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ANALYTICAL ISOTACHOPHORESIS

THE CONCEPT OF THE SEPARATION CAPACITY*

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SUMMARY

The concept of separation capacity in isotachophoresis has been investigated in order that the analytical separation possibilities may be predicted for given working conditions. A relationship has been derived between the amount and composition of a binary mixture under isotachophoretic separation and the amount of electricity which has to be passed through the system to obtain complete separation of the mixture. By using this relationship, values of the separation capacity have been calculated for three two-component mixtures analyzed by means of two isotachophoretic instruments and verified experimentally.

INTRODUCTION

Capillary isotachophoresis has been applied to the quantitative analyses of fertilizers[1,2], metallurgical baths[3], mineral waters[4], muscle extracts[5], silage extracts[6] and some metabolites of styrene, toluene and xylene in urine[7]. Earlier applications have also been reviewed[8,9]. However, it could be said that analytical isotachophoresis still awaits wider practical application.

In connection with the search for new applications, the question arises as to whether the appropriate separation or analysis can be performed for a given sample. The difference between the effective mobilities of the components under investigation is an important factor; if the mobilities do not differ sufficiently neither the separation nor the analysis can be performed. Some papers[4,10,11] have therefore been devoted to the selection of the operating conditions which optimize the difference between the effective mobilities of the components to be analyzed. However, the selection of the operating conditions[11] in this way was somewhat academic, being limited to the separation of model mixtures. Moreover, even when effective mobilities are sufficiently different, the required analysis may still not be possible.

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It is necessary to remember that we are attempting to separate given amounts of species (*cf.* ref. 12), and hence the problem can be restated in terms of whether a given amount (moles) of one component having a certain effective mobility can be separated from a given amount of another component having a different effective mobility. Further, each zone obtained by isotachophoresis occupies a definite volume in the column that is proportional to the amount of the component in the sample injected and inversely to the concentration of this component in the zone[13]. It follows that the zones occupy part of the column between the injection point and the detector and that the sum of the zone volumes must be less than the volume of the column between these two points. The volume of the column is thus a very important feature of an isotachophoretic instrument.

However, from a knowledge of the volume alone one cannot say anything about the separation possibilities, which are a characteristic not only of the equipment but also of the electrolyte system used, evaluated in terms of the actual sample. The concept of the separation capacity of the column (Arlinger[14]), *i.e.*, the maximum amount of the equimolar mixture of the two selected components that can be separated, illustrates better the analytical possibilities under given operating conditions. The separation capacity is evaluated by first preparing an equimolar mixture of the selected pair of components and then injecting increasing amounts of this mixture into the column. The largest amount of the mixture (in moles) that can just be separated determines the separation capacity of the column. On further increasing the amount injected a step appears in the record which corresponds to the mixed zone. The separation capacity so defined is valid only for the given conditions and for the particular pair of components. In order that such information may be generalised, *i.e.*, used for the calculation of the separation capacity of an isotachophoretic system in the analyses of other components, a theoretical background must be established for the concept of separation capacity. For this purpose the theory of the development of zones[15,16] should be applied. By employing a similar procedure to that described by Brouwer and Postema[15] we now show that the amount of electricity passed through the system is a general parameter describing the separation process. Moreover, based on this amount of electricity, general relationships can be derived that enable the separation capacity under given working conditions to be calculated, regardless of the geometry and the cross-sectional shape of the separation capillary used.

THEORETICAL

The concept of separation capacity as defined above is valid for both strong and weak electrolytes. The following approach, unless it is stated otherwise, is limited to strong univalent electrolytes and to the assumption of concentration-independent mobilities, since this allows fundamental relations to be derived simply and briefly without using too many symbols and intermediate calculations. The expansion to concentration-dependent mobilities and polyvalent strong and weak electrolytes will be discussed later. Moreover, the influence of diffusion on the spreading of the zone boundary is neglected and the latter boundary is considered to be a plane perpendicular to the direction of migration, having a surface area equal to the column cross-section.

Let us consider a sample containing N_A and N_B moles of ionic species A and

B with mobilities u_A and u_B, respectively. The sample is introduced into the column between the leading electrolyte (consisting of the leading ion, L, and of the counter-ion, R) and the terminating electrolyte (terminator ,T). Obviously, mobilities u_A and u_B must be less than that of the leading ion and greater than that of the terminator. Let us investigate now a system consisting of zone 1 of the leading ion (L), zone 2 of the pure component A, a mixed zone 3 containing components A and B, zone 4 of the pure component B and zone 5 of the terminator T. Since the concentrations of the components are a function of the position in the column, the concentrations will be studied at a given position. Passing this position, zone 2 has concentration $c_{A,2}$ of species A and zone 4 has concentration $c_{B,4}$ of the species B. The mixed zone 3 at this position contains components A and B in concentrations $c_{A,3}$ and $c_{B,3}$, respectively. The absolute values of the concentrations are given by the Kohlrausch relation and by concentration $c_{L,1}$. Additionally, since the mixed zone passes only stationary concentration boundaries, eqn. 1 holds (*cf.* ref. 17)

$$\frac{c_{A,3}}{c_{B,3}} = \frac{N_A}{N_B} \tag{1}$$

The conductivity of the mixed zone is given by

$$\varkappa_3 = F(u_A c_{A,3} + u_B c_{B,3} + u_R c_{R,3}) \tag{2}$$

where subscript R designates the values concerning the counter-ion and F is the Faraday constant. Taking into consideration strong univalent electrolytes, the following relation holds for neutrality

$$c_{A,3} + c_{B,3} = c_{R,3} \tag{3}$$

The velocities of the individual ionic species in the mixed zone are

$$v_{A,3} = E_3 \cdot u_A \tag{4}$$

$$v_{B,3} = E_3 \cdot u_B \tag{5}$$

and

$$E_3 = \frac{I}{S \cdot \varkappa_3} \tag{6}$$

is the gradient of the electric field in the mixed zone 3, I is the electric driving current and S is the cross-section of the separation capillary.

The front boundary of the mixed zone 3 moves at a speed determined by the ionic species which disappears in this position (*cf.* ref. 17), *i.e.*, by component B having the velocity $v_{B,3}$. The flow-rate of the ions of component A through the boundary, the surface area of which is identical to the capillary cross-section S, is

$$\frac{dN_A}{dt} = S(v_{A,3} - v_{B,3}) \cdot c_{A,3} \tag{7}$$

By combining eqns. 1–7, we obtain the relationship between the electric current I and resulting flow-rate dN_A/dt, which determines the speed of separation of component A from component B, in the form

$$I = F \cdot \frac{u_A + u_R + (u_B + u_R)(N_B/N_A)}{u_A - u_B} \cdot \frac{dN_A}{dt} \tag{8}$$

In order that the total amount N_A may be separated from the original mixture, *i.e.*, for the disappearance of the mixed zone 3, an amount of electricity, Q_S, must be passed. This so called "separation parameter" is given by integration of eqn. 8, giving

$$Q_S = F \cdot \frac{N_A u_A + N_B u_B + u_R (N_A + N_B)}{u_A - u_B} \tag{9}$$

It is to be noted that, under the above simplifications (an ideal injection between the leading and terminating electrolytes, and neglecting diffusion), the separation parameter, Q_S, is independent of the type of the leading and terminating ionic species and is a function of the ratio of the amounts of the components, the amount of the sample, the mobilities of its components and the mobility of the counter-ion.

On the passage of electricity not only separation of the components but also shift of the zones takes place along the column. Let us call "column hold-up", denoted Q_L, the amount of electricity corresponding to the current and the time required for the appearance of the sample front in the detector. In the case of a constant current, I, the relation $Q_L = I(t_1 - t_0)$ holds where $t_1 - t_0$ is the interval from the beginning of the separation at time t_0 to the passage of the first boundary through the detector at time t_1. Let us now determine the amount of component A, $N_{A,L}$, separated during the migration of zones from the injection point to the detector, *i.e.*, by the passage of the column hold-up Q_L

$$N_{A,L} = \frac{u_A - u_B}{u_A + u_R + (u_B + u_R)(N_B/N_A)} \cdot \frac{Q_L}{F} \tag{10}$$

It can be seen that the maximum amount separated, $N_{A,L}$, is independent of the total amount, N_A, in the sample (a sufficient amount must obviously be present in the sample, $N_A > N_{A,L}$) and is only a function of the ratio of the components in the sample, the corresponding mobilities and the column hold-up.

At first sight, $N_{A,L}$ may appear to be the total amount of component A that can be separated from the mixture $N_A + N_B$ and detected under the conditions given. Actually, a larger amount, $N_{A,max.}$, is separated and detected since the separation continues during the passage of zone 2 of pure component A through the detector. As can be seen from Fig. 1, the maximum separable amount is determined by the column hold-up Q_L, and by the amount of electricity that is necessary for zone 2 to pass the detector in such a way that the last amount of the mixed zone 3 may disappear in the detector. Fig. 1a shows the situation at time t_1, *i.e.*, after the amount of electricity Q_L has passed and the front of zone 2 has just reached the detector. Zone 2 is the zone of pure component A separated by the amount of electricity Q_L

from a mixed zone 3, and it contains the amount $N_{A,L}$ of component A. Fig. 1b depicts the situation at time t_2 where the second boundary, *i.e.*, the rear boundary of zone 2, has just reached the detector and the mixed zone 3 has just disappeared. Zone 2 is followed by zone 4 and the amount $N_{A,max.}$ in zone 2 is equal to the maximum separable amount of component A.

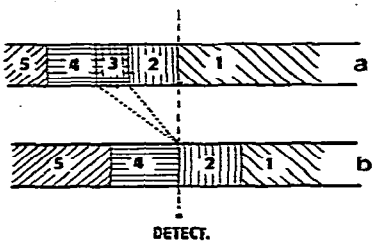

Fig. 1. The situation in the column after the passage of an amount of electricity equal to the column hold-up, Q_L (a), and in the case of the separation of the maximum amounts of the components (b).

The total amount of electricity, $Q_{max.} = I(t_2 - t_0)$, that has passed through the column from the beginning of the analysis to the entrance of the rear boundary of zone 2 into the detector is given by the sum of Q_L and the amount of electricity corresponding to the number of moles, $N_{A,max.}$, with regard to the transference number of component A into its zone, and can be expressed by the following relationship

$$Q_{max.} = Q_L + N_{A,max.} F \left(1 + \frac{u_R}{u_A}\right) \tag{11}$$

When $N_{A,L}$ and Q_L in eqn. 10 are replaced by $N_{A,max.}$ and $Q_{max.}$, respectively, and this equation is combined with eqn. 11, we obtain

$$N_{A,max.} = \frac{u_A - u_B}{(u_B + u_R)(N_B/N_A) + u_B(1 + [u_R/u_A])} \cdot \frac{Q_L}{F} \tag{12}$$

From eqn. 12 the amounts of components A and B that can be separated from one another under the given operating conditions, *i.e.*, the corresponding column hold-up, Q_L, are evaluated directly. Thus the mixture $N_{A,max.}$ and $N_{A,max.}(N_B/N_A)$ of components A and B, respectively, can be separated. In order to express the separation capacity, N_S, *i.e.*, for the equimolar mixture $N_A = N_B = N_S$, eqn. 12 is modified to give

$$N_S = \frac{u_A - u_B}{u_R(1 + [u_B/u_A]) + 2u_B} \cdot \frac{Q_L}{F} \tag{13}$$

The preceding relationships consider the absolute values of the mobilities of the individual components. This means that corrections must be made according to the Debye–Hückel–Onsager relationship if the tabulated limiting mobilities are to be used

in the calculations. This complication can, however, be eliminated by using the relative mobilities, which can then be considered to be independent of concentration (*cf.* ref. 18). In our case it is advantageous to select u_A as the reference mobility and the relative values are then $U_B = u_B/u_A$ and $U_R = u_R/u_A$. Under these conditions, eqns. 12 and 13 become

$$N_{A,max.} = \frac{1 - U_B}{(U_B + U_R)[N_B/N_A] + U_B(1 + U_R)} \cdot \frac{Q_L}{F} \quad (14)$$

and

$$N_S = \frac{1 - U_B}{U_R(1 + U_B) + 2U_B} \cdot \frac{Q_L}{F} \quad (15)$$

respectively.

The preceding relationships were derived for strong univalent electrolytes where there are no significant complex-forming interactions between the counter-ion and the species to be separated. It can easily be shown that, under the latter condition, the validity of all the relationships is maintained for strong polyvalent species and univalent weak electrolyte species when the amounts in moles and the ionic mobilities are replaced by the number of equivalents and the effective mobilities, respectively. In the case of polyvalent weak electrolytes, where one component passes the boundary of the mixed zone in the form of a larger variety of ions, the separation capacity can be calculated for a given situation by solving a modified system of the above equations with the aid of computer programme.

More complicated situations occur in the case of weak electrolytes in non-buffered systems and under the influence of complex-forming equilibria with counter-ions. In such cases the above relationships may be of no use since the tabulated mobility data do not correspond to the actual behaviour of the species in the isotachophoresis column. In such cases, the species separated may drastically influence each other, *e.g.*, enforced isotachophoresis[19], or they may change their charge and even show isoelectric behaviour (*cf.* ref. 20). Similarly, the model used may be invalidated in cases where other effects are superimposed, *e.g.*, adsorption in paper isotachophoresis[21,22].

EXPERIMENTAL

For verifying the relationships derived, three binary mixtures were chosen, consisting of the following pairs of anions, ClO_3^-–BrO_3^-, ClO_3^-–IO_3^- and ClO_3^-–trichloroacetate. These pairs were selected because sufficiently precise mobility data are available for these anions in the literature, the tabulated data are expected to be valid during the isotachophoretic separation and the data cover a broad range of mobility differences. The leading electrolyte chosen, 0.01 *M* NaCl, is expected to show no disturbing effects, and sufficiently precise data on sodium and chloride ions are available. Sodium chloride has already been used successfully as the leading electrolyte for the separation of strong anions[21]. 0.01 *M* glutamic acid served as the terminating electrolyte. All the above chemicals were of analytical grade (Lachema, Brno, Czechoslovakia).

Two types of isotachophoresis instrument were used, having different geom-

etries of the separation capillary and different detection systems. The first instrument, described previously[23], consisted of a block of organic glass into which electrode chambers, control valves, connecting channels and the separation capillary were inserted. The second apparatus used was an LKB 2127 Tachophor (LKB, Bromma, Sweden). In the first case, the separations were performed in a capillary of rectangular cross-section, dimensions 200 × 1.0 × 0.2 mm, created by a groove in the organic glass block and covered with PTFE foil pressed on to the block by means of a thermostatted plate. The plate was maintained at 25°. Detection was carried out by measuring the electric current gradient by means of two platinum contacts, penetrating into the groove for a distance of 0.04 mm. A stabilized current of 400 or 340 μA was used as the driving current, supplied from a high-voltage source. The high-voltage source and the detection device have been described previously[24]. A Servogor Model RE 571 recorder (Goerz, Electro, Vienna, Austria) was used. The total time of analysis ranged from 4 to 10 min. In the second case, the separations were carried out in a PTFE capillary (42 cm × *ca.* 0.5 mm I.D.), which was maintained at a constant temperature of 25°. The apparatus was equipped with a differential detector and with a UV detector operating at 254 nm. The separation was carried out at a constant current of 150 μA, and the total time of the analysis ranged from 8 to 16 min. The samples of standard mixtures were injected by a Hamilton syringe, the amount of electricity passed was calculated from the electric current (measured by means of an ammeter incorporated into the isotachophoretic instrument) and the time was measured by means of a stopwatch.

RESULTS AND DISCUSSION

The Q_L values for a flat-column system and for a Tachophor were determined as described previously to be 0.0708 and 0.120 C, respectively (the averages from five measurements). Values of N_S were calculated from relationship 13 using the determined values of Q_L, and for the pairs ClO_3^-–BrO_3^-, ClO_3^-–IO_3^- and ClO_3^-–Cl_3CCOO^- by using the limiting mobilities at 25° taken from the literature[25].

The determination of N_S values was carried out by injecting increasing amounts of equimolar mixtures of the pairs of components until the mixed zone appeared and started to grow. By extrapolating to zero length of the mixed zone, the maximum amount of the individual components in the mixture, N_S, that has just been separated is obtained. The measured dependences of the sizes of the mixed zones (in mC) on the injected amounts are shown in Fig. 2. The intercepts of the straight lines with the ordinate determine the values of N_S. The experimental and calculated values of N_S are compared in Table I. In each case, the experimental amounts were less than the calculated amounts.

In addition to the non-ideal situations, that have been neglected by the theoretical model, the deviations of the tabulated mobilities from their actual values are another source of errors. The values tabulated for the mobilities will be significant in those instances where the relative mobility U_B approaches 1. For example, in the case of a chlorate–bromate mixture a 2% deviation in the tabulated mobility represents a *ca.* 18% difference in the resulting theoretical amount, N_S. Taking into account all these factors, the agreement between the theoretical and experimental values in Table I is very good.

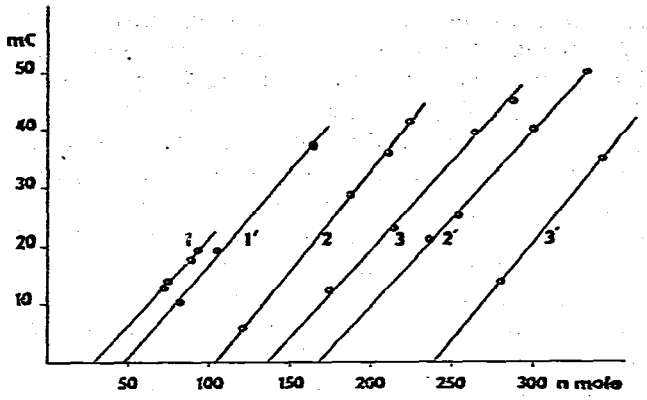

Fig. 2. The dependence of the size of the mixed zone on the amount of the equimolar mixtures of the pairs injected, $N_2 = N_4 = N_{inj.}$. The size of the mixed zone is expressed in terms of the amount of electricity (mC) required for the zone to pass the detector. The pairs separated were chlorate–bromate chlorate–iodate and chlorate–trichloroacetate, designated as 1,2 and 3, and 1′, 2′ and 3′, for the flat column and for the Tachophor, respectively.

TABLE I

COMPARISON OF THEORETICAL AND EXPERIMENTAL VALUES OF THE SEPARATION CAPACITY N_S

The values of mobility used for the calculation were: $50.1 \cdot 10^{-5}$, $64.6 \cdot 10^{-5}$, $55.7 \cdot 10^{-5}$, $40.5 \cdot 10^{-5}$ and $35.0 \cdot 10^{-5}$ cm²/V · s for sodium (counter-ion), chlorate, bromate, iodate and trichloroacetate, respectively[25].

Mixture	*Flat column, Q_L = 70.8 mC N_S (nmole)*			*Tachophor, Q_L = 119.9 mC N_S (nmole)*		
	Calc.	*Exptl.*	*Δ (%)*	*Calc.*	*Exptl.*	*Δ (%)*
Chlorate–bromate	31.9	28.2	11.6	54.1	46.5	4.1
Chlorate–iodate	108.8	104.1	4.3	184.3	167.1	9.3
Chlorate–trichloroacetate	147.3	135.5	8.0	249.5	237.7	4.7

CONCLUSIONS

The question as to whether a given separation of ionic species can be achieved by isotachophoresis can be examined with the help of the concept of separation capacity, which illustrates the separation possibilities under given conditions. It has been shown theoretically and verified experimentally that the separation capacity is directly proportional to the column hold-up, Q_L, under given conditions, *i.e.*, to the amount of electricity which passes through the column up to the time at which the sample front reaches the detector, and is independent of the length and cross-section of the separation capillary and of the magnitude of the driving current. Further, in cases where the mobilities of the ionic species to be separated can be considered as constants throughout the separation system, the separation capacity can be calculated by using relationship 15.

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