ISOTACHOPHORESIS OF COMPLEX IONS

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The present paper quantitatively describes four fundamental types of the migration of complex anions in an isotachophoretic arrangement, observed experimentally, and suggests the possibilities of utilizing the differences in the migration behaviour for analytical separations. Kinetically inert complexes form stable zones under isotachophoretic conditions and their migration behaviour does not differ from isotachophoresis of simple ions. Kinetically labile complexes are in the dissociation equilibrium with the free metal cations and the free ligand and their progressive decomposition occurs in the course of the migration, *i.e.*, the zone of the complex is not migrationally stable. By selecting operating conditions properly, in the first place the pH of the leading electrolyte, the migration stability of the zones of complexes can be influenced selectively in such a way that the decomposition of one of the complexes will be suppressed to a negligible extent while another complex will decompose totally in the course of the migration and will provide an equivalent zone of the free ligand. Various migration behaviour of the complexes can be applied in order that both required analytical separation may be obtained and the isotachophoregram may be evaluated quantitatively.

For successful electrophoretic separation of a mixture, sufficient differences are necessary in the effective mobilities of the components of this mixture. One of the methods of affecting selectively electrophoretic mobilities is based on the utilization of complex-forming equilibria. In zone electrophoresis[1], where the complex-forming agent is a constituent of the background electrolyte, even burdensome separations can be performed by selecting properly the complex-forming agent and other conditions, *e.g.*, complete separations of lanthanides on a cellulose acetate foil by employing α-hydroxyisobutyric acid[2] or by employing EDTA with a buffered concentration of the ligand[3]. Electrophoretic ion focusing, making use of reverse concentration gradients of H_3O^+ and a complex-forming agent[4], also makes it possible to perform difficult separations of mixtures of rare earth cations[5].

It is a specific feature of isotachophoresis that a zone consists only of a compound under separation and common counter ion, *i.e.*, there is no background electrolyte here and hence the possibility of making use of a complex-forming agent as a constituent of the background electrolyte is eliminated. Complex formation can, however, be utilized in isotachophoresis in some other ways[6].

The first possibility is based on using a suitable counter ion, forming kinetically labile complexes with the compounds under separation. Applications to the de-

termination of chlorides and sulphates in mineral waters[7] and to the determination of nitrates, sulphates and phosphates in liquid fertilizers[8], with the use of Cd^{2+} or Ca^{2+} as complexing counter ions, can serve as examples of this method.

The second possibility assumes that the compounds to be separated are taken for the analysis already in the form of complexes. As long as these are kinetically stable, they can be simply separated without any difficulties. The separation of complex anions $IrCl_nBr_{6-n}^{2-}$ ($n = 0, 6$) (ref.[9]), which was performed (for $n = 0-6$) even in a preparative arrangement[10], or the separation of anions $Cr(SCN)_n(CN)_{6-n}^{3-}$ ($n = 1-5$) (ref.[11]) by isotachophoresis in non-aqueous medium, are examples.

In the case of kinetically labile complexes the situation is complicated by the decomposition of these complexes in the course of the analysis, as described already in preliminary works[6,12,13]. Experiments have shown that EDTA complexes injected into an isotachophoretic system give, depending on the pH of the leading electrolyte, either a zone of the complex or a zone of the pure ligand, or both. The paper[14] reports that M(II)–EDTA complexes can be separated successfully only at higher pH values of 5–9.

To explain these observations, the problem was theoretically and experimentally analysed in detail[15]. This paper presents a classification and a qualitative description of fundamental types of isotachophoretic migration of complex ions, with respect to the possibilities of their analytical application.

EXPERIMENTAL

The measurement was performed in the isotachophoretic equipment described earlier[17], connected to a stabilized current power supply providing up to 400 μA at maximum voltage up to 16 kV. Solutions of 0·01M-HCl + 0·08M histidine (pH 6·9) and 0·015M-Cl^- (KCl + HCl) (pH 2·2) served as leading electrolytes. In the latter case, a maleic acid–potassium maleate buffering solution with the concentration of potassium ions and the pH identical with those of the leading electrolyte was placed in the anodic chamber (behind the membrane). Disodium salt of ethylenediaminetetraacetic acid (EDTA) and the other chemicals used were of analytical-reagent grade (Lachema, Brno). The model mixture of EDTA salts was prepared by dissolving weighed amounts of EDTA and chlorides or nitrates of the respective metals.

RESULTS AND DISCUSSION

As already mentioned, kinetically labile complexes decompose in the course of their migration in an isotachophoretic system. If, *e.g.*, an anionic complex MY^- (sign of electric charge has only a symbolic meaning here) is placed into an anionic isotachophoretic system, then a zone of complex MY^- (Fig. 1) will be formed between the zones of leading electrolyte L^- and terminator T^-. The zone of the complex contains, besides MY^-, certain amounts of free metal cation M^+ and free ligand Y^-, produced by the dissociation of the complex. Free cation M^+, with respect to the

sign of its charge, migrates in the direction opposite to that of the complex and leaves the zone through its rear boundary. The zone thus leaves behind a trace of the free metal cation, the concentration of which is proportional to the dissociation equilibrium of the complex. It means that the complex as well as its zone decompose progressively. Free ligand Y^-, the mobility of which is generally different from that of complex MY^-, also contributes to the decomposition as it moves out of the zone of the complex, too, and forms its own zone Y^-.

The above idea of the decomposing zone has led to the name "bleeding zone isotachophoresis"[13]. Analytical application of this technique assumes that such conditions be found under which the total decomposition of the complex in the separation run will be negligible. It follows from a physicochemical model[15] that the amount of the complex decomposed during the analysis depends, in addition to the parameters of the isotachophoretic column, particularly on the actual value of the stability constant of the complex. A strong dependence of this constant on the pH gives rise to that the pH value of the leading electrolyte is the main factor, by adjusting of which it is possible to decrease the degree of the complex decomposition below the given level ($\leqq 0{\cdot}1\%$). If the pH is selected correctly, the zone of the complex will migrate into the detector practically quantitatively and will appear as isotachophoretically stable.

A particular case will arise if the mobility of the free ligand is lower than that of the complex. Free metal cation M^+ as well as free ligand Y^- then leave the zone through its rear boundary behind which a zone of free ligand will be formed. That means that cation M^+, migrating into the zone of the terminator, passes through the zone

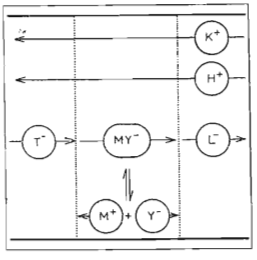

FIG. 1

Scheme of isotachophoretic migration of a kinetically labile complex.

K^+ and H^+ are common counter ions of the leading electrolyte, L^- and T^- are the leading and the terminating anions, respectively.

of the free ligand and re-combines with it. Complex MY^- is again formed, and its amount is proportional to the length of the ligand zone and the rate constant of the conversion metal → complex. The complex, which is thus again created in the zone of the ligand, returns back to its zone, and the total decomposition of the zone of the complex is thus retarded. Even a short zone of the free ligand behind the zone of the complex can cause that the zone of the complex, which would otherwise decompose due to its low stability, will thus migrate practically quantitatively and will behave as an isotachophoretic zone with enforced stability. In practice, this case when the ligand shows the mobility lower than that of the complex is particularly found in strongly acidic systems, where the mobility of free ligand can be decreased by protonation more than that of the complex.

Figs 2*a*,*b* show isotachophoregrams of analyses of a model mixture containing Al, Cu, Mn and Co in the form of EDTA complexes as examples. In the case of the leading electrolyte with the pH value of 6·9, being sufficiently high (Fig. 2*a*), the EDTA complexes of all four metals mentioned are isotachophoretically stable[14]. Free EDTA (here predominantly dissociated into the third degree – HY^{3-}) separates well from the complexes, which, however, do not separate from one another and create a mixed zone (step of M-EDTA in Fig. 2*a*). The effective mobilities of all four complexes are very close at the above pH since bivalent ions $[M(II)Y]^{2-}$ and $[Al(III)(OH)Y]^{2-}$ are the predominating form of their existence[16].

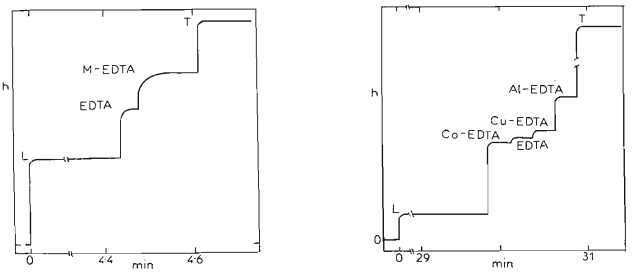

Fig. 2

Isotachophoregrams of the model mixture of EDTA complexes.

a) Sample: Cu(II)–EDTA, Co(II)–EDTA, Mn(II)–EDTA, Al(III)–EDTA, EDTA; 2·1 nmol each (0·7 μl of 0·003M solution). Leading electrolyte 0·01M-HCl + 0·08M histidine, pH 6·9; terminator: 0·01M glutamic acid; *I* 200 μA. *b*) Sample: Cu(II)–EDTA, Co(II)–EDTA, Mn(II)–EDTA, Al(III)–EDTA; 2·1 nmol each (0·7 μl of 0·003M solution). Leading electrolyte: 0·015M-Cl^- (HCl + KCl), pH 2·2; terminator: 0·01M glutamic acid; $I = 120$ μA.

In the acidic system (pH 2·2), the effective mobilities decrease significantly and selectively due to protonation, and a considerable decrease in the stability of some complexes occurs, making possible mutual separation of all four complexes and EDTA (Fig. 2*b*). Individual steps in the record correspond to zones of the different complexes and illustrate various types of the migration behaviour, as discussed above: Zone of kinetically stable Al(III)–EDTA complex which migrates without any decomposition and quantitatively. Zone of kinetically labile Cu(II)–EDTA complex showing a mobility lower than that of free EDTA. With respect to sufficient stability of the complex at given pH, the zone of the complex is isotachophoretically stable and migrates practically quantitatively. Zone of free EDTA, created by the total decomposition of the low stable Mn(II)–EDTA complex. The zone of this complex "bleeded out" completely during the analysis and the zone of the free ligand only reaches the detector. Zone of low stable Co(II)–EDTA complex with the mobility higher than is the mobility of the free ligand. Due to the EDTA zone migrating behind the zone of the complex, the complex is isotachophoretically enforced stable and migrates practically quantitatively.

From the example shown follows the possibility of using the bleeding zone technique for quantitative analysis of mixtures of cations in the form of anionic chelates under conditions when the components under analysis form isotachophoretically stable zones and are separated from each other.

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