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CONCENTRATION DISTRIBUTIONS IN FREE ZONE ELECTROPHORESIS

F. E. P. MIKKERS, F. M. EVERAERTS and Th. P. E. M. VERHEGGEN
Department of Instrumental Analysis, Eindhoven University of Technology, Eindhoven (The Netherlands)
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SUMMARY

The effect of electrophoretic migration on the concentration distributions in free zone electrophoresis is evaluated using a non-diffusional model. It is shown that sample constituents that have a mobility higher than that of the carrier constituent migrate with a concentration distribution that is diffuse at the front and sharp at the rear of the zone. The reverse holds for sample constituents that have a mobility lower than that of the carrier constituent. The conditions at which diffusional and migrational dispersion are of the same order of magnitude are discussed. It is shown that by a proper choice of operational conditions the adverse effect of a relatively large sample width can be reduced. Problems concerning retention behaviour and separability are discussed.

INTRODUCTION

When in zone electrophoresis longitudinal diffusion is the only mechanism of band spreading and migration occurs at a constant velocity, Gaussian concentration distributions are obtained[1,2]. The actual broadening, however, may exceed the diffusional broadening due to convection, electrodiffusion, electro-osmosis and reversible adsorption. Such non-idealities have been discussed in detail by Wieme[3] and Boyack and Giddings[4] and are collectively responsible for what has been called "electrophoretic dispersion". They can be dealt with by using pseudo-diffusion coefficients, which combine the adverse effects of this additional spreading[3].

In zone electrophoresis, however, frequently non-symmetrical concentration distributions are obtained. When adsorption processes can occur, non-linear adsorption isotherms or a hydrodynamic flow may explain the asymmetry[5–9]. The effect of an inhomogeneity of the electrical field on the zone profile has been discussed by several workers[10–15]. This phenomenon is closely related to the fact that in electrophoresis one frequently encounters boundary anomalies[5,16–18], in which the migration velocity is a function of concentration. It is generally assumed that in view of zone electrophoretic performance these boundary anomalies have to be avoided. This seems to be the result of the chromatographic principle that any effect which improves the definition of one boundary invariably causes deterioration of the other

boundary. Thus, in all chromatographic zonal separations the best resolution is obtained when these effects are absent and the zone boundaries are symmetrical. Although there is a close analogy between chromatographic and electrophoretic separation principles, some important methodological differences exist. Probably the greatest difference is that in electrophoresis Ohm's law must hold and that the resulting Kohlrausch relations[16–21] govern the electrophoretic process. Any changing of concentrations during an electrophoretic process are ruled by these relationships. As a result, on the one hand the occurrence of boundary anomalies can be used in a favourable way, while on the other hand problems in retention behaviour arise.

THEORETICAL

In all electrophoretic separation techniques changes of electrolyte constituent concentrations will occur owing to the action of an external electrical field. In zone electrophoresis a discrete sample zone is eluted by the so-called carrier electrolyte. Although gradient configurations (dimensional, thermal or electrolytic) are possible, we shall assume a separation compartment of uniform dimensions, operated at a constant temperature and filled with a homogeneous carrier electrolyte. This electrolyte consists of a carrier constituent A, which has the same electrical charge as the sample constituents, and a counter constituent B, to preserve electroneutrality. A small volume element of the separation compartment (Fig. 1), that originally was filled with the carrier electrolyte AB, will contain after an appropriate time of analysis a mixture of the carrier electrolyte and one or more sample constituent(s), C. After an even longer time, the sample will have left this volume element and the original situation will be restored again. Assuming the presence of only monovalent weak ionic constituents, two important Kohlrausch functions can be derived[19,21]:

$$\bar{c}_i = \omega_1 \quad \text{and} \quad \frac{\bar{c}_i}{r_i} = \omega_2 \tag{1}$$

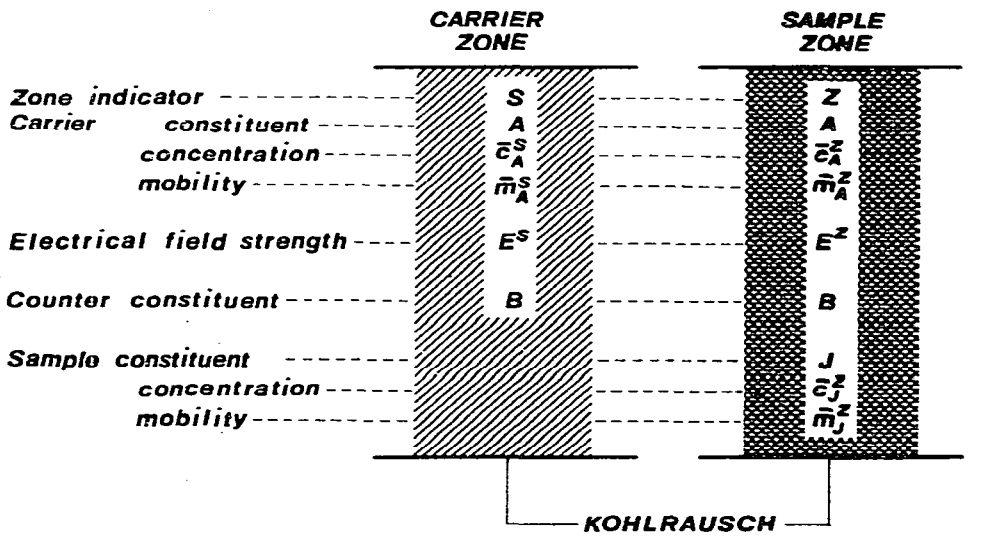

Fig. 1. A zone electrophoretic configuration.

where $\tilde{c}_i$ represents the molar concentration of constituent i and r_i is its ionic mobility relative to an appropriate reference constituent. Obviously the carrier constituent A offers the best reference mobility. It should be noted that concentrations and mobilities can most conveniently be taken as signed quantities[21]. Moreover, the use of relative mobilities will reduce the influence of temperature and activity effects.

The numerical values of the Kohlrausch functions, ω_1 and ω_2, are locally invariable with time. Thus, taking the carrier electrolyte as a frame of reference, it follows that for the situations shown in Fig. 1 it must hold that

$$\tilde{c}_A^S(x, t) = \tilde{c}_A^Z(x, t) + \sum_j k_j \tilde{c}_j^Z(x, t) \tag{2}$$

where

$$k_j = \left(\frac{r_j - r_B}{1 - r_B}\right)\left(\frac{1}{r_j}\right)$$

The summation indicates that within the volume element several sample constituents j can be present.

If a constant electrical driving current and the presence of only strong ionic constituents is assumed, it follows for the specific conductance, κ, that

$$\kappa^Z(x, t) = \kappa^S + \sum_j b_j c_j^Z(x, t) \tag{3}$$

where

$$b_j = F\, m_A\, (r_j - r_B)\left(1 - \frac{1}{r_j}\right)$$

F is the Faraday constant, m_A is the ionic mobility of the carrier constituent and c_j^Z the total concentration of the sample species j. Applying Ohm's law, we obtain for the electrical field strength, E:

$$E^Z(x, t) = \frac{E^S}{1 - \sum_j a_j c_j^Z(x, t)} \tag{4}$$

where

$$a_j = \frac{k_j}{c_A^S}(1 - r_j)$$

When only one sample constituent is present in the volume element, an important conclusion can be drawn from eqns. 2 and 4. If the sample constituent has a higher mobility than that of the carrier constituent, *i.e.*, $r_j > 1$, the electrical field strength in the volume element will always will be higher than that in the pure carrier electrolyte. For other mobility configurations, analogous relationships can be given:

$$\left.\begin{array}{ll} r_j > 1 & E^Z(x,t) < E^S \\ r_j = 1 & E^Z(x,t) = E^S \\ r_j < 1 & E^Z(x,t) > E^S \end{array}\right\} \tag{5}$$

The equation of continuity states for the electrophoretic process

$$\frac{\partial}{\partial t}\bar{c}_j(x,t) = \frac{\partial}{\partial x}\left[D_j \frac{\partial}{\partial x}\bar{c}_j(x,t) - v_j(x,t)\,\bar{c}_j(x,t)\right] \tag{6}$$

where D_j is the diffusion coefficient and v_j is the electrophoretic velocity of constituent j. Assuming a constant velocity, Gaussian concentration distributions are obtained, in which a symmetrical broadening of the sample zone occurs due to diffusion.

In electrophoresis one frequently encounters boundary anomalies in which the migration rate is a function of concentration. Virtanen[10] indicated that the electrophoretic velocity is not constant and gave an analytical solution for the equation of continuity, assuming that the velocity is linearly related to the sample constituent concentration. According to eqn. 4, this can only be approximate. The equations describing this effect are non-linear and the description of non-linear migration in which diffusional dispersion occurs is laboursome. The effect of boundary anomalies, however, can easily be deduced if one assumes that diffusional dispersion can be neglected. In this case, eqn. 6 reduces to

$$\frac{\partial}{\partial t}\bar{c}_j(x,t) = -\frac{\partial}{\partial x} v_j(x,t)\,\bar{c}_j(x,t) \tag{7}$$

If the presence of only one strong ionic sample constituent, C, is assumed, combination of eqns. 4 and 6 gives

$$\frac{\partial}{\partial t} c_C^z(x,t) = -m_A r_C E^S \frac{\partial}{\partial x}\left[\frac{c_C^z(x,t)}{1 - a_C c_C^z(x,t)}\right] \tag{8}$$

Introducing $\psi(x,t) = 1 - a_C c_C^z(x,t)$ this differential equation can easily be solved to give

$$\psi(x,t) = (\alpha x + \beta)^{-\frac{1}{2}}(t + \gamma)^{\frac{1}{2}} \tag{9}$$

The constants α, β and γ are determined by the actual boundary conditions.

During the migration process, several discontinuities can occur that are restricted in place and time. A complete mathematical treatment of all possible configurations will not be given here[22]. After an appropriate time of migration, however, the concentration distributions have a characteristic form. Fig. 2 gives these distributions for three possible cases of relative sample constituent mobilities.

When the sample constituent has a higher mobility than that of the carrier constituent, $r_C > 1$, the leading side of the sample zone always will be diffuse, whereas the rear will be sharp. This is caused by the fact that at the rear a stable moving boundary can be formed[21], whereas at the leading side the criterion for stability cannot be met. Although several time restrictions can occur during the migration process, the final distribution will be given by

$$c_C^z(x,t) = \frac{1}{a_C}\left(1 - \sqrt{\frac{x_{max.} - \Delta l_0}{x - \Delta l_0}}\right) \tag{10}$$

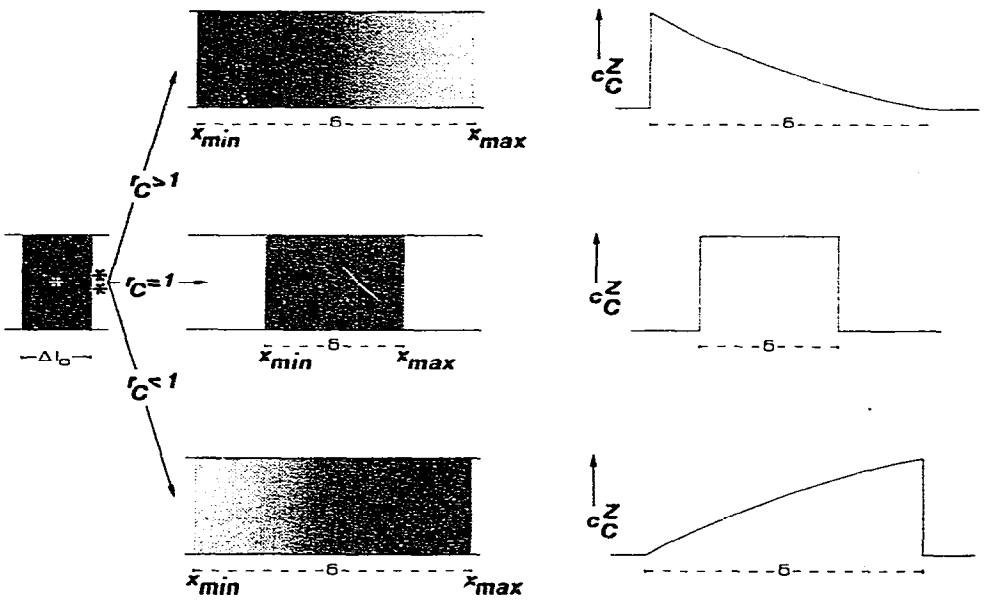

Fig. 2. Concentration distribution in zone electrophoresis as a function of the relative sample constituent mobility. *, Sampling compartment; **, separation compartment.

where Δl_0 is the initial width of the sample pulse and $x_{max.}$ is the maximal distance that the sample constituent has migrated in the given time interval. It follows that this maximal distance is given by

$$x_{max.} = m_A r_C E^S t + \Delta l_0 \tag{11}$$

The resulting electrical field strength profile can be evaluated directly from eqn. 4. For the peak width, δ, at time t it can be derived that

$$\delta = x_{max.} - x_{min.} = a_C \Delta l_0 c_C^{Z*} + 2\sqrt{(\Delta l_0 - m_C E^S t)\, a_C \Delta l_0 c_C^{Z*}} \tag{12}$$

where $x_{min.}$ is the minimal distance that the sample constituent has migrated in the given time interval and c_C^{Z*} is the concentration of the sample constituent in the sample.

When the mobility of the sample constituent is equal to the mobility of the carrier constituent, $r_C = 1$, the sample constituent is only diluted or concentrated over the stationary boundary between the sampling and separation compartments. If the sample again has been introduced as a block pulse, the concentration distribution will be given by

$$c_C^Z = c_A^S \left(\frac{\varphi}{1 + \varphi k_C}\right) \tag{13}$$

where φ is the sampling ratio, c_C^{Z*}/c_A^{Z*}, and k_C is given by eqn. 2. It follows that the zone concentration of the sample constituent is independent of time and that the maximal distance that the sample constituent has migrated is given by eqn. 11.

Moreover, it must be concluded that, after an initial elongation or shortening, the peak width is independent of time (Fig. 2). The electrical field strength in the sample zone will be equal to that in the carrier electrolyte.

When the sample constituent has a smaller mobility than that of the carrier constituent, $r_C < 1$, the leading side of the zone will be sharp, whereas the rear will be diffuse. In this instance the concentration distribution will be given by

$$c_C^Z(x, t) = \frac{1}{a_C}\left[1 - \sqrt{\frac{x_{min.}}{x + \Delta l_0(c_A^{S*}/c_A^S - 1)}}\right] \tag{14}$$

where c_A^{S*}/c_A^S is the dilution factor over the concentration boundary between the sampling and separation compartments. For the peak width δ it follows that

$$\delta = x_{max.} - x_{min.} = c_C^{Z*}\Delta l_0 a_C + 2\sqrt{c_C^{Z*}\Delta l_0 m_A r_C E^S a_C t} \tag{15}$$

With weak electrolytes the concentration distributions will be determined by the effective mobilities. Most of the previous considerations can be extended without problem to involve weak electrolytes.

DISCUSSION

In the above approach, diffusional effects were purposely neglected in order to emphasize the important influence of the electrophoretic migration process on the concentration distributions. In this way the asymmetry that frequently occurs in zone electrophoresis[23] can easily be explained as a result of the electrophoretic process. Obviously, in experimental practice the diffusional effect cannot be neglected and should be incorporated in the equation of continuity. The importance of diffusional and migrational dispersion, however, can easily be evaluated. Using the appropriate relationships, eqn. 15 can be rewritten in a more practical form:

$$\delta = \frac{c_C^{Z*}}{c_A^S} \cdot \Delta l_0 f(r) + 2\sqrt{\frac{c_C^{Z*}}{c_A^S} \cdot \Delta l_0 f(r)\, v_C t} \tag{16}$$

where $f(r)$ is a function of the ionic mobilities and v_C is the migration velocity of the sample constituent in the carrier electrolyte. Both $f(r)$ and Δl_0 will commonly show only a limited degree of freedom and both should be minimized. Neglecting the initial discontinuities, band spreading due to diffusion and to electrophoretic migration is of the same order of magnitude when

$$D \approx 0.1 \cdot \frac{c_C^{Z*}}{c_A^S} \cdot f(r)\, \Delta l_0 v_c \tag{17}$$

This relationship is illustrated in Fig. 3.

Taking a diffusion coefficient, D, of 10^{-5} cm^2/sec at a migration rate of 1 mm/sec and a initial band width of 1 mm, diffusion and migration will have a comparable adverse effect at a concentration ratio c_C^{Z*}/c_A^S of 10^{-2}. Below this value band spreading

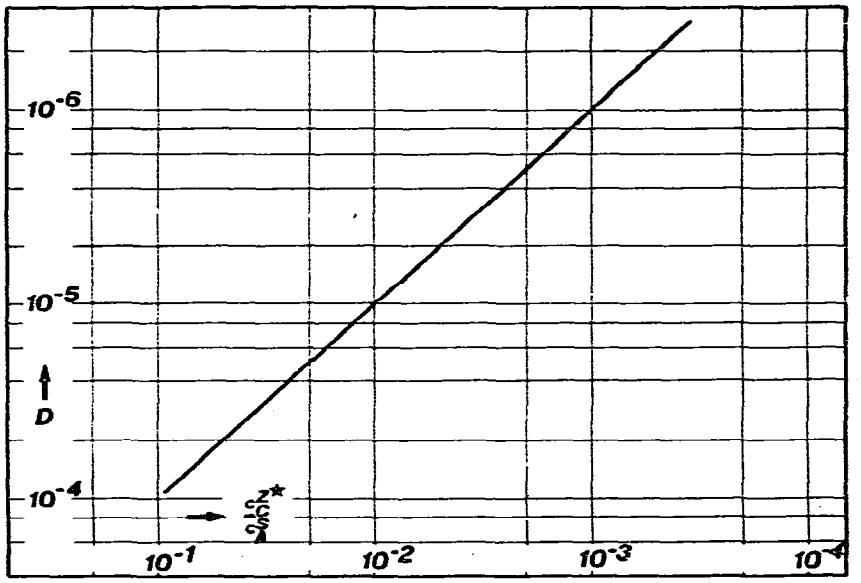

Fig. 3. Relationship between diffusional and migrational dispersion.

is due mainly to diffusion and above this value electrophoretic migration will mainly contribute. Assuming that zone electrophoretic separations are carried out in a narrow-bore tube of I.D. 0.2 mm (ref. 23) and using a carrier electrolyte at a concentration of 10 mM, the migrational effect will be appreciable when more than 3 pmole of the sample constituent are injected. Other forms of dispersion, through which the effective diffusion coefficient may exceed the linear thermal diffusion coefficient, will obscure the migrational dispersion and should be minimized. It should be noted, however, that the occurrence of boundary anomalies counteracts the influence of non-migrational dispersion. This has been shown to be especially true for isotachophoresis[24], but also holds for zone electrophoresis, although to a minor extent.

The adverse effect of a relatively large sampling width, Δl_0, can be counteracted by the concentrating capabilities of the electrolyte system. Choosing the condition $c_C^{Z*} \ll c_A^S$ and a high sampling ratio, φ, the sample constituent will be concentrated over the stationary concentration boundary between the sampling and separation compartments. This concentration is the result of the fact that in electrophoresis the Kohlrausch regulating function concept[19] cannot be overruled. It seems that this forms the most profound difference between chromatographic and electrophoretic separation principles. In experimental practice, this means that, in order to utilize the concentrating capabilities, the sample should *not* be equilibrated with the carrier electrolyte[23].

Fig. 4 shows the electrophoretic development of a sample constituent that has a higher mobility than that of the carrier constituent. From the concentration distribution after 1 sec it can be seen that the sample constituent concentrates over the stationary boundary between the sampling and separation compartments. After 5 sec of migration the zone still contains a homogeneous part, but the diffuse region is already clearly visible. After 10 sec the homogeneous part has just disappeared and complete elution starts. From this moment on, the concentration distribution accord-

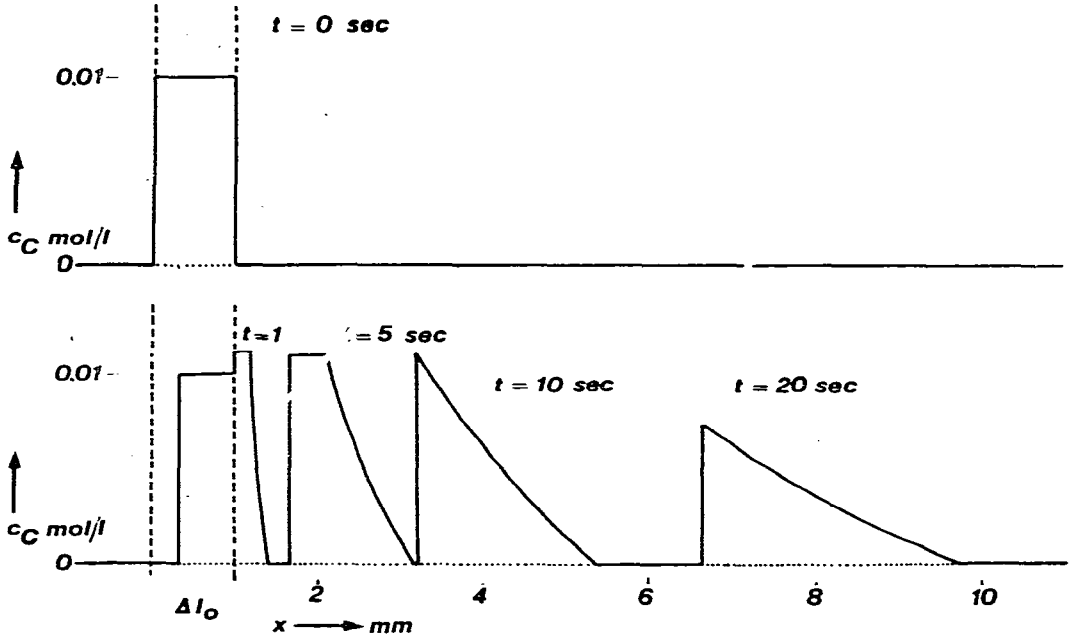

Fig. 4. Development of a zone electrophoretic process. c_c (mol/l) = Concentration of the sample constituent; x (min) = migration coordinate; t (sec) = time.

ing to eqn. 10 is present. Sample constituents that have a lower mobility than that of the carrier constituent can show a more complicated migration process, in which transient double peaks can occur[22]. Generally, diffusion will blurr the concentration profiles to less discrete forms as given in Figs. 2 and 4. Those cases for which r_C approaches unity will be particularly sensitive in this respect.

Obviously, the separation of multicomponent samples will develop in a complicated manner. This complexity is further increased as generally weak electrolytes will be applied. It has been shown[21] that, in isotachophoresis and moving boundary electrophoresis, the ratio of sample constituent mobilities in the mixed state is important when separability and separation efficiency are considered. The same holds for zone electrophoresis and generally the same optimization rationales[21] can be followed. The separation efficiency in zone electrophoresis, however, will be low in comparison with that in isotachophoresis owing to the continuous transport of carrier electrolyte.

In zone electrophoresis the zone characteristics will be mainly determined by the carrier electrolyte. Using a fixed point detection system[23], the time interval that the sample constituents need to reach the detector, *i.e.*, the retention behaviour, is strongly affected by the proper choice of the carrier electrolyte. Considering retention behaviour, it can be concluded that the difference in sample constituent mobilities is important. In experimental practice, a compromise between separation efficiency and retention behaviour has to be found. Obviously, pH and complex formation have a great influence on retention behaviour. Assuming a well buffered electrolyte system and the application of a small amount of sample, pH deviations and inhomogeneities in the electrical field can be neglected. For the retention time, t_R, it follows that

$$t_R \bar{r}_C = t_0 \tag{18}$$

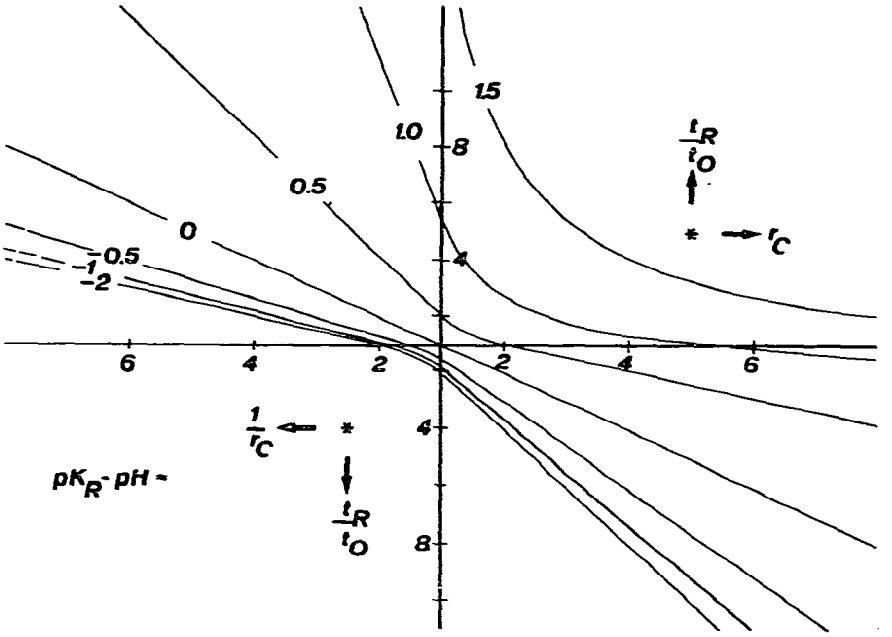

Fig. 5. Relative retention, t_R/t_0, as a function of the relative ionic mobility of the sample constituent, r_C. $pH_{carrier\ electrolyte} = pK_{carrier\ constituent}$. Parameter: $pK_R - pH$, the difference between the pK of the sample constituent and the pH of the carrier electrolyte.

where $\bar{r}_C$ is the relative effective mobility of the sample constituent and t_0 is the retention time of the carrier constituent. Fig. 5 shows the above relationship as a function of the relative ionic mobility of the sample constituent. The difference between the pK_a value of the sample constituent and the pH of the carrier electrolyte has been used as a parameter. The carrier constituent has been chosen for its optimal buffering capacity, *i.e.*, $pH^S = pK_A$. A constituent with a relative ionic mobility of 2 and a low pK_a value compared with the pH of the carrier electrolyte will have an inverse relative retention, t_R/t_0, of 4. This means that the sample constituent will migrate at a higher velocity than the carrier constituent. A sample constituent with a relative ionic mobility of 0.5, *i.e.*, $1/r_C = 2$, has a relative retention of unity. Obviously this sample constituent cannot be detected by conductimetric detection. For the molar response, Π, of a conductimetric detector it can be derived that

$$\Pi = F\left(1 - \frac{m_B}{m_C}\right)(\bar{m}_C - \bar{m}_A) \tag{19}$$

where F is the Faraday constant and $\bar{m}_C$ and $\bar{m}_A$ are the effective mobilities of the sample constituent and the carrier constituent. The ionic mobilities of the counter constituent and the sample constituent are given by m_B and m_C. To obtain a high response, the mobility of the counter constituent must be minimized and the difference in effective mobilities of the sample constituent and the carrier constituent must be maximized.

Sample constituents that have the same relative retention obviously cannot be separated. The appropriate formulations on separability have already been given in the criterion for separation[21].

A more detailed concept of retention and separability will not be given here, but it must be emphasized that the retention of each sample constituent is influenced by the physico-chemical parameters and concentrations of all constituents present. The effect of mutual interactions in electrophoretic separation techniques is more pronounced than in chromatographic separation techniques. This adverse effect of Ohm's law can be suppressed only by the application of very small amounts of sample.

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