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APPLICATION OF KINETICALLY LABILE COMPLEX EQUILIBRIA TO THE SEPARATION OF ANIONS BY ISOTACHOPHORESIS*

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SUMMARY

The kinetics of complex equilibria are briefly discussed from the viewpoint of their use in isotachophoresis. The possibility of separating some anions on the basis of the kinetically labile complex equilibria was studied and the composition of leading electrolytes suitable for this type of separation is discussed. A successful separation of some anions that differ in their stability constants with Ca^{2+} illustrates the separation of anions according to stability constants.

INTRODUCTION

An isotachophoretic separation of ionic species can be carried out in several ways because several factors that influence their effective mobilities are known (see, *e.g.*, ref. 1). If the ionic mobilities and/or the $\text{p}K$ values of the ionic species to be separated differ sufficiently, separations according to ionic mobilities and/or $\text{p}K$ values are possible¹⁻⁴. Different dependences of the effective mobilities of ionic species on ionic concentrations are also of practical use¹. Solvents other than water offer many possibilities for carrying out successful isotachophoretic separations^{1,5,6}.

Previously, little attention had been paid to the use of complex equilibria in isotachophoresis¹. The aim of this paper is to show that these equilibria, especially of the kinetically labile type, can be used for the separation of anions by isotachophoresis.

It is well known that the migration of ionic species in an electric field is accompanied by continuous breaking and attaining of equilibria that influence their effective mobilities. When the acid-base properties of ionic species are utilized for their separation by an electrophoretic technique, ionic forms of an ionic species migrate in a homogeneous zone because the acid-base equilibria are broken and attained almost

* Dedicated to Professor S. Stankoviansky on the occasion of his 70th birthday.

instantaneously. From the kinetic viewpoint, two extreme types of the complex equilibria are distinguished: kinetically labile and kinetically inert⁷. Owing to the kinetics of the complex equilibria, three types of electrophoretic behaviour of ionic species have been recognized in zone electrophoresis⁸. In isotachophoresis, an analogous behaviour of ionic species can be expected and comparison of the rate of attainment of complex equilibria with the velocity of migration of ions explains the different behaviour of ionic species:

(1) The rate of attainment of a complex equilibrium is much faster than the velocity of migration of ions. Kinetically labile complex particles are formed during migration. Ionic forms of an ionic species migrate as a homogeneous zone. If the complex formation is responsible for a separation effect, we can speak about the separation of ionic species according to stability constants. However, it should be mentioned that the stability constants need not be the only cause of the separation effect, as some concurrent reactions can also occur⁷.

(2) The rate of attainment of a complex equilibrium is much slower than the velocity of migration of ions. Inert complex particles are formed in this instance. An ionic species forming such complexes can be found in "independent" ionic forms. When the effective mobilities of the ionic forms differ, "independent" zones of the ionic species are observed. This type of complex equilibria has already been used in isotachophoresis (see ref. 1, p. 320).

(3) The rate of attainment of a complex equilibrium is equal to the velocity of migration. The complex particles formed are of an intermediate type, *i.e.*, neither labile nor inert. Electrodiffusion is characteristic of this type of equilibria⁹ and broadening of the zone boundaries occurs.

The advantages of kinetically labile complex equilibria for separation purposes in isotachophoresis are clear from the above discussion. The separation of anions using this type of complex equilibria was the purpose of this work.

Because of the kinetic lability of the equilibria involved, both the free metal ion concentration and the pH in a given zone must be kept constant. Thus, the effective mobilities of anionic species to be separated will be constant in the steady state. A leading electrolyte suitable for this type of separation of anionic species, therefore, has to consist of a pH buffering counter ion, a complex-forming cation (acting as a counter ion) and a leading anion. If differences in the effective mobilities of anionic species are due to the complex formation, their separation according to their stability constant with the cationic species in the leading electrolyte will be effected.

EXPERIMENTAL

An instrument for capillary isotachophoresis provided with both UV absorption and conductivity detectors, described in detail by Everaerts *et al.*¹, was used.

Chemicals used were of pro analysi purity. Morpholinoethanesulphonic acid (MES) was purified as recommended in the literature¹⁰. Mowiol 8-88 (Hoechst, Frankfurt, G.F.R.) was purified on Merck V mixed-bed ion exchanger (Merck, Darmstadt, G.F.R.). Chlorides of the complex-forming cations were used for the preparation of the leading electrolytes.

RESULTS AND DISCUSSION

The operational systems used are listed in Table I. The concentration of the leading anion (chloride, $10^{-2} M$) and pH (6.0) were constant, while the type of complex-forming cation and its concentration in the leading electrolyte were changed. The cation added to the leading electrolyte was chosen with respect to the anions investigated by using the qualitative rules valid in the chemistry of complex equilibria⁷.

TABLE I
OPERATIONAL SYSTEMS USED FOR THE SEPARATION OF ANIONS

Parameter	Electrolyte	
	Leading	Terminating
Anion	Cl ⁻	MES
Concentration	$10^{-2} M^*$	$ca. 5 \cdot 10^{-3} M$
Counter ion	Histidine ⁺	Tris ⁺
Metal ion	Ca ²⁺ (Cd ²⁺ , Al ³⁺)	—
Concentration	$0.2 \cdot 10^{-3} M$	—
pH	6.0	$ca. 6$
Additive	0.05% Mowiol	—

* The concentration of the leading anion (chloride) was kept constant in all experiments.

In order to show the effect of complex formation on the effective mobilities of the anions, their relative step heights* were plotted against the cations added to the leading electrolytes (Fig. 1). The relative step heights of the anions using a leading electrolyte containing no metal ions are given as a reference (Fig. 1a). The effect of complex formation on the relative step heights (*i.e.*, on the effective mobilities) of the anions is clearly visible (Figs. 1b–1d). In these instances, the concentration of the cations in the leading electrolytes was $10^{-3} M$. The effective mobilities of tartrate, oxalate, fluoride and citrate were lower than that of the terminating anion (MES) using a leading electrolyte containing Al³⁺ (Fig. 1d).

The influence of the concentration of a complex-forming cation on the relative step heights of the same group of anions is illustrated in Fig. 2. Ca²⁺ was added to the leading electrolyte at concentrations of 10^{-3} and $2 \cdot 10^{-3} M$. The effect of concentration on the relative step heights of the anions forming strong complexes with Ca²⁺ is remarkable. Such a decrease in the effective mobilities of these anions suggests that only lower steps of the complex formation are of importance under these experimental conditions. When the Ca²⁺ concentration in the leading electrolyte was higher than $5 \cdot 10^{-4} M$, an anomalous behaviour of oxalate (broken lines in Figs. 1 and 2) was observed. Several zones (one zone would be expected) between the zone of the leading anion and the zone of the terminating anion appeared, while the step height of the terminating zone was unexpectedly high. These phenomena were not observed when Al³⁺ or Cd²⁺ was added at the same concentration instead of Ca²⁺ to the leading electrolyte. This implies that precipitation of calcium oxalate may be the cause of these disturbances.

* The step heights of the zones were measured relative to the zone of perchlorate, as described by Everaerts *et al.* (ref. 1, p. 307).

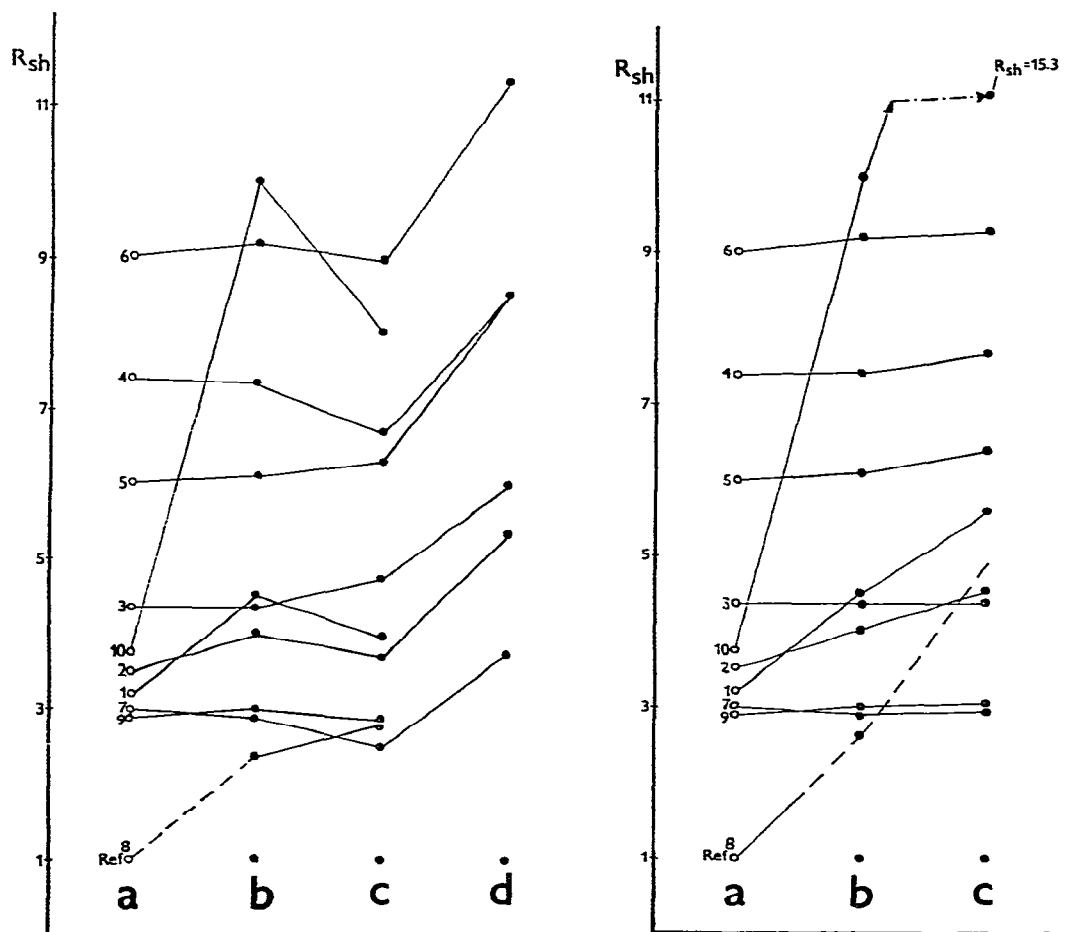


Fig. 1. Relative step heights (R_{sh}) of some anions for different complex-forming cations added to the leading electrolyte at pH 6.0. (a) Without addition of a cation; (b) $10^{-3} M Ca^{2+}$; (c) $10^{-3} M Cd^{2+}$; (d) $10^{-3} M Al^{3+}$. Further information on the operational systems is given in Table I. 1 = Tartrate; 2 = α -ketoglutarate; 3 = succinate; 4 = acetate; 5 = pyruvate; 6 = lactate; 7 = formate; 8 = oxalate; 9 = fluoride; 10 = citrate; Ref = perchlorate.

Fig. 2. Relative step heights (R_{sh}) of the same group of anions as in Fig. 1, for different concentrations of Ca^{2+} in the leading electrolyte at pH 6.0. (a) Without addition of Ca^{2+} ; (b) $10^{-3} M Ca^{2+}$; (c) $2 \cdot 10^{-3} M Ca^{2+}$. For further information on the operational systems used, see Table I.

The kinetically labile complex equilibria can be used for the separation of anions by isotachopheresis, as is illustrated in Fig. 3. A group of anions that are difficult to separate using a leading electrolyte without complex forming additives (Fig. 3A) can easily be separated at the same pH using a leading electrolyte containing $2 \cdot 10^{-3} M Ca^{2+}$ (Fig. 3B). The complete separation of formate, tartrate, α -ketoglutarate and citrate can be ascribed to the formation of complexes of these anions with Ca^{2+} .

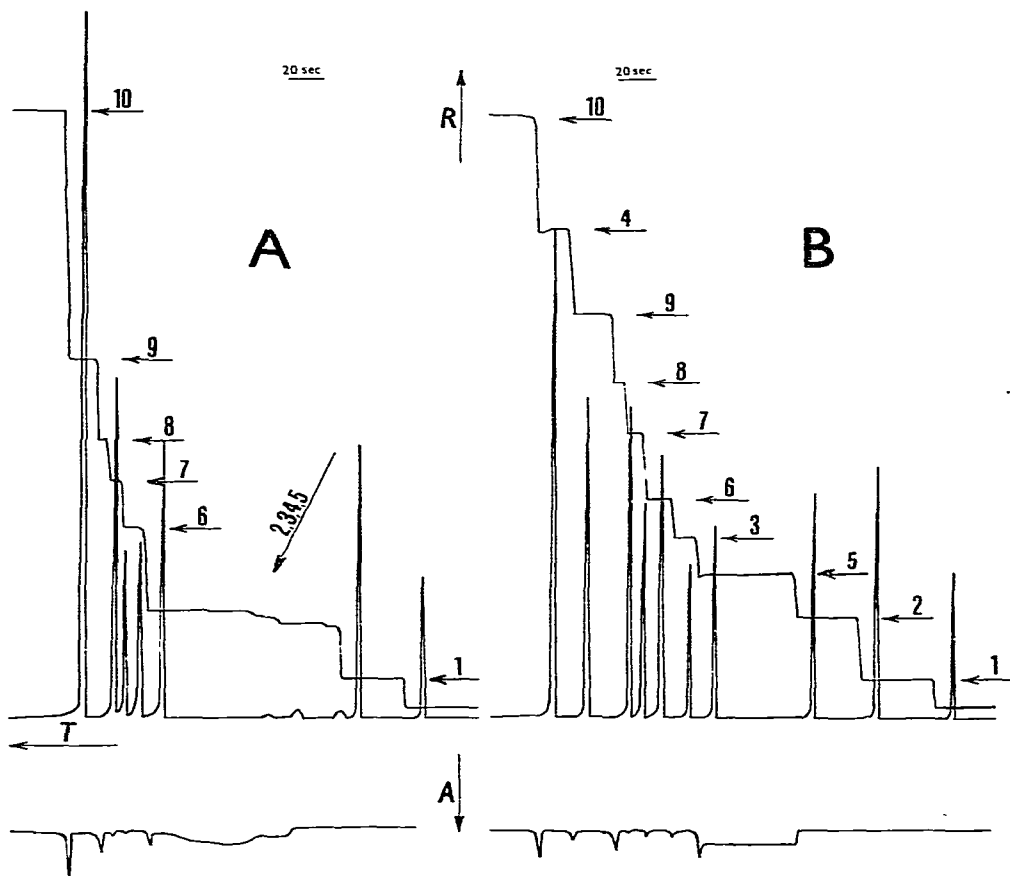


Fig. 3. Isotachopherograms for the separation of a group of anions at pH 6.0. (A) Without a complex-forming cation in the leading electrolyte; (B) $2 \cdot 10^{-3} M$ Ca^{2+} in the leading electrolyte. A $1\text{-}\mu\text{l}$ volume of the sample (1 = perchlorate; 2 = formate; 3 = tartrate; 4 = citrate; 5 = α -ketoglutarate; 6 = acetate; 7 = lactate; 8 = 2-hydroxybutyrate; 9 = capronate) was injected in both instances. MES^{10} was the terminating anion. A = Increasing UV absorption; R = increasing resistance; T = time. The current was stabilized at $0.05 A\text{ cm}^{-2}$.

CONCLUSIONS

The kinetically labile complex equilibria can be used to solve some problems in the separation of anions in isotachopheresis. Owing to the kinetics of these equilibria, the free metal ion concentrations in the zones must be kept constant. By addition of a complex-forming cation to the leading electrolyte this condition is fulfilled.

The relative step heights (*i.e.*, the effective mobilities) of some anions are influenced in a different manner by both the type of complex-forming cation used and its concentration in the leading electrolyte.

The separation of formate, tartrate, α -ketoglutarate and citrate at pH 6.0 using a leading electrolyte containing Ca^{2+} at $2 \cdot 10^{-3} M$ concentration clearly shows that the separation of anions according to stability constants in isotachopheresis is possible.

Because the influence of complex formation on the effective mobilities of anions is reproducible and characteristic for each of them, it is of value for the identification of components from the response of a universal detector in isotachopheresis.

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