol. 2, Longman, London, 1973, p. 207.
4 F. Kohlrausch, *Ann. Phys. (Leipzig)*, 62 (1897) 209.
5 J. Kendall, *Phys. Rev.*, 21 (1923) 389.
6 D. Peel, in E. Reid (Editor), *Methodological Developments in Biochemistry*, Vol. 2, Longman, London, 1973, p. 205.
7 M. Gazith and A. Roy, *Electrochem. Technol.*, 2 (1964) 85.
8 H. D. Freyer and K. Wagener, *Angew. Chem., Int. Ed. Engl.*, 6 (1967) 757.
9 J. O. N. Hinckley, *Clin. Chem., in press.*
10 D. A. MacInnes and L. G. Longsworth, *Chem. Rev.*, 11 (1932) 171.
11 L. G. Longsworth, *J. Amer. Chem. Soc.*, 66 (1944) 449.
12 R. J. Routs, *Doctoral Thesis*, Eindhoven Technological University, Eindhoven, 1971.
13 J. W. Westhaver, *J. Res. Nat. Bur. Stand.*, 38 (1947) 169.
14 B. P. Konstantinov and O. V. Oshurkova, *Sov. Phys. Tech. Phys.*, 11 (1966) 693.
15 R. J. Routs, *Electrolyte Systems in Isotachophoresis and Their Application To Some Protein Separations*, Solna Skriv- & Stenograftjanst AB, Solna, Sweden, 1971, p. 37.
16 R. Hall and J. O. N. Hinckley, written communication, 1971.
17 J. O. N. Hinckley, written communication, 1973.
18 G. Brouwer and G. A. Postema, *J. Electrochem. Soc.*, 117 (1970) 874.
19 H. Weber, *Sitz. Preuss. Akad. Wiss. Berl. Sitzungsber.*, (1897) 936.
20 L. Arlinger and R. Routs, *Sci. Tools*, 17 (1970) 21.