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Note

Simple calculation of the sample zone pH in isotachophoresis

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The evaluation of the sample zone pH is a basic problem in the study of the steady state in isotachophoresis (ITP). This parameter determines all the chemical variables of the zone, and must therefore be known for the prediction and optimization of ITP separations. The usual method of calculation of the zone pH is computer assisted and provides satisfactory results[1-5]. A more simple procedure, without the aid of a computer, can be used for the case of monoacid species by making some approximations. Such an approach was first investigated by Schumacher and Studer[6], and then by Martin and Everaerts[7]. A generalization of their results is presented here, and practical relationships, as well as their range of validity, are proposed for calculation of the sample zone pH.

THEORETICAL

Beckers and Everaerts reported the sets of equations which need to be considered for accurate computer simulations of cationic[8] and anionic[9] buffered systems. These equations will be used here in a simplified form.

Buffered anionic systems
Let us consider the steady state migration of leading, sample and counter-ion species, denoted L, A and C, respectively. Assuming these species are monoacidic, we can write

$$m_L = m_L^0 \cdot \frac{[L^-]}{[L]_t}, \quad m_A = m_A^0 \cdot \frac{[A^-]}{[A]_t}, \quad m_C = m_C^0 \cdot \frac{[CH^+]}{[C]_t} \tag{1}$$

where m_i^0 and m_i are the absolute and effective mobilities of species i, $[i]$ is the concentration of ionic species i and $[i]_t$ is the total concentration of species i. The mobilities are here regarded as algebraic parameters having the same sign as that of the charge of the species. The conductivity, K_i, of a zone i is then expressed by

$$K_i = F([CH^+]^i m_C^0 - [i^-] m_i^0 + [H^+]^i m_H^0 - [OH^-]^i m_{OH}^0) \tag{2}$$

where F is Faraday's constant and $[j]^i$ is the concentration of species j in zone i. In

most cases, $[H^+]$ and $[OH^-]$ can be neglected and eqn. 2 reduces to:

$$K_i = F([CH^+]^i m_C^0 - [i^-]m_i^0) \tag{3}$$

Introducing eqn. 1 into eqn. 3, we obtain:

$$K_i = F([C]_t^i m_C^i - [i]_t m_i) \tag{4}$$

The ITP steady state condition and Ohm's law can be combined into the expression[2]:

$$\frac{K_L}{m_L} = \frac{K_A}{m_A} \tag{5}$$

Using eqn. 4 for $i = A$ and L, eqn. 5 becomes

$$\frac{m_A}{m_L} = \frac{[C]_t^A m_C^A - [A]_t m_A}{[C]_t^L m_C^L - [L]_t m_L} \tag{6}$$

which can be rearranged to:

$$[L]_t - [A]_t = ([C]_t^L m_C^L / m_L) - ([C]_t^A m_C^A / m_A) \tag{7}$$

Combining the counter-ion mass-balance equation[2]

$$\frac{[C]_t^A}{[C]_t^L} = \frac{1 - m_C^L / m_L}{1 - m_C^A / m_A} \tag{8}$$

with eqn. 7, we obtain:

$$[L]_t - [C]_t^L = [A]_t - [C]_t^A \tag{9}$$

This equation states that the difference between the total concentrations of the sample and counter-ion species is the same for any sample zone and for the leading one. It generalizes the conservation of uncharged weak electrolyte already stated for moving boundary systems[10].

Let us now introduce the acidity constant of each species (in the subsequent equations, i is A or L)

$$k_i = \frac{[i^-][H^+]}{[iH]}, \qquad k_C = \frac{[C][H^+]}{[CH^+]} \tag{10}$$

in which activities are approximated by concentrations. The conservation of the species can be written as:

$$[i]_t = [i^-] + [iH] \qquad [C]_t^i = [C]^i + [CH^+]^i \tag{11}$$

With the same assumptions as in eqn. 3, the electroneutrality principle states that, for a zone i:

$$[i^-] = [CH^+]^i \qquad (12)$$

Combining eqns. 10–12 we obtain:

$$\frac{[C]_t^L}{[L]_t} = \frac{1 + k_C/[H^+]_L}{1 + [H^+]_L/k_L} \quad \text{and} \quad \frac{[C]_t^A}{[A]_t} = \frac{1 + k_C/[H^+]_A}{1 + [H^+]_A/k_A} \qquad (13)$$

Using eqn. 13, eqn. 9 becomes

$$[L]_t - [L]_t \cdot \frac{1 + k_C/[H^+]_L}{1 + [H^+]_L/k_L} = [A]_t - [A]_t \cdot \frac{1 + k_C/[H^+]_A}{1 + [H^+]_A/k_A} \qquad (14)$$

which can be rearranged into a quadratic equation with respect to $[H^+]_A$:

$$\left\{ \frac{1}{k_A} + \frac{[H^+]_L}{k_A k_L} + \frac{[L]_t}{[A]_t}\left(\frac{k_C}{k_A[H^+]_L} - \frac{[H^+]_L}{k_A k_L} \right) \right\}[H^+]_A^2 +$$

$$+ \frac{[L]_t}{[A]_t}\left(\frac{k_C}{[H^+]_L} - \frac{[H^+]_L}{k_L} \right)[H^+]_A - k_C\left(1 + \frac{[H^+]_L}{k_L} \right) = 0 \qquad (15)$$

This equation can be solved in order to express $[H^+]_A$ in terms of $[H^+]_L$, the acidity constants and the ratio $[L]_t/[A]_t$. It must be borne in mind that the latter ratio can be evaluated without knowledge of the pH of the sample zone, as it has been stated that[2]:

$$\frac{[L]_t}{[A]_t} = \frac{1 - m_C^0/m_A^0}{1 - m_C^0/m_L^0} \qquad (16)$$

Although the solution of eqn. 15 needs no more than a basic calculation, the general solution will not be given here, for it would result in a confused relationship. In most practical cases, the leading species is a strong acid, i.e., k_L is large, and eqn. 15 reduces to

$$(\eta + 1)[H^+]_A^2 + \eta k_A[H^+]_A - k_C k_A = 0 \qquad (17)$$

with:

$$\eta = \frac{k_C}{[H^+]_L} \cdot \frac{[L]_t}{[A]_t} = \frac{k_C}{[H^+]_L} \cdot \frac{1 - m_C^0/m_A^0}{1 - m_C^0/m_L^0} \qquad (18)$$

The positive solution of eqn. 17 gives the hydrogen-ion concentration of the sample zone:

$$[H^+]_A = k_A \cdot \frac{-\eta + \sqrt{\eta^2 + 4(\eta + 1)k_C/k_A}}{2(\eta + 1)} \tag{19}$$

This result was given previously by Martin and Everaerts[7] in their basic paper on displacement electrophoresis.

Fig. 1 shows the dependence of pH_A on pH_L for sample species of various pk_A. In order to maintain a satisfactory buffering potential, the useful range of pH_L is about one pH unit on both sides of pk_C[9]. According to whether the first or the second power term can be neglected in eqn. 17, two asymtotes are observed in Fig. 1, one being hardly different from $pH_A = pH_L$. For low pH_L values, η becomes negligible with respect to unity and eqn. 17 reduces to

$$[H^+]_A^2 = k_A k_C \tag{20}$$

i.e.:

$$pH_A = \tfrac{1}{2}(pk_A + pk_C) \tag{21}$$

For high pH_L values, the second power term becomes negligibly small and eqn. 17 reduces to
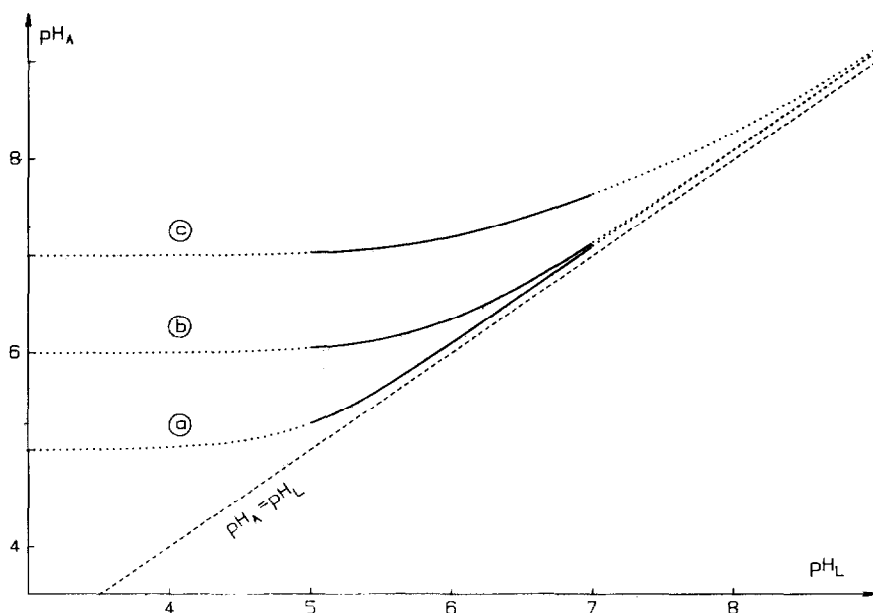
$$[H^+]_A = [H^+]_L \cdot \frac{[A]_t}{[L]_t} \tag{22}$$

Fig. 1. Dependence of the sample zone pH on pH_L (eqn. 19). Leading electrolyte: 10 mM Cl$^-$ ($m^0 = -79.1$), histidine ($pk = 6.0$, $m^0 = 29.6$) (m^0 in 10^{-5} cm^2 V^{-1} s^{-1}, from ref. 11). Sample: arbitrary species ($m^0 = -40$) (a) $pk = 4$, (b) $pk = 6$, (c) $pk = 8$. Dotted lines: extension to experimentally irrelevant electrolyte systems, considering the buffering capacity.

i.e.:

$$pH_A = pH_L + \log\frac{1 - m_C^0/m_A^0}{1 - m_C^0/m_L^0} \tag{23}$$

A further insight into eqn. 17 leads to the limits of validity of eqns. 21 and 23:

$$pH_L < [pk_C \text{ and } \tfrac{1}{2}(pk_A + pk_C)] \qquad \text{for eqn. 21}$$

$$pH_L > [pk_A \text{ and } \tfrac{1}{2}(pk_A + pk_C)] \qquad \text{for eqn. 23}$$

The two limits correspond to the separation of anionic species in increasing order of their pk_A values for eqn. 21 and according to their absolute mobilities for eqn. 23, as defined by Beckers and Everaerts[9].

Buffered cationic systems

For a cationic system, the same treatment can be applied. With LH^+, AH^+/A and CH/C^- as leading, sample and counter-ion species respectively, the quadratic equation obtained is

$$\frac{[H^+]_A^2}{k_C} - \frac{[L]_t}{[A]_t} \cdot \frac{[H^+]_L}{k_C} \cdot [H^+]_A - k_A\left(1 + \frac{[L]_t}{[A]_t} \cdot \frac{[H^+]_L}{k_C}\right) = 0 \tag{24}$$

where k_A and k_C denote the acid dissociation constants of A and C:

$$k_A = \frac{[A][H^+]}{[AH^+]}, \qquad k_C = \frac{[C^-][H^+]}{[CH]} \tag{25}$$

The solution of eqn. 24 is

$$[H^+]_A = \frac{\eta + \sqrt{\eta^2 + 4k_A/k_C(\eta + 1)}}{2/k_C} \tag{26}$$

with:

$$\eta = \frac{[H^+]_L}{k_C} \cdot \frac{[L]_t}{[A]_t} = \frac{[H^+]_L}{k_C} \cdot \frac{1 - m_C^0/m_A^0}{1 - m_C^0/m_L^0}$$

Two simplified relationships can be used for low and high pH_L values:

$$pH_A = pH_L + \log\frac{1 - m_C^0/m_L^0}{1 - m_C^0/m_A^0} \quad \text{for } pH_L < [pk_A \text{ and } \tfrac{1}{2}(pk_A + pk_C)] \tag{27}$$

$$pH_A = \tfrac{1}{2}(pk_A + pk_C) \qquad \text{for } pH_L > [pk_C \text{ and } \tfrac{1}{2}(pk_A + pk_C)] \tag{28}$$

Eqn. 27 corresponds to the separation of cationic species according to their absolute mobilities, whereas eqn. 28 corresponds to a separation in decreasing order of the pk_A values. The separation of metal cations without complex-forming agents is an example of the first case[8].

Buffer-free anionic systems

As regards buffer-free systems, *i.e.*, when H^+ is the only counter ion, the equation set becomes more simple. For L^- and AH/A^- as leading and sample species, one obtains

$$[H^+]_A^2 + k_A[H^+]_A - k_A\omega[H^+]_L = 0 \qquad (29)$$

with

$$\omega = \frac{1 - m_H^0/m_L^0}{1 - m_H^0/m_A^0}$$

from which it can be deduced that:

$$[H^+]_A = \frac{k_A}{2}(-1 + \sqrt{1 + 4\omega[H^+]_L/k_A}) \qquad (30)$$

Of course, in these calculations, $[H^+]$ is not neglected, as it is equal to $[L^-]$.

A very good approximation is given by the relationships:

$$pH_A = \tfrac{1}{2}(pH_L + pk_A - \log \omega) \quad \text{for } pH_L < pk_A + \log 4\omega$$

$$\qquad (31)$$

$$pH_A = pH_L - \log \omega \qquad\qquad \text{for } pH_L > pk_A + \log 4\omega$$

These relationships apply to the particular case of a buffer-free anionic system[6] for which the various absolute mobilities are assumed to be equal ($\omega = 1$).

No relationship will be given for unbuffered systems, *i.e.*, when the counter ion has no acid–base properties, because experimentally in these cases the sample zone pH is not well defined, and liable to instabilities.

DISCUSSION

At present, no device allowing on-line measurement of the sample zone pH in the steady state is commercially available. However, some workers have compared off-line measurements of the zone pH with their computer-simulated values, and demonstrated the reliability of the calculations[1,12]. In this work, the pH value given by eqn. 19 was directly compared with the value computed from our own simulation program. This program gives step heights in good agreement with experimental ones[5]. As the zone pH determines the extent of dissociation of all the species and thereby the conductivity of the zone, the computed pH is considered to be accurate.

The comparison is shown in Fig. 2, where the dependence of pH_A on pH_L is plotted within the range pH 2.5–7 using appropriate counter ions. As soon as pH_L
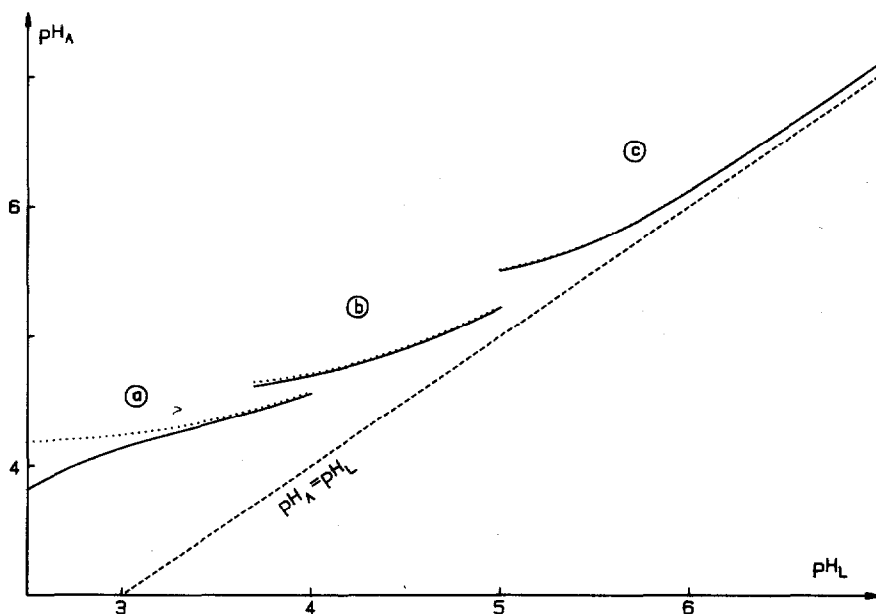
Fig. 2. Dependence of the sample zone pH on pH_L for various electrolyte systems. Comparison between computer simulation (continuous line) and eqn. 19 (dotted line). Leading ion: 10 mM Cl$^-$ (m^0 = −79.1). Counter ions: (a) pH_L = 2.5–4, β-alanine (pk = 3.55, m^0 = 36.7); (b) pH_L = 3.7–5, ε-aminocaproic acid (pk = 4.37, m^0 = 28.8), (c) pH_L = 5–7, histidine (pk = 6.0, m^0 = 29.6). Sample ion: acetate (pk = 4.76, m^0 = −42.4). m^0 (in 10^{-5} cm^2 V^{-1}s^{-1}) and pk values at 25°C taken from ref. 11.

is greater than 3.5 (for a leading ion concentration of 10 mM), there is no significant difference between the two calculated values of pH_A. The approximations made in eqns. 3 and 12 are fully justified for pH_L > 3.5, which accounts for the good fit of the two curves. Moreover, the approximation of activities by concentrations seems to have a negligible influence. Similar results were obtained with various sample species or high pH_L values, so eqn. 19 can be used in the range pH_L 3.5–11 and for any monoacid sample species.

As regards buffer-free systems, pH_L is closely related to the leading ion concentration, as [H$^+$] = [L$^-$], and the useful pH_L range is 2–3. Lower pH_L values would lead to perturbations due to the high mobility of H$^+$, and higher values would correspond to insufficient conductivities of the electrolyte. As [H$^+$] was taken into account in the presented calculations, eqn. 30 can be used in almost every case of buffer-free systems.

CONCLUSIONS

The zone pH is a key parameter as it allows the calculation of the effective mobilities and the conductivity of the zone, which are very useful in the prediction and optimization of separations[1,5]. For monoacid species, and provided that the acidity constants and absolute mobilities are known for any intervening species, a straightforward calculation of the sample zone pH is possible for most of the practical

pH range of ITP experiments. As regards buffered systems, and considering the usual leading ion concentrations (about 10 mM), eqns. 19 and 26 can be used for anionic and cationic separations respectively, in the range pH_L 3.5–11. For buffer-free anionic systems, eqn. 30 (or a similar one for cationic separations) can be used in almost every case, but it is well known that these systems are of limited utility.

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