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CALCULATION AND MEASUREMENT OF CONCENTRATIONS
IN ISOTACHOPHORESIS

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SUMMARY

A set of equations is developed to describe isotachophoretic electrolyte systems in the steady state. pH measurements are done in capillaries and sucrose gradients to check the results of calculations based on these equations. The experimental values show good agreement with the theory.

INTRODUCTION

Isotachophoresis has already proved to be an electrophoretic method with a high resolving power^{1,2}. The principle of this technique is that zones of ionogenic compounds, separated according to their mobilities and equilibrium constants, will move with the same speed. Their concentrations are then adjusted to the concentration of the first ion zone, called the leading electrolyte. The last ion zone is formed by the terminating electrolyte.

The theory of isotachophoresis has been dealt with by several authors. In a theoretical treatment of disc electrophoresis, ORNSTEIN³ derived some equations for the separation of ions in the first isotachophoretic phase of this procedure. In this first phase, the discs are stacked upon each other and move with the same speed. In the second phase, they migrate according to the principles of zone electrophoresis. The equations are valid between pH 4 and 10, where the influence of protons and hydroxyl ions on the conductivity can be neglected.

SCHUMACHER AND STUDER⁴ calculated pH increments in an isotachophoretic system for monovalent weak electrolytes, but where no buffer ions are taken into consideration. They attempted to verify this theory with experiments on paper. MARTIN AND EVERAERTS^{5,7} calculated the pH of isotachophoretic systems for univalent ions including H⁺ and OH⁻. They found, like ORNSTEIN, a difference in pH between two consecutive zones. This pH shift varied from a few tenths of a pH unit to several units. BROUWER AND POSTEMA⁶ described the very separation process by considering the ions before the steady state in isotachophoresis is reached (when all zones move with the same speed).

The purpose of this paper is to derive general equations that can be applied to

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most isotachophoretic systems. The concentrations of the ions in the moving zones will be calculated and the results will be compared to actual measurements.

THEORETICAL

Ion concentrations in isotachophoresis

Let us consider a system of two zones, which are moving according to isotachophoretic principles. The first zone contains A^- , ..., $A^{\alpha-}$ and the second B^- , ..., $B^{\beta-}$ ions. The general positive counter-ions are P^+ , ..., $P^{\pi+}$ (Fig. 1). Choosing a

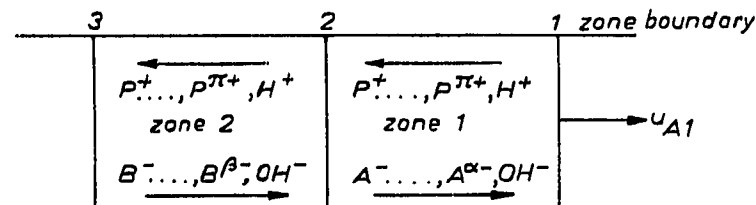


Fig. 1. Isotachophoretically moving zones of the ions A^- , ..., $A^{\alpha-}$ and B^- , ..., $B^{\beta-}$. P^+ , ..., $P^{\pi+}$ is the buffering counterion.

particular concentration of A and a certain pH in zone 1, the concentration of P can be calculated from the equilibrium equations. The concentrations of B and P in the second zone will adjust to the concentrations in the first zone, in such a way that they will create a voltage gradient large enough to give the second zone the same speed as the first one. In order to calculate the concentrations of B and P in zone 2, one can assume the following conditions:

The balance of the electric current. The electric current is constant throughout the system. The current in zone 1 is the same as in zone 2.

The balance of mass. The concentration of the counter-ion within one zone is constant in the steady state. This means that the amount of P transported into a zone is equal to the amount that moves out of the zone.

The electroneutrality principle. The amounts of positive and negative charges are equal in the same zone.

Equilibrium equations. The acid and base equilibria determine the partial ion concentrations.

In the derivation of the following equations it is assumed that: (a) the diffusion effects are negligible; (b) the solutions are dilute, *i.e.*, no activity coefficients have to be included; (c) the area through which the current passes is constant; (d) the effect of electroendosmosis is negligible; (e) no hydrostatic flow exists.

The balance of electric current. For an explanation of the symbols used the reader is referred to the LIST OF SYMBOLS.

According to assumption (c) above, we have

$$I_1 = I_2 \quad (1)$$

The electric conductivity in zone 1 is

$$\begin{aligned} \lambda_1 &= c_{OH^-} m_{OH^-} z_{OH^-} + c_{H^+} m_{H^+} z_{H^+} + \sum_{i=0}^{\alpha} z_{A_i} c_{A_i} m_{A_i} + \sum_{i=0}^{\pi} z_{P_i} c_{P_i} m_{P_i} \\ &= c_{OH^-} m_{OH^-} + c_{H^+} m_{H^+} + \sum_{i=0}^{\alpha} i c_{A_i} m_{A_i} + \sum_{i=0}^{\pi} i c_{P_i} m_{P_i} \end{aligned} \quad (2)$$

Ohms law gives

$$I_1 = G_1 \lambda_1 \quad (3)$$

$$I_2 = G_2 \lambda_2 \quad (4)$$

Substitution of (4) and (3) in (1) gives:

$$G_1 \lambda_1 = G_2 \lambda_2 \quad (5)$$

Substitution of (2) in (5) results in:

$$G_1 (c_{\text{OH}1} m_{\text{OH}1} + c_{\text{H}1} m_{\text{H}1} + \sum_{i=0}^{\alpha} i c_{\text{A}1i} m_{\text{A}1i} + \sum_{i=0}^{\gamma} i c_{\text{P}1i} m_{\text{P}1i}) =$$

$$G_2 (c_{\text{OH}2} m_{\text{OH}2} + c_{\text{H}2} m_{\text{H}2} + \sum_{i=0}^{\beta} i c_{\text{B}2i} m_{\text{B}2i} + \sum_{i=0}^{\pi} i c_{\text{P}2i} m_{\text{P}2i}) \quad (6)$$

As the temperature in zone 1 is higher than in zone 2 (ref. 8), the net mobilities $m_{\text{P}1i}$ and $m_{\text{P}2i}$ will be different.

In an isotachophoretic system all zones have the same speed:

$$u_{\text{A}1} = u_{\text{B}2} \quad (7)$$

$$u_{\text{A}1} = G_1 \cdot \sum_{i=0}^{\alpha} m_{\text{A}1i} \cdot \frac{c_{\text{A}1i}}{c_{\text{A}1}^1} \quad (8)$$

Combination of (7) and (8):

$$\frac{G_2}{G_1} = \frac{c_{\text{B}2}^1 \cdot \sum_{i=0}^{\alpha} m_{\text{A}1i} c_{\text{A}1i}}{c_{\text{A}1}^1 \cdot \sum_{i=0}^{\beta} m_{\text{B}2i} c_{\text{B}2i}} \quad (9)$$

Substituting (9) in (6) gives:

$$\frac{c_{\text{A}1}^1}{c_{\text{B}2}^1} = \frac{\sum_{i=0}^{\alpha} m_{\text{A}1i} c_{\text{A}1i} c_{\text{OH}2} m_{\text{OH}2} + c_{\text{H}2} m_{\text{H}2} + \sum_{i=0}^{\beta} i m_{\text{B}2i} c_{\text{B}2i} + \sum_{i=0}^{\pi} i m_{\text{P}2i} c_{\text{P}2i}}{\sum_{i=0}^{\beta} m_{\text{B}2i} c_{\text{B}2i} c_{\text{OH}1} m_{\text{OH}1} + c_{\text{H}1} m_{\text{H}1} + \sum_{i=0}^{\alpha} i m_{\text{A}1i} c_{\text{A}1i} + \sum_{i=0}^{\gamma} i m_{\text{P}1i} c_{\text{P}1i}} \quad (10)$$

This is the extended form of the KOHLRAUSCH⁹ regulating function. It gives a relation between the ion concentration in the first and the second zone.

The balance of mass. Let us consider the total mass balance of the counterion P in zone 2. If the zone boundary 2 (Fig. 1) is moving forward with a speed $u_{\text{A}1}$ and the total concentration of the counterion in zone 1 is $c_{\text{P}1}^1$ then the mass transport of P into zone 2 due to this movement is $u_{\text{A}1} c_{\text{P}1}^1$

Furthermore, there is an electrophoretic transport of P-ions, which is equal to $G_1 \cdot \sum_{i=0}^{\gamma} c_{\text{P}1i} m_{\text{P}1i}$.

The total mass transport of P into zone 2 is therefore:

$$u_{\text{A}1} c_{\text{P}1}^1 + G_1 \sum_{i=0}^{\gamma} c_{\text{P}1i} m_{\text{P}1i} \quad (11)$$

When the steady state is reached the mass transport of P through boundary 2 and 3 will be equal:

$$u_{A1}c^1_{P1} + G_1 \sum_{i=0}^{\pi} c_{P1i}m_{P1i} = u_{B2}c^1_{P2} + G_2 \sum_{i=0}^{\pi} c_{P2i}m_{P2i} \quad (12)$$

Insertion of (7) and (8) will result in:

$$c^1_{P1} - c^1_{P2} = -c^1_{A1} \frac{\sum_{i=0}^{\pi} m_{P1i}c_{P1i}}{\sum_{i=0}^{\alpha} m_{A1i}c_{A1i}} + c^1_{B2} \frac{\sum_{i=0}^{\pi} m_{P2i}c_{P2i}}{\sum_{i=0}^{\beta} m_{B2i}c_{B2i}} \quad (13)$$

The electroneutrality equations. The balance of charge for zone 1 is:

$$c_{OH1} + \sum_{i=0}^{\alpha} ic_{A1i} = c_{H1} + \sum_{i=0}^{\pi} ic_{P1i} \quad (14)$$

The charge balance for zone 2 is:

$$c_{OH2} + \sum_{i=0}^{\beta} ic_{B2i} = c_{H2} + \sum_{i=0}^{\pi} ic_{P2i} \quad (15)$$

Eqns. 14 and 15 will be different if we consider a system of positive ions A and B and negative ion P which is not the case for the eqns. 10 and 13:

$$c_{OH1} + \sum_{i=0}^{\pi} ic_{P1i} = c_{H1} + \sum_{i=0}^{\alpha} ic_{A1i} \quad (16)$$

$$c_{OH2} + \sum_{i=0}^{\pi} ic_{P2i} = c_{H2} + \sum_{i=0}^{\beta} ic_{B2i} \quad (17)$$

Equilibrium equations. From the equilibrium equations for A, B and P it is possible to derive the following equations:

$$c_{A1i} = \frac{c_{A10}}{(c_{H1})^i} \prod_{j=1}^i k_{Aj} \quad (18)$$

$$c_{B2i} = \frac{c_{B20}}{(c_{H2})^i} \prod_{j=1}^i k_{Bj} \quad (19)$$

$$c_{P1i} = \frac{c_{P10}(c_{H1})^i}{\prod_{j=1}^i k_{Pj}} \quad (20)$$

$$c_{P2i} = \frac{c_{P20}(c_{H2})^i}{\prod_{j=1}^i k_{Pj}} \quad (21)$$

Application of the equations to a system with a divalent terminating ion

Let us consider a system with a divalent, negatively charged, terminating ion and a monovalent, leading and buffering ion. The influence of protons and hydroxyl ions is neglected as a first approximation. Eqn. 10 will then take the form:

$$\frac{c_{A1}^1}{c_{B2}^1} = \frac{m_{A11}c_{A11}}{m_{B21}c_{B21} + m_{B22}c_{B22}} \cdot \frac{m_{B21}c_{B21} + 2m_{B22}c_{B22} + m_{P21}c_{P21}}{m_{A11}c_{A11} + m_{P11}c_{P11}} \quad (22)$$

By application of the electroneutrality principle:

$$c_{A11} = c_{P11} \quad (23)$$

$$c_{B21} + 2c_{B22} = c_{P21} \quad (24)$$

Substitution of (23) and (24) in (22) gives:

$$\frac{c_{A1}^1}{c_{B2}^1} = \frac{m_{A11}}{m_{A11} + m_{P11}} \cdot \frac{(m_{B21} + m_{P21})c_{B21} + 2(m_{B22} + m_{P21})c_{B22}}{m_{B21}c_{B21} + m_{B22}c_{B22}} \quad (25)$$

Combination of the mass balance (13) and electroneutrality rule (23), (24) results in:

$$c_{P1}^1 - c_{P2}^1 = -\frac{c_{A1}^1 \cdot m_{P11}}{m_{A11}} + \frac{c_{B2}^1 m_{P21} (c_{B21} + 2c_{B22})}{m_{B21}c_{B21} + m_{B22}c_{B22}} \quad (26)$$

The equilibrium equations are:

$$c_{A11} = \frac{(c_{A1}^1 - c_{A11})}{c_{H1}} \cdot k_{A1} \quad (27)$$

$$c_{B21} = \frac{(c_{B2}^1 - c_{B21} - c_{B22})}{c_{H2}} \cdot k_{B1} \quad (28)$$

$$c_{B22} = \frac{(c_{B2}^1 - c_{B21} - c_{B22})}{c_{H2}^2} \cdot k_{B1} \cdot k_{B2} \quad (29)$$

$$c_{P11} = \frac{(c_{P1}^1 - c_{P11})}{k_{P1}} \cdot c_{H1} \quad (30)$$

$$c_{P21} = \frac{(c_{P2}^1 - c_{P21})}{k_{P1}} \cdot c_{H2} \quad (31)$$

If two of the nine parameters in the seven equations (25–31) are known, the other seven can be calculated. Thus if the pH and the total concentration of the leading ion are chosen, all other ion concentrations, including the pH in the terminating electrolyte, are fixed. Consequently we are able to calculate the net mobility¹⁰ of the terminating ion, which is defined as

$$mm_B = \frac{\sum_{l=0}^B c_{B2l} \cdot m_{B2l}}{c_{B2}^1} \quad (32)$$

Polyvalent electrolyte systems

Dealing with systems of multivalent ions, where proton and hydroxyl ion-influence is not neglected, an even more nonlinear set of equations will be obtained than those described above. The use of data techniques is necessary to obtain all the roots.

With increasing nonlinearity, the equation will have an increasing number of roots, which are physically incorrect solutions for the system considered. The correct solution is obtained by rejecting all roots containing negative, imaginary and obviously unrealistic concentrations.

EXPERIMENTAL

Materials and methods

The equations derived in the preceding section can be checked by the determination of the pH changes between isotachophoretically moving zones. These measurements, and temperature measurements⁸ by thermocouples, were done in a capillary tube apparatus. A second series of pH determinations was done on a preparative scale in a sucrose-gradient in a glass column. VESTERMARK¹² has already reported results of pH measurements in sucrose-gradients.

All the chemicals used are commercially available and of analytical grade.

The capillary apparatus

A set of six PTFE capillaries was mounted in a water-bath thermostat between two electrode compartments (Fig. 2). The inner/outer diameters of the tubes are 0.45/0.75 mm and the length is approximately 1.5 m. The cathode compartment consists of a perspex block, containing six reservoirs for the terminating electrolyte. The anode compartment is a reservoir for the leading electrolyte. A cellulose acetate membrane is placed over the latter and is stretched by an exactly-fitting polyethylene tube. This construction excludes hydrostatic flow and prevents endosmotic effects to a great extent.

A Baird-Atomic voltage supply, model 1512, was used. All measurements were

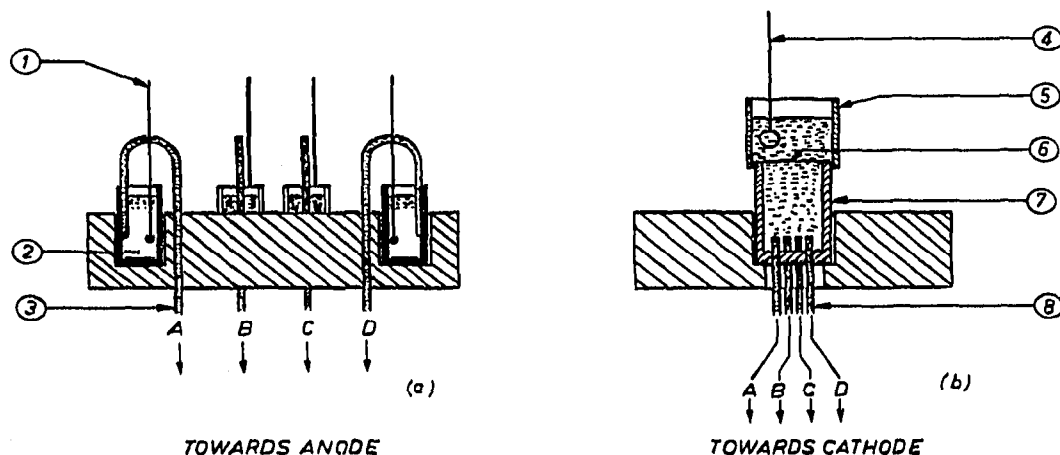


Fig. 2. The electrode compartments of the capillary apparatus. (a) Terminator block. 1 = cathode; 2 = reservoir for terminating electrolyte; 3 = capillary. (b) Leading electrolyte block. 4 = anode; 5 = polyethylene tube; 6 = membrane; 7 = compartment for leading electrolyte; 8 = capillary.

done with a constant voltage of 5 kV. In each experiment, one of the terminator-reservoirs was filled with 0.02 *M* picric acid to indicate how far the analysis had proceeded. The pH in each terminator-reservoir was increased with Tris to a value which was about 0.5 pH units below the pH of the leading electrolyte.

When an experiment was finished, the electrolyte from two capillaries was collected and its pH measured. Then the pH of the content of two other tubes and, finally, of all five tubes together, was determined. The results were averaged together with those of a second identical experiment. The standard deviation was in all cases less than 0.03 pH units.

The isotachophoresis apparatus used for measurement of the temperatures of the zones is basically the same as the one described by EVERAERTS AND VERHEGGEN¹¹, which has a thermocouple glued to the capillary wall to act as a heat detector.

The column apparatus

An isoelectric focusing column (LKB 8100) was used for measurements on a larger scale. Part of the platinum wire of the anode (Fig. 3) was removed to increase the migration distance. A constant flow of 10 ml of leading electrolyte per hour was maintained around the anode, to prevent the migration of protons and electrode products to the cathode. To prevent convection, the leading ion was introduced into the column in a sucrose gradient. The inner tube (Fig. 3), and the bottom part of the outer tube of the column, is filled with leading electrolyte in a 40% sucrose solution. A sucrose gradient, from 40 to 10%, is then layered on top of this solution. Finally, the terminating electrolyte is introduced. The temperature was kept at 25° by a water thermostat. Two power supplies (LKB 4471D) were connected in series and delivered

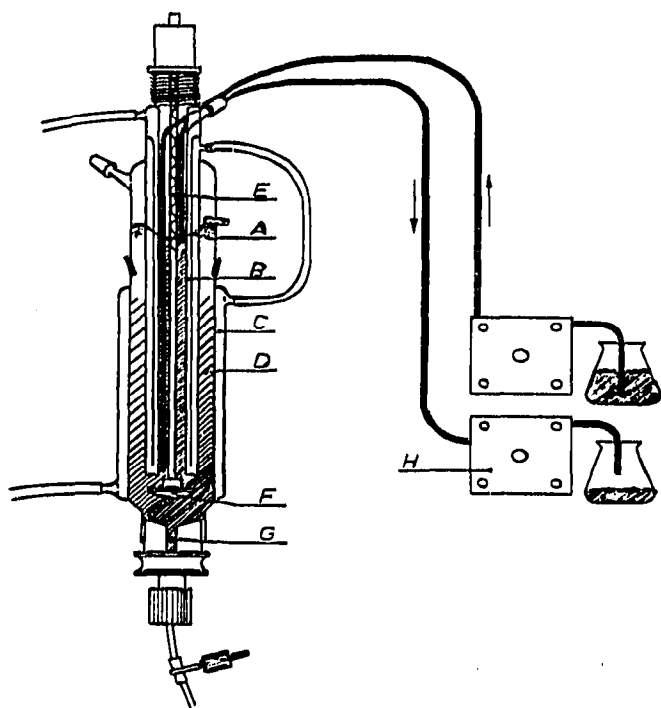


Fig. 3. Column apparatus. A = cathode; B = inner cooling jacket; C = outer cooling jacket; D = annular separation chamber; E = anode; F = valve; G = outlet; H = buffer circulation pump.

a constant voltage of 1600 V. The current decreased from 10 to 2 mA during the time of the analysis. The analysis time was 3 to 4 h, after which the electrolyte was pumped out of the column. Fractions of 2 ml were collected and the pH determined for each fraction. Fig. 4 shows the result of such an experiment.

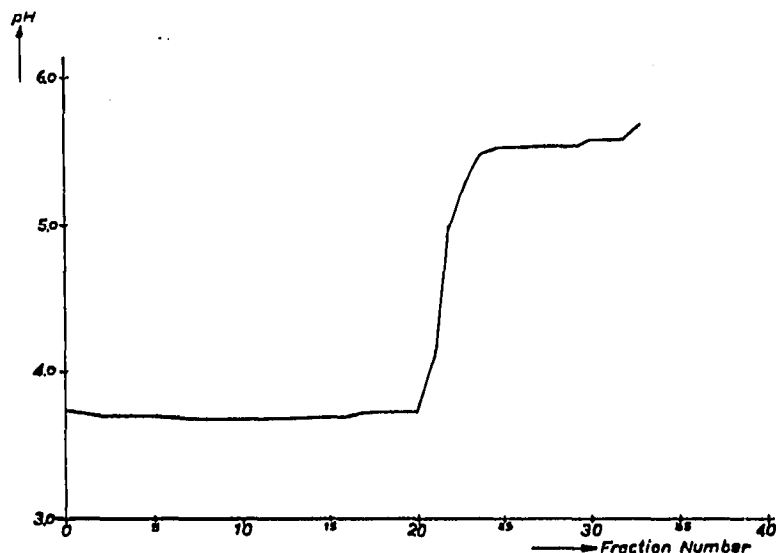


Fig. 4. The pH-change of the zones in an isotachopheretic separation of chloride and carbonate in a sucrose gradient. The buffering counterion is benzidine.

RESULTS

In the first series of experiments a solution of hydrochloric acid and histidine was used as the leading electrolyte. For the capillary experiments the concentrations were: 0.01 M HCl and 0.014 M histidine, which gave a pH of 5.75. In this system the pH of eleven weak acids, which were used as terminators, was determined. The temperature of the thermostatted bath was kept at 25°.

There is good agreement between the theoretical* and experimental values of the pH in the terminator zones, as can be seen in the first two columns of Table I.

The next two columns in Table I show the total concentrations of the terminating ion and the counter-ion in the terminator zone, as they were calculated from eqns. 10 and 13, respectively. The net mobilities are tabulated in the next column.

It is possible to calculate the electrical resistance in a zone from the partial ion concentrations. A plot of this parameter against the experimental recorder step-height of temperature measurement^{7,8} results in Fig. 5. Theoretically, there should be a linear relation between the heat produced and the resistance, which in fact is the case for low resistances (Fig. 5). The deviation from the linearity at high temperatures can be explained by the fact that, in our calculation, the mobility dependence on temperature is not taken into account. Moreover the temperature measured by the thermocouple is not linear with respect to the temperature in the tube, because the heat transport by conductance, convection and radiation, changes with temperature.

The same histidine-HCl electrolyte system was used for the pH measurements

* All theoretical values were calculated with the GE 265 computer. All pK and mobility data are taken from literature (refs. 14-17).

TABLE I

THEORETICAL AND EXPERIMENTAL VALUES OF pH, CONCENTRATION, NET MOBILITY, RESISTANCE AND STEP HEIGHT IN AN ISOTACHOPHORETIC SYSTEM WITH HISTIDINE-HCl AS LEADING ELECTROLYTE

The experimental values are obtained from measurements in a capillary apparatus.

Ion species	$pH_{theor.}$	$pH_{exp.}$	$c^{1}B_2$	$c^{1}P_2$	mm_H	ρ ($\Omega\text{ cm} \times 10^3$)	h (mm)
Chloride	5.75	5.75	0.0100	0.0145	78	1.12	0
Oxalate	5.76	5.78	0.0050	0.0143	72	1.20	20.1
Tartrate	5.79	5.80	0.0048	0.0141	62	1.38	47.2
Formate	5.79	5.81	0.0094	0.0139	56	1.50	51.5
Citrate	5.82	5.81	0.0043	0.0139	56	1.51	60.0
Succinate	5.85	5.81	0.0052	0.0138	52	1.61	68.0
Malonate	5.85	5.83	0.0055	0.0137	51	1.64	72.1
Acetate	5.89	5.87	0.0087	0.0132	39	2.03	109.4
α -Hydroxybutyrate	5.89	5.87	0.0085	0.0130	36	2.16	125.2
Phosphate	5.87	5.87	0.0077	0.0127	34	2.27	140.4
Carbonate	6.39	6.41	0.0089	0.0133	23	3.55	187.3
Diethylbarbiturate	6.88	6.74	0.0077	0.0121	6	11.80	—

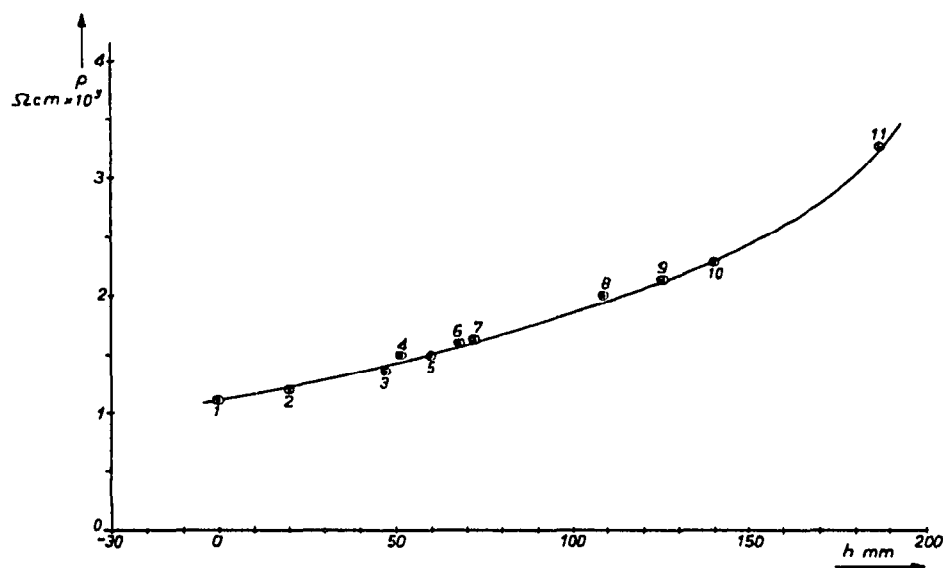


Fig. 5. The theoretical specific resistance of eleven isotachophoretically moving acid zones is plotted against the signal of a thermocouple which measures the temperature of these zones. 1 = chloride, 2 = oxalate, 3 = tartrate, 4 = formate, 5 = citrate, 6 = succinate, 7 = malonate, 8 = acetate, 9 = α -hydroxybutyrate, 10 = phosphate, 11 = carbonate.

in the glass column. For practical reasons the pH in the leading electrolyte was not the same in each experiment. The results are listed in the first three columns of Table II. Differences can be found between theoretical and experimental values for some acids. This could be due to diffusion and mixing effects during the sampling procedure. The influence of the original pH in the terminator solution on the pH of the isotachophoretically migrating terminator zone was studied in a few experiments. There was no difference in the experimental pH values as shown in Table II irrespective of whether histidine oxalate at pH = 5.1, or oxalic acid at pH = 1.95 was used as original

TABLE II

THEORETICAL AND EXPERIMENTAL VALUES OF THE pH IN TWO ISOTACHOPHORETIC SYSTEMS, ONE WITH HISTIDINE-HCl AND THE OTHER WITH BENZIDINE-HCl AS LEADING ELECTROLYTE

The experimental values were obtained from measurements in a sucrose gradient.

Ion species	Leading electrolyte: histidine-HCl			Leading electrolyte: benzidine-HCl		
	pH _{Cl}	pH _{theor.}	pH _{exp.}	pH _{Cl}	pH _{theor.}	pH _{exp.}
Oxalate	5.88	5.89	6.00	3.88	4.09	4.06
Formate	5.88	5.92	5.92	3.78	4.19	4.22
Succinate	6.10	6.16	6.21	3.70	4.36	4.34
Tartrate	6.11	6.13	6.19	3.88	4.12	4.12
Phosphate	5.93	6.04	6.14	3.72	3.87	4.10
Citrate	5.91	5.97	6.10	4.20	4.36	—
Malonate	5.85	5.94	5.90	3.81	3.89	4.00
Acetate	5.78	5.92	5.91	3.80	4.65	4.62
α-Hydroxybutyrate	5.86	5.98	6.05	3.70	4.58	4.28
Carbonate	5.89	6.43	6.40	3.70	5.52	5.52
Diethylbarbiturate	5.90	6.92	7.06	3.75	5.98	5.60

terminating electrolyte. Also, carbonate solutions at pH 9.1 and 7.0 gave the same pH values.

A second electrolyte system was used based on benzidine and hydrochloric acid. The reasons for the choice of benzidine were threefold. Firstly, the use of benzidine as a buffer ion enables the validity of the equations for a system with a divalent counter ion to be checked. Secondly, most of the weak acids which are used as terminators have their pH values within the buffering region of benzidine. Thirdly, because benzidine is a buffer at low pH, it can be used to prove that the equations are still valid even if a relatively large part of the current is carried by H⁺.

On the other hand benzidine is not very stable, only slightly soluble in water and is poisonous. The pH values of the citrate and phosphate zones in the column could not be measured exactly, because large amounts of white precipitate were formed in the column, probably because of the higher ionic strength of these com-

TABLE III

THEORETICAL AND EXPERIMENTAL VALUES OF THE pH, CONCENTRATIONS AND NET MOBILITY IN AN ISOTACHOPHORETIC SYSTEM WITH BENZIDINE-HCl AS LEADING ELECTROLYTE.

The experimental values were obtained from measurements in a capillary apparatus.

Ion species	pH _{theor.}	pH _{exp.}	c ¹ _{B2}	c ¹ _{P2}	nm _B
Chloride	3.35	3.35	0.00100	0.0055	78
Oxalate	3.56	3.42	0.00682	0.00475	46
Formate	3.97	3.85	0.00999	0.00453	35
Succinate	3.65	3.62	0.00803	0.00434	35
Tartrate	3.75	3.75	0.00631	0.00429	33
Phosphate	3.60	3.70	0.00720	0.00417	32
Citrate	3.78	3.74	0.00676	0.00399	28
Malonate	4.16	4.00	0.00864	0.00375	21
Acetate	4.47	4.20	0.00958	0.00346	15
α-Hydroxybutyrate	4.44	4.19	0.00444	0.00330	14
Carbonate	5.36	4.80	0.00536	0.00303	4

ponents. Tables III and II show the theoretical and experimental pH values in the capillary and column system, respectively. There are differences between calculated and experimental values, which will be discussed below.

In the preceding two series of experiments, pH values were measured in the terminating zones, using the same pH in the leading electrolyte. In a third series of experiments the influence of the pH of the leading electrolyte (0.03 *M* acetate) on the pH of the terminator zone (cacodylate) was studied over a wider pH range. The pH of the leading electrolyte was varied by changing the counter ion (Tris) concentration. The results are plotted in Fig. 6. Three experimental points seem to fit the theoretical curve. In two cases there is a small deviation from the curve.

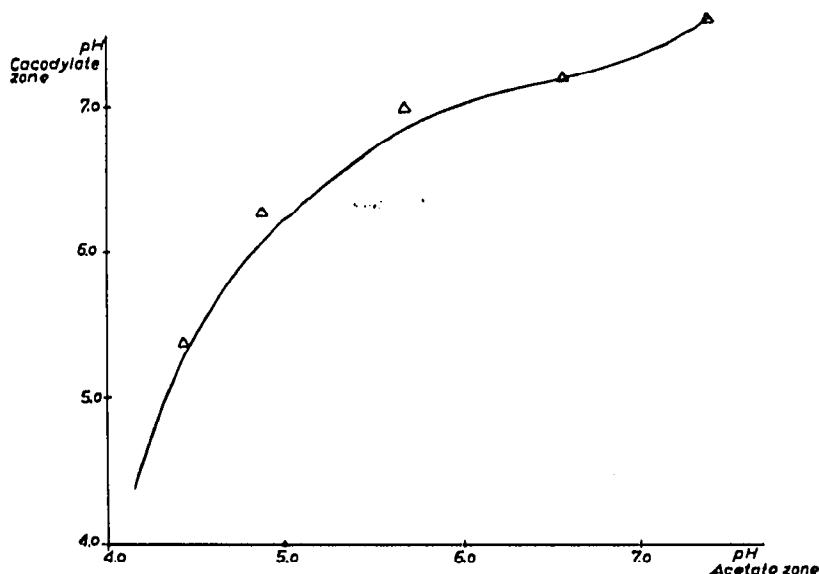


Fig. 6. The relation between the pH in the leading electrolyte and in the terminator zone for an isotachophoretic system. Acetate (0.03 *M*) is the leading ion, cacodylate is the terminator. The concentration of the counterion Tris determines the pH of the leading electrolyte. The Δ -symbol is used for the experimental results; the curve being the one determined theoretically.

DISCUSSION

The purpose of this paper was to derive equations applicable to most isotachophoretic systems and to compare the results of these equations with experimental measurements. The results of the pH measurements seem to confirm the theory. However, one should bear in mind that only a limited number of electrolyte systems has been tested here. Small differences between calculated and measured pH values are probably due to variations in mobility and *pK* data taken from literature. Moreover, temperature differences between the leading electrolyte zone and the terminator zone should be taken into account. The original pH in the terminator solution is not decisive for the pH created in the isotachophoretically moving terminator zone.

Mobilities and *pK* values are the most important parameters for the separation of ions. When working in a pH range near the *pK* values (\pm one pH unit) of the intermediate ions it is clear that small differences in *pK* values will influence the isotachophoretic separation more than small differences in the mobilities.

The pH chosen for the leading electrolyte is important for the separation of the sample ions. Fig. 7 shows clearly that phosphate and formate, for example, will move in separate zones, whatever pH between 7.5 and 5.75 is chosen in the leading electrolyte. Acetate and phosphate, however, are not separated or are very difficult to separate around pH 6.5. It also should be mentioned that the order of the net mobilities of two compounds (*e.g.* phosphate and acetate) can be changed by varying the pH of the leading electrolyte.

The results of the calculations and experiments, as described above, give the impression that the pH in an anionic system always rises from one zone to the next according to the net mobility in those zones. However, it is possible for the pH in two succeeding zones not to rise. In a system which contains weak and strong electrolytes with comparable mobilities, a pH drop can be expected.

Fig. 7 illustrates this effect. At pH 7.5, phosphate will move in front of picrate, but at a higher pH. This pH-drop might cause some difficulties in the separation, when the pH of the leading electrolyte is decreased to 7. When phosphate ions mix with the picrate ions owing to diffusion or convection effects, they experience a lower pH and consequently acquire a lower net mobility which in turn prevents them from migrating back into the phosphate zone.

This effect is disturbing when complex mixtures are separated with the aid of spacers^{1,2,13}. If the spacers are strongly acidic and are used for separations of compounds with higher pK values (*e.g.* proteins), they cannot separate and will migrate together with these compounds in one zone.

Another case when the equations are not applicable is this: Let us consider an anionic system at a high pH, say 12. This pH will give a constant migration of hydroxyl ions from the terminator zone through the preceding zones. It is no longer

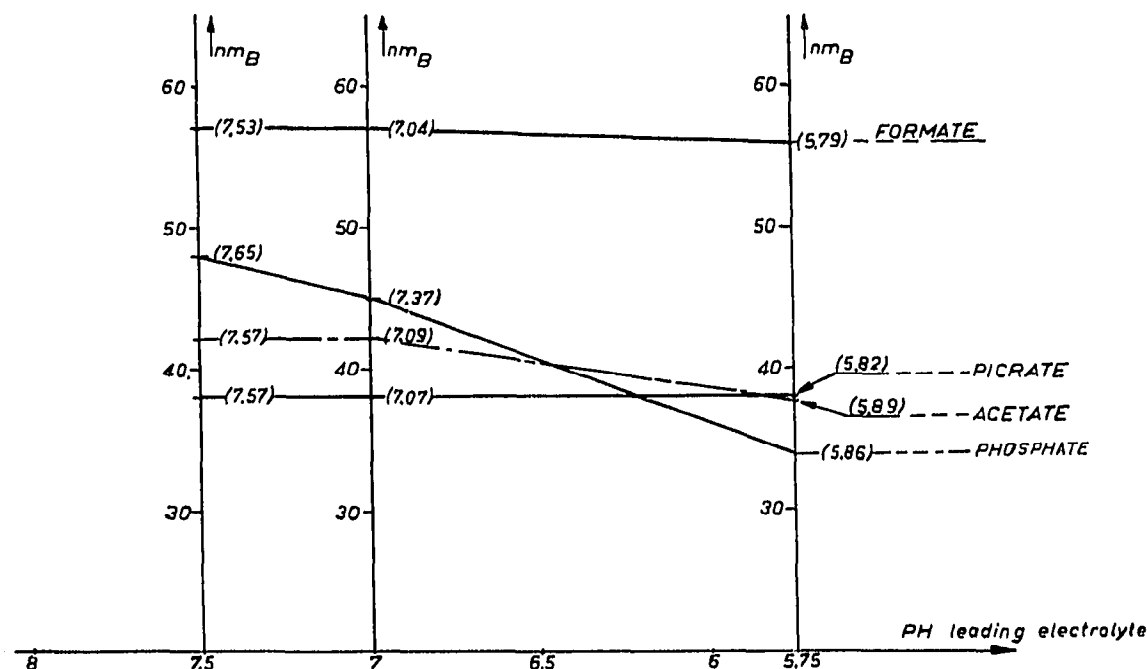


Fig. 7. The net mobilities of some acids are plotted against the pH of the leading electrolyte. Tris is the counterion at pH = 7.5 and pH = 7. Histidine is the counterion at pH = 5.75. The values in parenthesis show the pH of the zone of the ion species considered.

necessary then for the sample ion zones to follow each other directly with the same speed, since the hydroxyl ions will carry a considerable part of the current. It is difficult to predict at which pH the conditions for an isotachophoretic system are no longer fulfilled. The degree of isotachophoresis depends on the ratio of the current transported by hydroxyl ions and by the anions in question. As long as the conductivity of the hydroxyl ions is low (*e.g.* 1%), compared to the conductivity of the sample ions, the equations can be applied. For cationic systems protons will give a corresponding effect at low pH's.

LIST OF SYMBOLS, INDICES AND ABBREVIATIONS

Symbols

c	partial ion concentration (mol cm ⁻³)
c^1	total concentration (mol cm ⁻³)
G	voltage drop (V cm ⁻¹)
h	stepheight (mm)
I	current (A)
i	ionisation degree
j	ionisation degree
k	equilibrium constant
m	mobility (cm ² V ⁻¹ sec ⁻¹)
nm	net mobility (cm ² V ⁻¹ sec ⁻¹)
r	resistance (Ω)
u	velocity (cm/sec)
α, β, π	maximal ionisation degrees for ion A, B and P
λ	conductivity (Ω^{-1} cm ⁻¹)
ρ	specific resistance (Ω cm)

Indices

A ₁ i	ion A in zone 1 with charge i
A ₁	ion A in zone 1
OH ₁	hydroxyl ion in zone 1

Abbreviations

pH _{exp.}	experimentally determined pH value
pH _{theor.}	theoretically determined pH value
PTFE	polytetrafluoroethylene
Tris	trishydroxymethylaminomethane

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