Short communication

New method to determine stability constants of lanthanide–EDTA complexes by isotachophoresis

Takeshi Hirokawa*, Qinglu Mao

Applied Physics and Chemistry, Faculty of Engineering, Hiroshima University, Kagamiyama 1, Higashi-hiroshima 739, Japan

Abstract

The zone reduction in the isotachophoretic migration of several lanthanide–EDTA complexes at low pH was investigated. The typical leading electrolyte was 20 mM HCl buffered by triglycine at pH 3.1 and the terminating electrolyte was 20 mM ammonia solution buffered by acetic acid at pH 4.8. It was observed that a good linear relationship exists between the stability constants and the zone reduction due to the bleeding effect (liberating free metal ions). This relationship is the basis of a new method for the evaluation of the stability constants of the lanthanide complexes. © 1997 Elsevier Science B.V.

Keywords: Stability constants; Isotachophoresis; Lanthanide complexes; Metal complexes

1. Introduction

Besides the separation and analysis of ionic substances, isotachophoresis is also useful for the measurement of their mobility and dissociation constants [1]. Even for the kinetically labile complexes (ion pairs), stability constants (log $K < \sim 4$) can be evaluated by analyzing the dependence of the observed effective mobility of sample metal ions on the concentrations of the complex-forming agents. In such cases, the metal ions interact with the counter complexing ions migrating from the leading electrolyte. The method cannot be successfully adapted to the stable complexes having large log K values. This is because a drastic change of effective mobility of metal ions occurs in the low ligand concentration region and the experimental reproducibility of the observed effective mobility is not good. To avoid this problem, the pH of the leading electrolyte should

be lowered to obtain a small conditional stability constant of the complex. However, at the low pH condition, the sample does not always migrate isotachophoretically depending on its effective mobility. Typical examples of the above situation can be found in the case of metal–EDTA complexes which have large log K except for alkaline metal cations [2,3]. The isotachophoretic zone of the metal–EDTA complex anion (LnY^-) gradually liberates free metal cations during migration at sufficiently low pH due to zone-bleeding phenomena and thus the zone length is reduced. The zone reduction has been shown to depend on the conditional stability constant and the decomposing zones are predicted to be isotachophoretically stable and can be utilized for quantitative analysis under proper operating conditions [2,4].

This paper first demonstrates a good linear relationship between the zone reduction and the stability constants of the lanthanide–EDTA complexes which can serve as a good example of the prediction given

*Corresponding author.

by Refs. [2,4]. This relationship is the basis of a new method for estimating the stability constants of the lanthanide complexes by measuring the zone reduction.

2. Experimental

The experiments were performed under the conditions listed in Table 1. The leading electrolyte mainly used was 20 mM HCl–triglycine (pH 3.1). The terminating electrolyte was 20 mM ammonia solution buffered by acetic acid at pH 4.8. The other leading electrolytes and terminating electrolytes in Table 1 were used in appropriate combinations. The samples were prepared from 10 mM stock solutions of eight kinds of lanthanide chlorides LnCl$_3$ (LnCl$_3$, Ln=La, Ce, Pr, Nd, Sm, Eu, Gd and Tb) and added with an equimolar amount of EDTA. The pH of the sample solution was adjusted to 4.8 by the addition of acetic acid, which was the same as the terminating electrolyte. The isotachopherograms were recorded by a high-frequency contactless conductivity detection system (HFCCD) and the separation compartment was a ZKI-001 (Labeco, Slovakia).

3. Results and discussion

The isotachopherogram of LaY$^-$ (Fig. 1a) was obtained by using the leading electrolyte (pH 3.1) in Table 1. The LaY$^-$ zone could not be observed where only 15 nmol LaY$^-$ was injected at this pH value. The calibration line obtained from these pherograms is shown in Fig. 1b together with those obtained by using different leading electrolytes.

Table 1
Experimental conditions of the operational electrolyte system

1. *Leading electrolytes for anions*	(Anolytes)
(1) 20 mM HCl–triglycine	pH 3.1
(2) 20 mM HCl–β-alanine	pH 3.6
(3) 20 mM HCl–ε-aminocaproic acid	pH 4.4
(4) 20 mM HCl–histidine	pH 6.0
2. *Terminating electrolytes for anions*	(Catholytes)
(1) 20 mM ammonia solution–acetic acid	pH 4.8
(2) 20 mM ammonia solution–caproic acid	pH 4.8
(3) 20 mM ammonia solution–MOPS	pH 6.0

Since the intercept of these calibration lines was dependent on the pH of the leading electrolyte and no zone reduction was observed at pH 6, it can be concluded that this phenomena was caused by the decomposition of LaY$^-$ due to a small conditional stability constant at low pH. The concentration of the ITP zones is unique corresponding to the charge and the effective mobility, therefore, quantitative analysis in isotachophoresis can be achieved by utilizing a linear relation between the zone passing time and the amount of sample injected. This calibration line usually passes through the origin. In the isotachophoresis of LnY$^-$, however, it does not pass through the origin due to the bleeding effect. As shown in our experiments, the LnY$^-$ zone cannot be observed at a relatively low pH until the amount of the injected sample exceeds a threshold value.

Calibration lines of other lanthanide ions were also plotted in Fig. 2 where the pH of the leading electrolyte was 3.1. The zone reduction was obtained from the intercepts of the calibration lines. The relation between the stability constants (log K) and log [zone reduction/nmol] was checked by plotting the data in Fig. 3. A good linear relationship between them is found which can be explained by the following theoretical consideration.

Gebauer et al. [2,4] gave a theoretical explanation of the stability of the isotachophoretic zone of EDTA complexes. They described how the instability of the complex zone is due to decomposition of the complex, liberating metal ions. Fig. 4 illustrates the decomposition of the isotachophoretic zone of LaY$^-$. The degree of instability is proportional to the concentration of free metal ions in the complex zone and to the load of leading electrolyte.

The experiment assumes that the dissociation equilibrium of the EDTA complex is rapid enough in comparison with electrophoretic migration and has the following form:

$$LnY^- = Ln^{3+} + Y^{4-} \qquad (1)$$

Although the formed Y^{4-} in the above equilibrium in fact reacts immediately with H$^+$ to form H$_x$Y^{x-4} depending on the pH, Eq. (1) is sufficient for the present purpose. In the isotachophoretic LnY$^-$ zone, it can be assumed that the concentration of free metal ion [Ln^{3+}] is equal to that of EDTA ion [Y^{4-}]. The
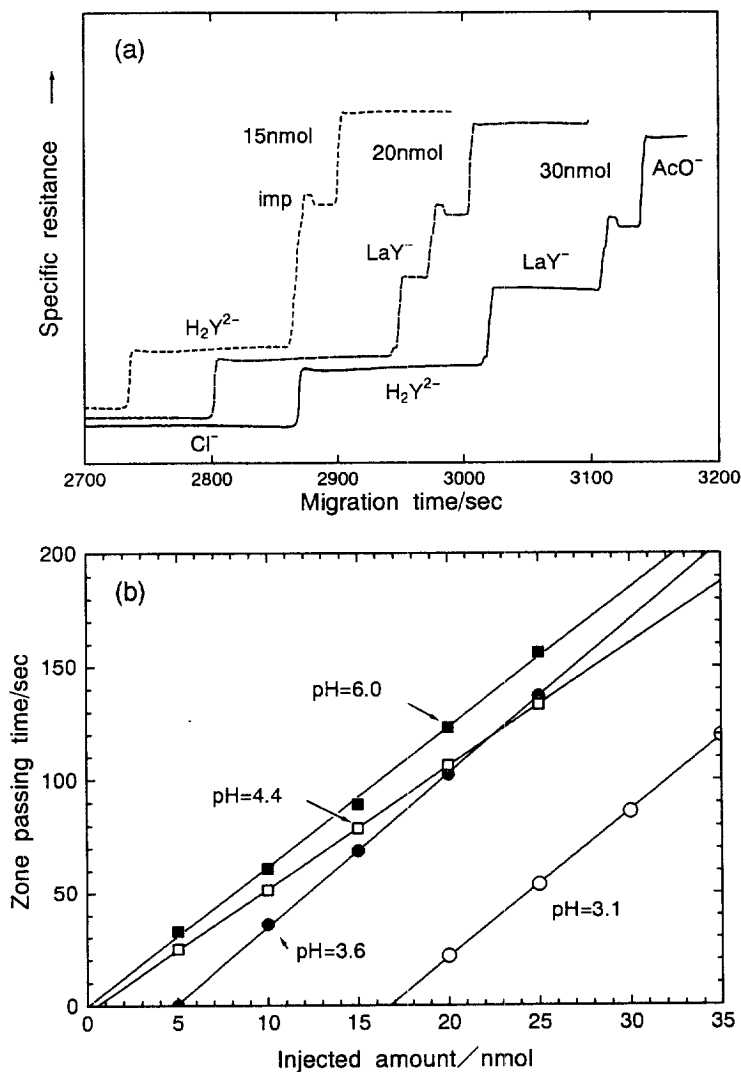
Fig. 1. (a) Isotachopherograms of LaY⁻ at injection volumes of 15, 20 and 30 nmol while the leading electrolyte is 20 m*M* HCl–triglycine (pH 3.1). (b) Calibration line of LaY⁻ zone versus injection amount under different leading electrolytes (LaY⁻, log $K = 15.46$).

definition of the stability constant therefore can be rewritten as Eq. (2). The expression of log K has the form of Eq. (3):

$$K = \frac{[LnY^-]}{[Ln^{3+}][Y^{4-}]} = \frac{[LnY^-]}{[Ln^{3+}]^2} \qquad (2)$$

$$\log K = \log [LnY^-] - 2 \log [Ln^{3+}] \qquad (3)$$

While the concentration of [LnY⁻] is almost

constant for different lanthanide ions (~10 m*M* according to our simulation), stability constant log K should be proportional to the concentration of free metal ion, [Ln³⁺]. Furthermore, since the zone instability in terms of zone reduction must be directly related with the concentration [Ln³⁺], it is very reasonable to expect a linear relation between log K and log [zone reduction] as confirmed in Fig. 3. The correlation coefficient is 0.990 and the slope of the line is −1.97 which corresponds well with the
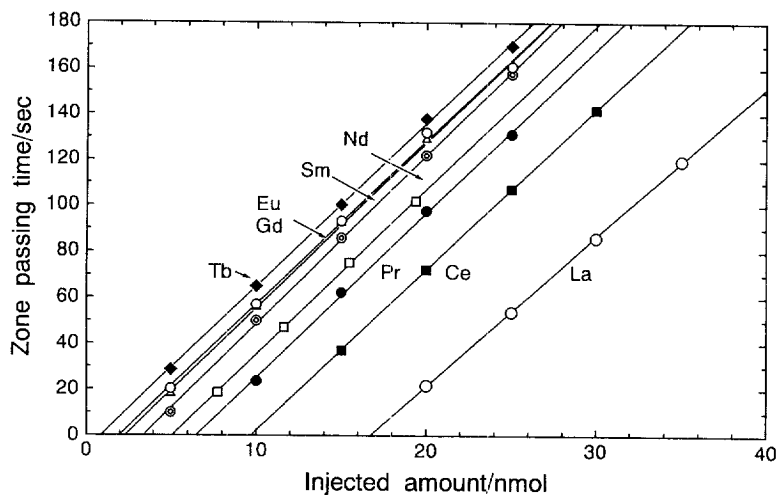
Fig. 2. Calibration lines of eight LnY^- zones versus injection amount for the leading electrolyte (pH 3.1).

predication value of -2 given by Ref. [2]. Then, a general expression for the relationship can be deduced as:

$$\log K = -2 \log[\text{zone reduction}] + b \qquad (4)$$

where b is dependent on the pH of the leading electrolytes. This relationship suggests that stability constants can be evaluated from the zone reduction. As clearly shown in Ref. [2], this relation is universally suitable for kinetically labile anionic complexes under the conditions of isotachophoretic migration. In this work, we only investigated the isotachophoretic behavior of the lanthanide–EDTA complexes which can be considered as kinetically
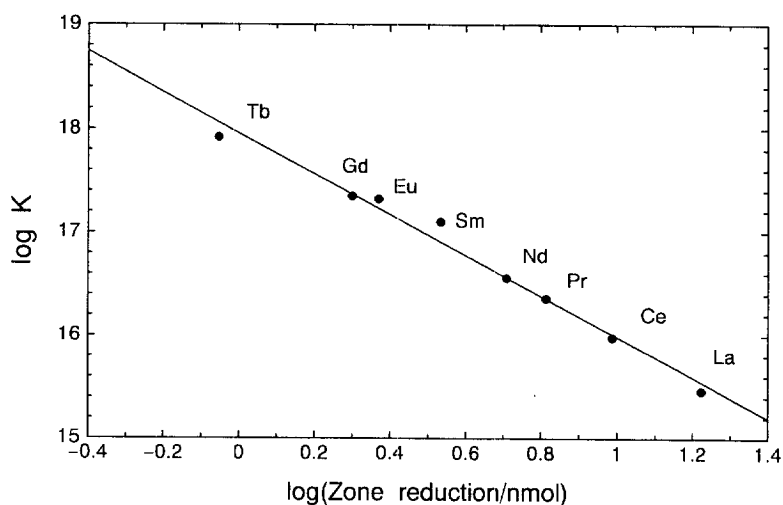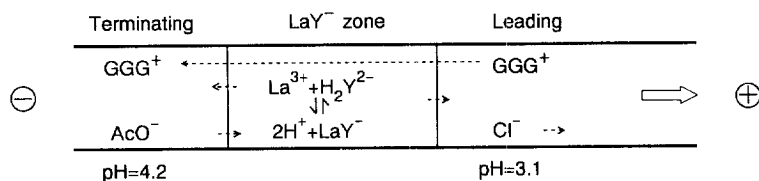

Fig. 3. Relationship between the stability constants and LaY^- zone reduction for the eight lanthanide–EDTA complexes while the pH of the leading electrolyte is 3.1 and the solution ionic strength is 0.1.
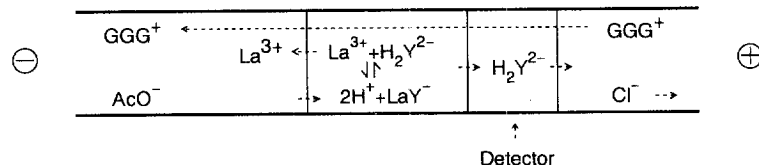
Fig. 4. Illustration of the decomposition of isotachophoretic zone of LaY$^-$ anion at a relativly low pH value.

labile ones at low pH. Also, it seems that only the zone reduction of lanthanide anionic complexes appears to be practically useful example for the estimation of their stability constants because of its systematic behavior.

Although the dynamic range of stability constants was not so large, smaller stability constants can be obtained by using a leading electrolyte with high pH. It is obvious, however, that the larger stability constants may be difficult to evaluate, because ITP experiments at low pH are subject to much limitation.

Acknowledgements

References

[1] T. Hirokawa, Y. Kiso, Anal. Sci. 8 (1992) 737.
[2] P. Gebauer, P. Bocek, M. Deml, J. Janak, J. Chromatogr. 199 (1980) 81.
[3] H. Yoshida, I. Nukatsuka, S. Hikime, Bunseki Kagaku 28 (1979) 382.
[4] P. Gebauer, P. Bocek, J. Chromatogr. 299 (1984) 321.