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ISOTACHOPHORESIS OF KINETICALLY LABILE COMPLEXES

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SUMMARY

This paper deals both theoretically and experimentally with the behaviour of kinetically labile anionic complexes under the conditions of isotachophoretic migration. It has been shown that in an isotachophoretic zone of an anionic metal–ligand complex, owing to the dissociation equilibrium free metal cations and free ligand anions are present, which, in proportion to the signs and values of their mobilities, migrate out of the zone of the complex. The migrating zone of the complex, leaving behind a trace of the free metal cation, appears as a bleeding zone and decomposes slowly in the course of the migration. A mathematical description of the decomposing zone is elaborated, giving the decomposition rate of the complex depending on the conditional complex stability constant, operational parameters of the column and the composition of the leading electrolyte. It has been demonstrated that in both main cases of mutual movement of the complex and the free ligand (the ligand overtakes the complex or lags behind it) the decomposition of the complex during the migration can be negligible under properly selected operating conditions (mainly the composition of the leading electrolyte), and the zone will appear isotachophoretically stable and can be utilized for quantitative analysis. Model experiments with NTA and EDTA complexes agree with the theory. The model described makes it possible both to predict the isotachophoretic behaviour of anionic complexes under the given operating conditions and to select these conditions so that the stability of the zones of the complexes under investigation may be influenced selectively in order to obtain the required separation.

INTRODUCTION

Every electrophoretic separation is based on differences in the mobilities of the components under investigation. These differences are often so small that a direct separation cannot be carried out. The utilization of complex-forming equilibria provides one of the possibilities of affecting the migration behaviour of the components in such a way that the required separation may be achieved.

A number of workers have studied the utilization of complex-forming equilibria in zone electrophoresis (for a review see ref. 1). Of interest is the use of a background electrolyte containing citric[2] or lactic[3] acid, with which a number of metal cations, including rare earths, were separated successfully. The influence of a complex-

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forming agent can be so strong that it may result in a migration in the opposite direction, *e.g.*, the separation of Cu, Cd, Bi and Hg in hydrochloric acid medium[4,5]. The separation of metal cations in the form of EDTA complexes in ligand-buffered systems[6] was elaborated both theoretically and experimentally and was utilized, *e.g.*, for the separation and the determination of rare earth metals[7].

Electrophoretic ion focusing[8] is a special technique employing complex-forming equilibria. It is based on the establishment of gradients of H_3O^+ and the complex-forming agent in reverse directions. Using chloride, acetate, EDTA and NTA, cations of analytical groups II–IV[9] and rare earth metals[10] were separated.

In isotachophoresis attention has so far been paid above all to kinetically inert complexes[1]. Both analytical counter-flow isotachophoresis[11,12] and its preparative continuous arrangement[13] have been applied to separate $IrCl_nBr_{6-n}^{2-}$ ($n = 0$–6) complex anions. Further, *e.g.*, $Cr(SCN)_n(CN)_{6-n}^{3-}$ ($n = 0$–6) anions were separated by isotachophoresis in non-aqueous media[14,15].

Labile complex-forming equilibria have already been employed in isotachophoresis to affect the mobilities of the anions under separation where the leading electrolyte used contained a suitable complex-forming counter cation[16]. Under such conditions, during the isotachophoretic run the counter cation continuously enters the migrating zones and forms kinetically labile complexes with the various anionic species. Applications have been elaborated for the determination of chlorides and sulphates in mineral waters[16] and for the determination of nitrates, sulphates and phosphates in liquid fertilizers[17], with Cd^{2+} and Ca^{2+}, respectively, as complex-forming counter ions.

There is little information available on the migration behaviour of kinetically labile complexes sampled in the isotachophoretic operational electrolyte system where the leading electrolyte used does not contain a complex-forming counter ion. Theoretical and experimental studies carried out at this Institute[18] with EDTA complexes serving as model species have shown that EDTA complexes injected into an isotachophoretic system may provide, depending on the pH, either a zone of pure complex or a zone of pure ligand, or both. These investigations have led to the suggestion of a promising variant of isotachophoresis, the so-called "bleeding zone technique"[19].

A recently published paper[20] gives experimental observations on the isotachophoretic behaviour of EDTA complexes. It was shown that the separation of M^{II}–EDTA complexes is possible only at higher pH values of 5–9, and on decreasing the pH to 4 the effective mobilities of the complexes converge to the effective mobility of the free ligand.

The aim of this work was to provide a comprehensive theoretical description of the migration behaviour of kinetically labile anionic complexes and to verify the theory experimentally on the example of NTA and EDTA complexes.

THEORETICAL

Let us consider a metal cation M^+ and a ligand Y^- which form with one another the only negatively charged complex MY^- according to the equilibrium

$$M^+ + Y^- \rightleftharpoons MY^- \qquad (1)$$

where the signs of the particles are presented regardless of the number of elemental charges involved. The stability constant of the complex MY^- is given by the relationship

$$K' = K \cdot \frac{\alpha_{MYH}}{\alpha_{YH}\,\alpha_{MOH}} = \frac{c_{MY}}{c_M c_Y} \tag{2}$$

where c_i is the analytical concentration of the component i; the coefficients α of the side-reactions of protonations of the ligand and the complex and of the formation of hydroxo complexes of the metal are included in the conditional constant, K' (ref. 21).

Let us now introduce the complex MY^- into an anionic isotachophoretic system which follows the condition $\bar{u}_Y < \bar{u}_L$, $\bar{u}_{MY} < \bar{u}_L$ (where $\bar{u}_i$ is the effective mobility of component i and the subscript L designates the anion of the leading electrolyte). Let H^+ and K^+ be common counter ions in all of the zones, the mutual ratio of which is determined by the concentration and pH of the leading electrolyte.

After a certain migration time the situation occurs when between the zone of leading electrolyte L^- and that of terminator T^- migrates a zone of complex MY^- with concentration c_{MY} adjusted to the leading electrolyte. Complex MY^- is in its zone in the dissociation equilibrium according to eqn. 1. Assuming $c_{MY} \gg c_Y$, the contribution of Y^- to the conductivity of the zone is negligible and MY^- migrates almost isotachophoreticly. For the migration velocity of the leading ion L^- and that of the complex MY^- holds the relationship

$$E_L \bar{u}_L = E_{MY} \bar{u}_{MY} \tag{3}$$

where E_i is the electric gradient in zone i. If the protonation of complex MY is neglected, *i.e.*, $\alpha_{MYH} = 1$, the condition of constant density of electric current throughout the zones can be written as follows:

$$E_L(z_L c_L \bar{u}_L + c_K u_K + c_H u_H)_L = E_{MY}(z_{MY} c_{MY} \bar{u}_{MY} + c_K u_K + c_H u_H)_{MY} \tag{4}$$

where z_i is the charge of ion i and subscripts L and MY designate the zone of the leading electrolyte and that of the complex, respectively. If the complex protonation is neglected as mentioned above, the ratio of the concentrations of K^+ and H^+ remains constant, *i.e.*,

$$\frac{c_{K,MY}}{c_{K,L}} = \frac{c_{H,MY}}{c_{H,L}} = \frac{z_{MY} c_{MY}}{z_L c_L} \tag{5}$$

By combining eqns. 3, 4 and 5, the following relationship can be derived for the adjusted concentration, c_{MY}:

$$c_{MY} = c_L \cdot \frac{\bar{u}_{MY}}{\bar{u}_L} \cdot \frac{\bar{u}_L z_L + \frac{1}{c_L}(c_K u_K + c_H u_H)_L}{\bar{u}_{MY} z_{MY} + \frac{1}{c_L} \cdot \frac{z_{MY}}{z_L}(c_K u_K + c_H u_H)_L} \tag{6}$$

Considering eqn. 2, then for the concentration of cation M^+ and ligand Y^- in the zone of complex MY^- we have

$$c_M = c_Y = \sqrt{\frac{c_{MY}}{K'}} \tag{7}$$

The situation in the zone of complex MY^- is illustrated schematically in Fig. 1. Cation M^+ migrates in the opposite direction to that of the zone of complex MY^- and leaves this zone through its rear boundary. The zone thus leaves behind a trace of the free cation M^+, the concentration of which is proportional to the dissociation equilibrium of the complex — the complex decomposes during the migration and its zone seems to bleed (*cf.*, "bleeding zone isotachophoresis"[19]).

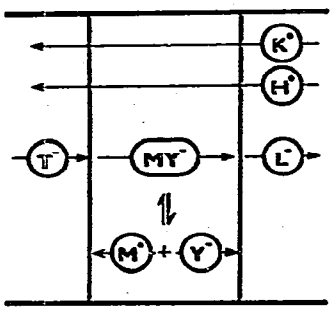


Fig. 1. Diagram of isotachophoretic migration of a kinetically labile complex.

Anion Y^- moves principally in the same direction as complex MY^-; however, its migration relative to that of the zone of the complex is given by the difference in the effective mobilities of both components. With $\bar{u}_Y > \bar{u}_{MY}$ the free ligand overtakes complex MY^- and leaves the zone through its front boundary; when $\bar{u}_Y < \bar{u}_{MY}$ the ligand in the zone of the complex is retarded and leaves the zone via its rear boundary.

The following section will discuss both of these possibilities separately.

(*a*) $\bar{u}_Y > \bar{u}_{MY}$

In this case the zone of complex MY^- is considered, with front boundary α and rear boundary β (Fig. 2a) and with free ligand Y^- inside it. Ligand Y^- migrates more rapidly than complex MY^- and leaves the zone via its front boundary α. Let us now follow the processes proceeding in the layer $\alpha'\alpha$ at the front boundary. Within time t, n_α moles of cation M^+ will leave the layer under investigation via the plane α', which is determined by the relationship

$$n_\alpha = c_M S_k v_M t = c_M S_k E_{MY}(\bar{u}_M + \bar{u}_{MY})t \tag{8}$$

where S_k is the cross-sectional area of the isotachophoretic column and v_M is the migration velocity of cations M^+ through the zone of the complex. The complex-forming equilibrium in layer $\alpha'\alpha$ is thus disturbed and its re-establishment is accom-

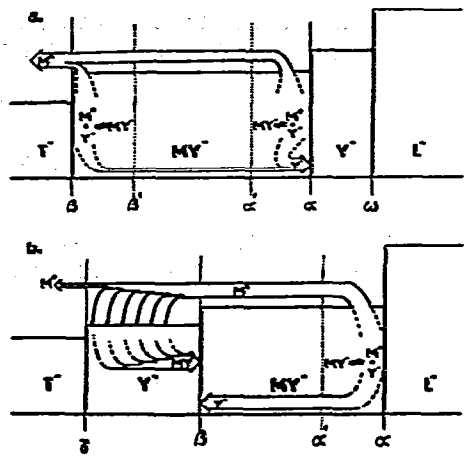


Fig. 2. Isotachophoretic migration behaviour and decomposition of kinetically labile complex MY^- for the mobility of free ligand Y^- higher or lower than that of the complex: (a) $\bar{u}_Y > \bar{u}_{MY}$; (b) $\bar{u}_Y < \bar{u}_{MY}$.

panied by the decomposition of n_α moles of complex MY^- into n_α moles of cation M^+ and n_α moles of ligand Y^-. The whole process has a dynamic character, since cation M^+ leaves layer $\alpha'\alpha$ continuously and migrates into the zone of the complex. This results in the dynamic decomposition of the complex at the front boundary α. Ligand Y^- released by the decomposition of complex MY^- (together with the ligand coming from the zone through plane α') will separate from complex MY^-. It migrates through boundary α and forms zone $\alpha\omega$ of the pure ligand Y^- (Fig. 2a). The concentration $c_{Y,Y}$ is again adjusted to the leading electrolyte L^-.

The decomposition of the complex at the rear boundary β of the zone of complex MY^- will proceed analogously, with the only difference that the complex-forming equilibrium will be disturbed by the migration of free ligand Y^- into zone $\alpha\beta$ (Fig. 2a). By analogy with eqn. 8, we can write for the amount of the complex decomposed at boundary β within time t

$$n_\beta = c_Y S_k E_{MY}(\bar{u}_Y - \bar{u}_{MY})t \tag{9}$$

The resulting decomposition rate of the complex, dn_{MY}/dt, will then be given by the superposition of the decomposition rates of the complex at both boundaries of the zone, α, β:

$$\frac{d}{dt}(n_\alpha + n_\beta) = S_k E_{MY}\,[c_M(\bar{u}_M + \bar{u}_{MY}) + c_Y(\bar{u}_Y - \bar{u}_{MY})] \tag{10}$$

and with regard to eqn. 7 then

$$\frac{dn_{MY}}{dt} = S_k E_{MY} \sqrt{\frac{c_{MY}}{K'}}(\bar{u}_M + \bar{u}_Y) \tag{11}$$

The number of the moles of the complex decomposed within the total analysis time, t_a, is then (considering that $E_{MY} = v_{MY}/\bar{u}_{MY}$ and $L_k = t_a v_{MY}$, where L_k is the length of the column) given by

$$n_r = S_k L_k \left(\frac{\bar{u}_M + \bar{u}_Y}{\bar{u}_{MY}}\right) \sqrt{\frac{c_{MY}}{K'}} \tag{12}$$

If the electric current is expressed as $I = E\varkappa S_k$ and $\varkappa = F(\bar{u}_{MY}c_{MY}z_{MY} + u_K c_K + u_H c_H)_{MY}$ is the specific conductivity, by rearrangement according to eqns. 3 and 4 the following expression is obtained:

$$E_{MY} = \frac{I}{S_k F} \cdot \frac{\bar{u}_L}{\bar{u}_{MY}} \cdot \frac{1}{(\bar{u}_L c_L z_L + u_K c_K + u_H c_H)_L} \tag{13}$$

where F is the Faraday constant. By combining eqns. 11 and 13 and by the rearrangement we obtain

$$n_r = \frac{t_a I \bar{u}_L}{F(\bar{u}_L z_L c_L + u_K c_K + u_H c_H)_L} \left(\frac{\bar{u}_M + \bar{u}_Y}{\bar{u}_{MY}}\right) \sqrt{\frac{c_{MY}}{K'}} \tag{14}$$

where the easily measurable amount of electricity passed through the column from the start until the appearance of the first zone in the detector, the column hold-up[22], $t_a I$, is included instead of the column volume, $S_k L_k$.

Eqn. 12 makes it possible to determine the factors that influence the amount of decomposed complex. They are as follows:

(i) The conditional stability constant of the complex, K', the value of which for a given complex is strongly dependent on pH;

(ii) the mobilities of individual components, also dependent on pH;

(iii) the concentration of the complex in its zone, c_{MY}, determined by eqn. 6; and

(iv) the column volume, $S_k L_k$, which can be expressed (*cf.*, eqn. 14) by combining the electric current, I, analysis time, t_a, and the composition of the leading electrolyte.

By estimating the values of the above factors, and by using eqn. 14, calculations can be made to show how the selected complex will behave under the given operating conditions: whether complex MY^- will decompose to a considerable extent during the separation run or whether the zone of the complex will reach the detection cell virtually quantitatively, *i.e.*, whether the complex will form a virtually stable isotachophoretic zone.

(*b*) $\bar{u}_Y < \bar{u}_{MY}$

This case can be expected in acidic systems where the migration of free ligand Y^- may be retarded by protonation much more than that of complex MY^-. Both of the components formed by the decomposition of complex MY^- —cation M^+ and ligand Y^-— will then leave the zone of MY^- through the rear boundary. The processes proceeding during the complex decomposition at the zone front boundary are

illustrated in Fig. 2b. From layer $\alpha\alpha'$ at the front boundary both cation M^+ and ligand Y^- pass through plane α' into zone $\alpha\beta$. The same amounts of both M^+ and Y^- will be formed by the decomposition of the complex and will pass through plane α' per unit of time. However, the equilibrium concentrations of M^+ and Y^- in zone $\alpha\beta$, c_M and c_Y, respectively, will be established according to the inverse ratio of their speeds related to zone $\alpha\beta$ as a reference frame. It holds that

$$\frac{c_M}{c_Y} = \frac{\bar{u}_{MY} - \bar{u}_Y}{\bar{u}_{MY} + \bar{u}_M} \tag{15}$$

By analogy with eqn. 8 we can write for the amount of the complex decomposed within time t at the front boundary α

$$n_\alpha = c_M S_k v_M t = c_M S_k E_{MY}(\bar{u}_M + \bar{u}_{MY})t = c_Y S_k E_{MY}(\bar{u}_{MY} - \bar{u}_Y)t \tag{16}$$

By combining eqns. 2 and 15, the following relationship is obtained for conditional constant, K':

$$K' = \frac{c_{MY}}{c_M^2} \cdot \frac{\bar{u}_{MY} - \bar{u}_Y}{\bar{u}_{MY} + \bar{u}_M} \tag{17}$$

and by combining eqns. 16 and 17 we obtain for the decomposition rate

$$\frac{dn_{MY}}{dt} = S_k E_{MY} \sqrt{\frac{c_{MY}}{K'}(\bar{u}_{MY} + \bar{u}_M)(\bar{u}_{MY} - \bar{u}_Y)} \tag{18}$$

By analogy with eqn. 12 the resulting relationship is then obtained:

$$n_r = S_k L_k \sqrt{\frac{c_{MY}}{K'}} \cdot \frac{\sqrt{(\bar{u}_{MY} + \bar{u}_M)(\bar{u}_{MY} - \bar{u}_Y)}}{\bar{u}_{MY}} \tag{19}$$

determining the molar amount of complex MY^- decomposed during the migration along the column length, L_k, at the front of the zone in the case when the ligand has a lower mobility than the complex, $\bar{u}_Y < \bar{u}_{MY}$.

Now let us reconsider the processes taking place at the rear boundary of the zone of the complex (Fig. 2b). While free metal cation M^+ leaves zone $\alpha\beta$ for the terminator zone, a zone of free ligand Y^-, $\gamma\beta$, is created behind boundary β (under the assumption that $\bar{u}_Y > \bar{u}_T$). Thus cation M^+ passing through boundary β enters zone $\gamma\beta$ of free ligand Y^- and as a result of the recombination process complex MY^- is again formed. Owing to its higher mobility, complex MY^- is forced to join its own zone $\alpha\beta$. Hence this process counteracts the decomposition of the complex and decreases the decomposition rate.

The speed at which cation M^+ will pass the zone of the ligand is given by the relationship

$$v_{M,Y} = \frac{dl}{dt} = (\bar{u}_Y + \bar{u}_M) E_Y \tag{20}$$

Passing the zone, cation M^+ will gradually be converted again into complex MY^-, proportionally to the tendency to establish the equilibrium according to eqn. 1. For the conversion rate of the process $M^+ + Y^- \xrightarrow{k} MY^-$ it holds that

$$-\frac{dc_M}{dt} = kc_M c_Y - \frac{k}{K'} \cdot c_{MY} \tag{21}$$

Eqn. 21 holds for non-equilibrium concentrations of all of the components at a fixed point of zone $\gamma\beta$ at any given distance, l, from boundary β. Assuming that $c_M c_Y \gg c_{MY}/K'$, the second term in eqn. 21 can be neglected and, after combination with eqn. 20 we can write

$$-\frac{1}{c_M} \cdot \frac{dc_M}{dt} = \frac{kc_Y}{(\bar{u}_Y + \bar{u}_M) E_Y} \cdot \frac{dl}{dt} \tag{22}$$

After rearrangement (considering that $E_Y = v_Y/\bar{u}_Y$) and by integration we obtain

$$[\ln c_M]_{c_{Mo}}^{c_{Mk}} = -\frac{kc_Y \bar{u}_Y}{(\bar{u}_Y + \bar{u}_M) v_Y} [l]_0^{l_Y} \tag{23}$$

where l_Y is the length of zone $\gamma\beta$ of ligand Y^- and c_{Mo} and c_{Mk} are the concentrations of free cation M^+ at boundary β and γ, respectively.

By expressing eqn. 23 in explicit form:

$$c_{Mk} = c_{Mo} \exp\left\{-kl_Y \frac{c_Y}{v_Y} \cdot \frac{\bar{u}_Y}{\bar{u}_Y + \bar{u}_M}\right\} \tag{24}$$

it follows that with increasing length of ligand zone $\gamma\beta$ the concentration of cation M^+ in this zone decreases exponentially. Fig. 3 illustrates concentration profiles for cation M^+ in the zone of ligand Y^- for various values of the rate constant, k. Estimates of numerical values were taken as the other parameters in eqn. 24 for the calculation.

It can be seen from Fig. 3 that a key factor determining the amount of the metal captured in the zone of ligand Y^- is the rate constant, k. If the value of this constant is sufficiently high, even a short zone of free ligand Y^- can cause the zone of the complex, which would otherwise (in accord with eqn. 19) completely decompose during the analysis, to migrate virtually intact and again behave as a stable isotachophoretic zone (enforced stability).

(c) $\bar{u}_Y = \bar{u}_{MY}$

For completeness, even the case when the effective mobilities of complex MY^- and free ligand Y^- are identical must be considered. Under these conditions free ligand Y^- formed by the decomposition does not separate from complex MY^- and during the migration a mixed zone with an increasing concentration of Y^- is formed. Owing to the increasing concentration of Y^- in the mixed zone, the concentration of M^+ in this zone will decrease during the analysis, in agreement with eqn. 2, and

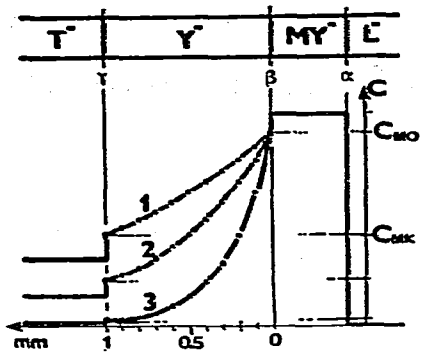


Fig. 3. Calculated concentration profiles of metal cation M^+ in the column for various values of the rate constant, k: (1) $k = 10^2\ M^{-1}\ sec^{-1}$; (2) $k = 2 \cdot 10^2\ M^{-1}\ sec^{-1}$; (3) $k = 5 \cdot 10^2\ M^{-1}\ sec^{-1}$.

therefore the decomposition rate of the complex will also decrease. The total molar amount of the complex decomposed during the analysis will then be less than the amount calculated for the initial decomposition rate by using eqn. 12.

EXPERIMENTAL

The experiments were carried out in an isotachophoretic column made of a block of Perspex with a separation capillary of rectangular cross-section (1.0 × 0.2 mm). A gradient detector, composed of two platinum contacts placed in the capillary *ca.* 0.05 mm apart, was applied to the detection. The column was connected to a high-voltage source of stabilised d.c. current supply providing a maximum current of 400 μA at a voltage of 16 kV. The entire equipment has been described in detail earlier[23,24].

All of the measurements were performed at a driving current of 140 μA and the temperature was maintained at 294°K. A non-buffered solution of a mixture of potassium chloride and hydrochloric acid with a total concentration of chlorides in the range 0.005–0.020 *M* served as the leading electrolyte. Its pH varied over the range 2.0–4.0. To prevent possible disturbing effects caused by penetration of the artefacts formed in the electrode chambers in the separation capillary, buffer solutions of potassium acetate–acetic acid (or potassium maleate–maleic acid) with the same concentration of potassium ions and with the same pH as those of the leading electrolyte were placed in the anodic chamber (behind the membrane). Solutions of glutamic and benzoic acids, respectively, of suitable concentrations served as a terminator.

Nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA) disodium salt and the other chemicals used were of analytical-reagent grade (Lachema, Brno, Czechoslovakia). Volumes of 1.0–15.0 μl of the solutions at a concentration of 0.003 *M* were injected for analysis. Solutions of complexonates were prepared by mixing standard NTA or EDTA solutions with a solution of chloride or nitrate of the respective metal.

The pH of the solutions was measured with a Radelkis OP 203 pH meter with the use of a glass electrode and a saturated calomel electrode.

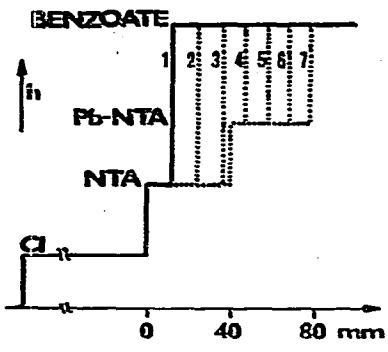
Experiments with a counter flow of the leading electrolyte were carried out by

using a simple arrangement in which an extra reservoir of the leading electrolyte was connected via a narrow-bore capillary to the buffering volume of the column. The counter flow-rate was then controlled by setting up a suitable height of this reservoir above the level of the terminating electrolyte.

RESULTS AND DISCUSSION

To verify the suggested model experimentally, complexes of copper and lead with nitrilotriacetic acid were selected in case (a). At a pH of the leading electrolyte of 2–3 these complexes partially decompose during the analysis and therefore represent suitable models for our purposes.

A direct consequence of eqn. 12 is that the amount of the complex decomposed is independent of the amount injected for the analysis, if the amount injected is sufficiently large and if constant operating conditions are maintained. Fig. 4 illustrates experimental isotachophoregrams for various amounts of Pb–NTA injected. It can be seen that with small amounts injected the complex decomposed totally during the analysis and only the step of the ligand appeared in the record. The respective step-length was proportional to the amount of the complex injected (ascending part of curve 1 in Fig. 5). As the amount injected exceeds the n_r value, *i.e.*, the maximum amount of the complex which can decompose during the analysis, in addition to the step of the ligand also the step corresponding to zone of the complex (*cf.*, curve 4 in Fig. 4) appeared in the isotachophoregram. With an even greater amount injected, the step-length of the complex extended (curve 2 in Fig. 5) while the step-length corresponding to the ligand zone remained constant (plateau of curve 1 in Fig. 5). The maximum amount of the complex, n_r, which can decompose under the given operating conditions can be determined from Fig. 5 as the point of intersection of straight line 2 with the *x*-axis.



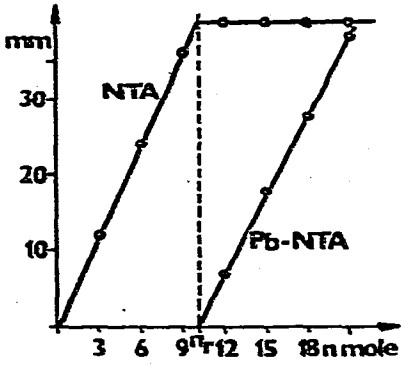


Fig. 4. Isotachophoregrams of various amounts injected (1.0–7.0 μl, curves 1–7) of 0.003 *M* Pb–NTA. Leading electrolyte, 0.008 *M* Cl^- (KCl + HCl); pH = 3.0; I = 140 μA.

Fig. 5. Dependence of the step-length (mm) of the ligand and the complex on the amount of Pb–NTA injected (nmole). NTA, curve 1; Pb–NTA, curve 2 (see text).

Let us now examine how the amount, n_r, will vary with changes in some of the operating conditions.

The principal factor in eqn. 12 determining the stability of the complex is the conditional stability constant, K'. It is strongly dependent on pH in the zone of the

complex. Fig. 6 shows experimentally determined amounts of decomposed Pb–NTA complex, n_r, at various pH values of the leading electrolyte and, for comparison, also a full-line curve representing theoretical values according to eqn. 12. The necessary values of the effective mobilities of Pb–NTA and NTA were determined experimentally by evaluation of the heights of the steps in the isotachophoregram[24], and the values of other constants were taken from tables[25,26]. The step of the pH between the leading electrolyte and the zone of the complex was estimated by an orientation calculation[27] to be *ca.* 0.8 pH unit. It can be seen from Fig. 6 that the experimental values are in good agreement with the theoretical values.

According to eqn. 12, the amount of the decomposed complex, n_r, is proportional to the square root of the complex concentration in its zone, c_{MY}. To verify this dependence, Pb–NTA decomposition was investigated under various concentrations of the leading electrolyte at a constant pH of 3.0, the complex concentration in the zone then being calculated according to eqn. 6. Fig. 7 illustrates the dependence thus obtained, which confirms that the value of n_r is directly proportional to the square root of the complex concentration.

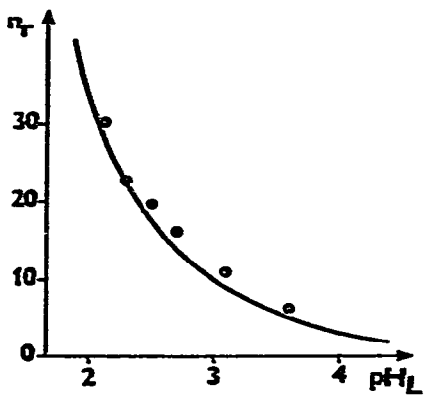


Fig. 6. Calculated curve and experimental values for the dependence of the amount of decomposed Pb–NTA, n_r (nmole), on the pH of the leading electrolyte, pH$_L$.

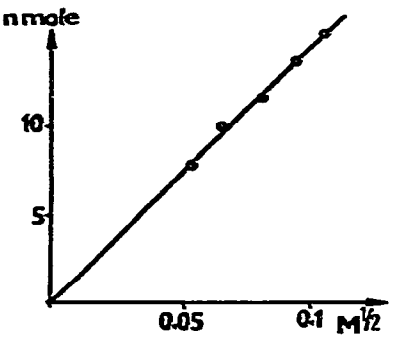
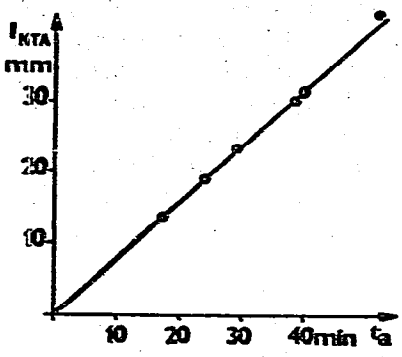


Fig. 7. Experimental dependence of the amount of Pb–NTA decomposed (nmole) on the square root of the complex concentration in the zone ($M^{1/2}$).

From eqn. 12 it follows additionally that the amount of the decomposed complex is directly proportional to the column volume, $S_k L_k$, or to the amount of the electricity, $t_a I$, passed through the column (*cf.* eqn. 14). To verify this experimentally an extension of the migration time, t_a, was obtained by employing a counter flow of the leading electrolyte at a constant driving current, I. The dependence of the step-length corresponding to the zone of free ligand (which is directly proportional to the amount of the decomposed complex, *cf.* Fig. 5) on the analysis time, t_a, is shown in Fig. 8. The measurement was carried out for the Cu–NTA complex at a pH of the leading electrolyte of 2.3. It can be seen that experimental results agree with the theory.

In order to select a suitable experimental model for case (b) of the migrating zone where $\bar{u}_Y < \bar{u}_{MY}$, dependences on pH were measured for the mobilities of EDTA



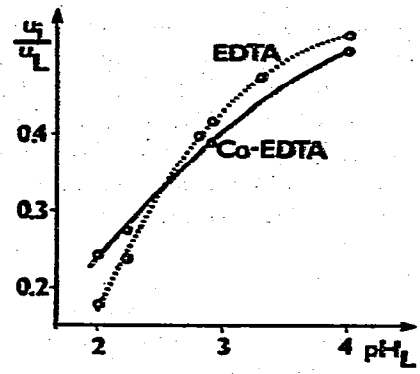


Fig. 8. Experimental dependence of the step-length of the ligand, l_{NTA}, on the analysis time, t_a.

Fig. 9. Experimental dependence of the relative mobilities of EDTA and Co(II)–EDTA on pH of the leading electrolyte (Cl^-).

and its complexes. It can be seen from Fig. 9, showing the curves obtained for EDTA and Co(II)–EDTA complex, that at pH values lower than 2.5 the mobility of the free ligand is less than the mobility of the complex. Fig. 10 shows the isotachophoregrams of Co–EDTA complex at various pH values of the leading electrolyte. It can be seen that at pH 3.0 the complex partially decomposes and a zone of EDTA is formed in front of the Co–EDTA zone (Fig. 10a). At pH 2.0 (see Fig. 10b) the complex forms a stable zone which does not decompose during the analysis owing to a short EDTA zone migrating behind it. This short EDTA zone is formed at the beginning of the analysis by the decomposition of a small portion of the complex and/or it can be ensured by adding EDTA to the sample prior to separation.

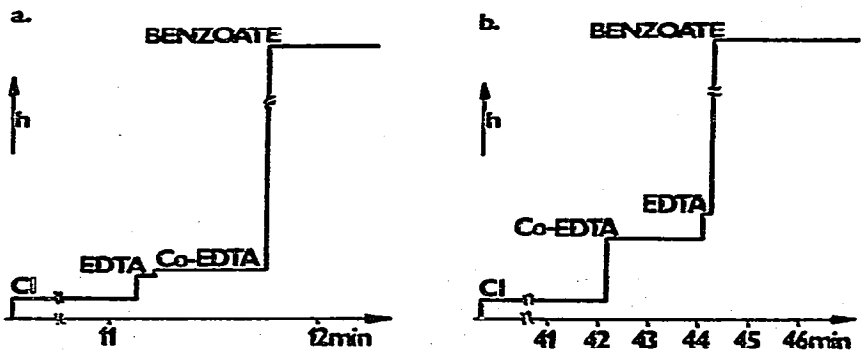


Fig. 10. Isotachophoregrams of Co(II)–EDTA complex for various pH values of the leading electrolyte (0.015 *M* Cl^-): (a) pH = 3.0; (b) pH = 2.0. Amount injected: 15 nmole of Co(II)–EDTA; $I = 140\,\mu A$.

Fig. 11 illustrates the isotachophoregram of a mixture modelling the composition of an aluminium bronze (67 wt.-% Cu, 15 wt.-% Al, 8 wt.-% Fe and 8 wt.-% Ni) as an example of a possible analytical application where various types of migration behaviour are demonstrated. Cu(II)–EDTA complex has a lower mobility than has EDTA at the given pH and is still sufficiently stable and demonstrates an analytical

application of case (a). Ni(II)–EDTA complex shows a higher mobility than does EDTA and migrates in the form of an enforced stable zone, described under case (b). Moreover, there are steps corresponding to zones of the kinetically inert complexes Al–EDTA and Fe(III)–EDTA.

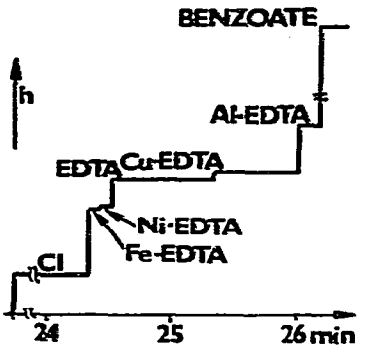


Fig. 11. Isotachophoregram of a model mixture (aluminium bronze). Leading electrolyte: 0.01 *M* Cl^-; pH = 2.2; $I = 140\,\mu A$.

CONCLUSIONS

Kinetically labile anionic complexes show different types of migration behaviour under the isotachophoretic conditions $\bar{u}_T < \bar{u}_Y$, $\bar{u}_{MY} < \bar{u}_L$ depending on the mutual relationship between $\bar{u}_Y$ and $\bar{u}_{MY}$.

In the case of $\bar{u}_Y > \bar{u}_{MY}$ the free metal and the free ligand released by the dissociation of the complex migrate from the zone and cause gradual decomposition of the complex. While the free ligand leaves the zone of the complex through its front boundary, where it forms its own zone, the free metal leaves through its rear boundary into the zone of the terminator and thus causes "bleeding" of the zone of the complex. The decomposition rate of the complex is dependent on the conditional stability constant of the complex, the working parameters of the column and the composition (particularly pH) of the leading electrolyte (see eqn. 12). With a suitable selection of the conditions the decomposition of the complex is negligible and its zone then behaves as isotachophoreticly stable.

When $\bar{u}_Y < \bar{u}_{MY}$, the decomposition of the complex is evoked by the migration of both free metal and free ligand through the rear boundary of the zone of the complex. The free metal, passing the zone of the free ligand, which is established at the rear boundary, again partially re-combines with the ligand into the complex, which migrates back to its zone. The decomposition of the complex, described by eqn. 19, can be retarded by this process to such an extent that the zone of the complex will migrate as a zone with enforced isotachophoretic stability. The percentage retardation of the decomposition is determined by the rate of the reversed metal to complex reaction and is an exponential function of the length of the zone of the free ligand behind the zone of the complex (*cf.* eqn. 24).

Model experiments, performed with NTA and EDTA complexes, are in agreement with the theory. The model described makes it possible both to estimate the

isotachophoretic behaviour of anionic complexes under the given operating conditions and to select operating conditions such that the stability of the zones of the complexes under investigation may be influenced selectively in order to obtain the required separation.

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