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CONTINUOUS FREE FOCUSING ELECTROPHORESIS OF METAL IONS IN A STEPWISE GRADIENT SYSTEM OF COMPLEXING AGENT

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SUMMARY

Theoretical considerations indicated the possibility of the focusing electrophoretic separation of metal ions using a stepwise gradient of complexing agent concentration. Separations of cations in several prepared samples were performed in an apparatus for continuous free electrophoresis. The separation performance and focusing effect of this system predicted theoretically were confirmed. Each component seeks its own concentration step in the complexing agent.

INTRODUCTION

In the analysis of mixtures of metal ions, group separations and separations of individual components are often necessary, and electrophoresis is an important approach in both instances. The differences in the mobilities of free (hydrated) metal ions, particularly ions in the same group in the Periodic Table and which have similar chemical properties, are usually so small that it is very difficult to separate them by electrophoresis. However, electrophoretic separations of metal ions are much more effective in electrolyte solutions with complexing agents such as citric acid[1,2], EDTA[3,4] and NTA[5]. In 1957, Schumacher[6,7] developed focusing electrochromatography for metal ions. This is a method for the rapid and efficient separation of microamounts of metal ions with a focusing effect.

A stepwise gradient of complexing agent concentration might be expected to improve the electrophoretic separation of metal ions, particularly in the separation of samples of higher concentration.

THEORETICAL

Schumacher[6,7] reported a theoretical study of the concentration focusing of metal ions in ligand systems. Jokl[8,9] derived and investigated an equation for the mobility curve of metal ions in ligand buffer solutions. The following simplified the-

oretical considerations attempt only to indicate the possibility of effecting the focusing electrophoresis of metal ions using a stepwise gradient of complexing agent concentration and the approach for realizing the system.

In the following, it is assumed that a metal ion forms a 1:1 complex with a ligand, that there is only one kind of complexing agent complexing metal ions in the system and that the concentration of complexing agent, the electric field and the temperature are independent of time.

Let a metal ion M form a complex ML with a ligand L. The complexation equilibrium can be expressed by the equation

$$\beta = \frac{[ML]}{[M][L]} \tag{1}$$

where β is the stability constant.

When the metal ion is in complexation equilibrium, its migration velocity U can be described by

$$U = \frac{[ML] U_{ML} + [M] U_M}{[ML] + [M]} \tag{2}$$

Application of eqn. 1 to eqn. 2 yields

$$U = U_{ML} + (U_M - U_{ML}) \frac{1}{1 + \beta[L]} \tag{3}$$

where U_M, U_{ML} and β are characteristic constants. U therefore changes with $[L]$. U_M is positive and U_{ML} is negative if the complex is negatively charged. Let us assume $|U_M| = 2|U_{ML}|$; it then follows that the following relationship exists between $[L]$ and β at the isoelectric point:

$$[L] = \frac{2}{\beta} \tag{4}$$

From eqn. 4, it can be predicted that the separation and focusing of metal ions having different stability constants can be attained by establishing an $[L]$ gradient across the electrophoresis bed between two electrodes. The gradient can be linear, as used by earlier workers, or stepwise. The latter might have advantages over the former in separating samples with higher concentration and in establishing separation conditions.

$[L]$ is a function of the total concentration $[L']$ of the complexing agent and of pH:

$$[L] = \alpha[L'] \tag{5}$$

where α is the fraction of the complexing agent as the ligand L.

Fig. 1 shows how the separation and focusing of metal ions in a stepwise $[L']$ gradient system at constant pH take place. This is the fundamental consideration of
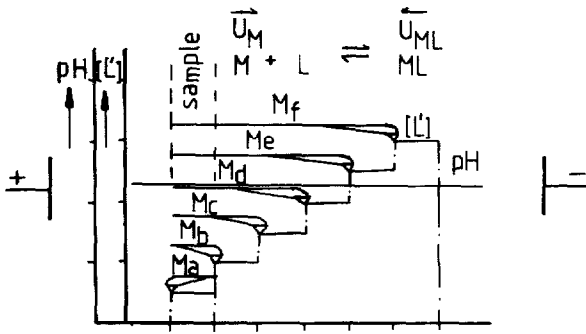
Fig. 1. Schematic diagram of separation and focusing in a stepwise [L'] gradient system.

the method in this paper. Under the influence of an electric field, the components with positive velocities migrate towards the cathode, whereas those with negative migration velocities move towards the anode. Let us consider a component with positive charge. When it arrives at the front of a concentration step where its velocity becomes zero or changes direction, it will stop there and focusing will take place. At first glance it might be thought that the component should turn back towards the anode when its velocity has changed direction. However, it must not be forgotten
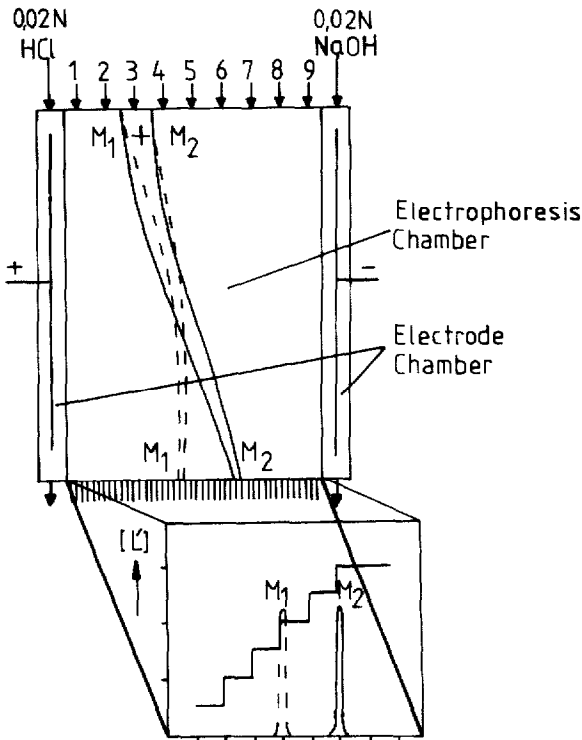

Fig. 2. Schematic diagram of the separation and focusing of components M_1 and M_2 on an apparatus for continuous free electrophoresis.

that the velocity of the component on the left-hand side of the front is positive. It is obvious that the ion that has arrived at the front cannot leave the front but only move in a zig-zag fashion. Each component, having a different stability constant from the others, seeks a concentration step. It should be emphasized again that the rate of migration of the component at the front need not be exactly zero. The most important factor is that the velocity direction on one side is opposite to that on the other side of the front. In fact, a linear gradient exists at the interface of two steps.

Fig. 2 illustrates schematically the separation process and the focusing of components 1 and 2 on a suitable apparatus. Separation occurs as the electrolyte solutions flow through the chamber, and at the same time ionic components are diverted towards the electrode of opposite charge. In this way each ionic species travels along its own path and emerges at the end at different outlet intervals.

EXPERIMENTAL

Instrumentation

The electrophoretic device was constructed according to Wagner and co-workers[10,11]. The movement of solution in the device for continuous free electrophoresis is illustrated in Fig. 2. The apparatus has 9 inlets and 74 outlets, connected with the 9 initial solution reservoirs and 74 collection tubes, respectively. The electric current flows across the electrophoresis chamber betweeen the two electrode chambers, and the electrolyte solutions flow vertically through the chamber. The substances under study are introduced at inlet 3 by mixing them in the electrolyte solution. The flow of solution is effected by a peristaltic pump at the outlets. The fractions are collected in 74 collection tubes.

Reagents

The complexing agents were cyclohexanediaminetetraacetic acid (CDTA), ethylene glycol bis(2-aminoethyl) ether N,N,N′,N′-tetraacetic acid (EGTA) and propylenediaminetetraacetic acid (PDTA).

The anode solution was 0.02 M hydrochloric acid and the cathode solution was 0.02 M sodium hydroxide solution.

Procedure

Nine initial solutions were prepared in accordance with the requirements in Fig. 3–6 for the separation example.

The total concentration [L′] of complexing agent of a certain step for focusing a particular component was approximately calculated by the equation

$$[L'] = \frac{2}{\alpha\beta} \tag{6}$$

where α depends on the pH in the system; α and β can be found in handbooks dealing with complex chemistry.

The diameters of all reservoirs and the solution levels in them must be kept the same. The residence time of the electrolyte solutions in the electrophoretic chamber was controlled by controlling the flow-rate. After suitable amounts of solutions
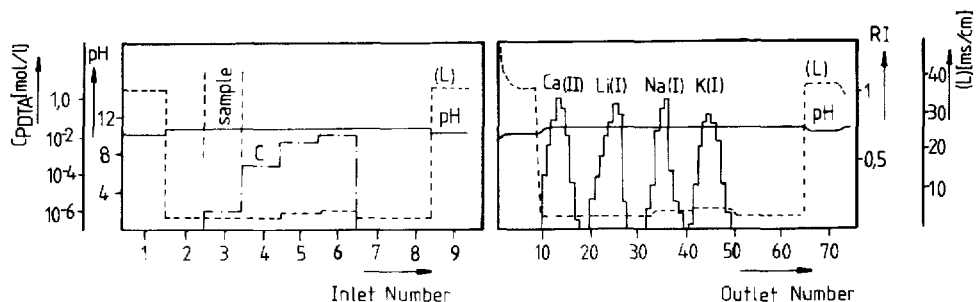
Fig. 3. Separation of alkali metal ions. Initial solutions: 1, 9, 0.5 M ammonium acetate + ammonia to pH 10.5; 2, 7, 8, 0.03 M ammonium acetate + ammonia to pH 10.7; 3, 1 · 10^{-6} M PDTA, 50 ppm Li(I), 100 ppm Na(I), 50 ppm K(I), 1 ppm Ca(II), 0.03 M ammonium acetate + ammonia to pH 10.7; 4, 3.7 · 10^{-4} M PDTA, 0.03 M ammonium acetate + ammonia to pH 10.7; 5, 6 · 10^{-3} M PDTA, 0.03 M Ammonium acetate + ammonia to pH 10.7; 6, 1 · $10^{-2}M$ PDTA, 0.03 M ammonium acetate + ammonia to pH 10.7. Voltage, 470 V; residence time, 180 sec. In this and subsequent figures, RI indicates the relative concentration of metal ions and (L) the electrical conductivity of the electrolyte solution.

had been collected the electrophoresis was stopped. The pH and conductivity (L) of the collected solution in each collection tube were then measured. The relative concentrations of metal ions to be separated were determined (Li, Na, K and Ca by flame emission spectroscopy and the other elements by flameless atomic-absorption spectroscopy). The data obtained were plotted against outlet number.

RESULTS AND DISCUSSION

Fig. 3 shows the separation of alkali metal ions from alkaline earth metals and from one another with PDTA as complexing agent. Ammonium acetate–ammonia buffer solution was used. All alkaline earth metals and some transition metal ions were grouped together with calcium and are not shown.

Calcium moved towards the anode because it had a negative velocity with the concentration of complexing agent in the first step, whereas all alkali metal ions
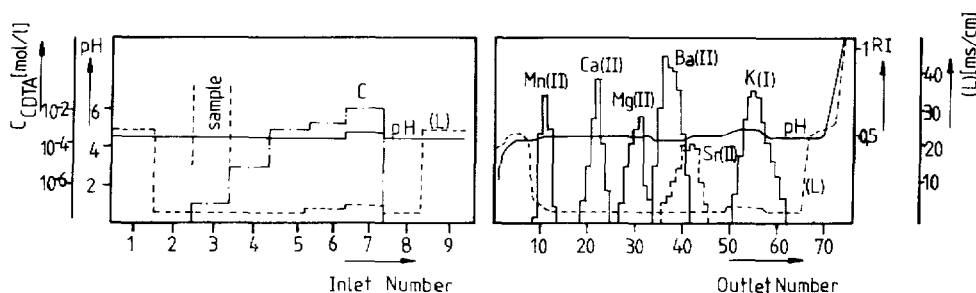

Fig. 4. Separation of alkaline earth metals. Initial solutions: 1, 9, 0.5 M sodium acetate, 0.05 M acetic acid; 2, 8, 0.05 M sodium acetate, 0.05 M acetic acid; 3, 1 · 10^{-7} M CDTA, 0.05 M sodium acetate, 0.05 M acetic acid; 100 ppm K(I), 1 ppm Mg(II), Ca(II), Sr(II), Ba(II), Mn(II); 4, 1 · 10^{-4} M CDTA, 0.05 M sodium acetate, 0.05 M acetic acid; 5, 1 · 10^{-3} M CDTA, 0.05 M sodium acetate, 0.05 M acetic acid; 6, 2 · 10^{-3} M CDTA, 0.05 M sodium acetate, 0.05 M acetic acid; 7, 2 · 10^{-2} M CDTA, 0.05 M sodium acetate, 0.05 M acetic acid. Voltage 400 V; residence time, 240 sec.
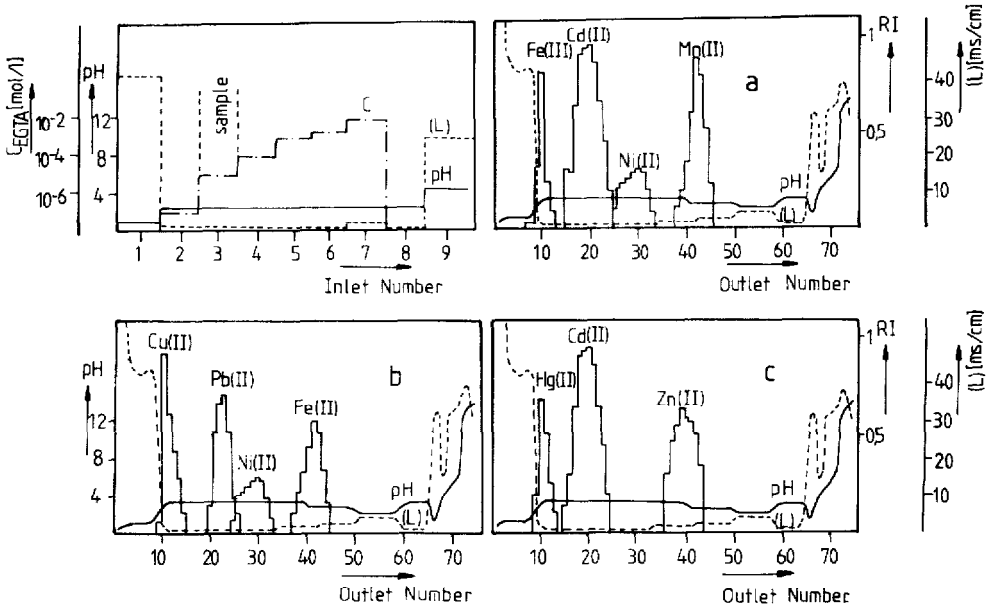
Fig. 5. Separation of transition metals. Initial solutions: 1, + HCl to pH 1.2 + KCl to (L) = 35; 2, 1 ·
10^{-7} M EGTA + HCl to pH 2.5; 3, 1 ppm Fe(III), Cd(II), Mn(II), 2 ppm Ni(II)/1 ppm Cu(II), Pb(II),
Fe(II), 2 ppm Ni(II)/5 ppm Hg(II), 1 ppm Cd(II), 5 ppm Zn(II)/1 · 10^{-5} M EGTA + HCl to pH 2.5; 4,
1 · 10^{-4} M EGTA + HCl to pH 2.5; 5, 1 · 10^{-3} M EGTA + HCl to pH 2.5; 6, 3 · 10^{-3} M EGTA +
HCl to pH 2.5; 7, 1 · 10^{-2} M EGTA + HCl to pH 2.5; 8, + HCl to pH 2.5; 9, 0.05 M sodium acetate,
0.05 M acetic acid + KCl to (L) = 27.5. Voltage, 700 V; residence time, 135 sec.
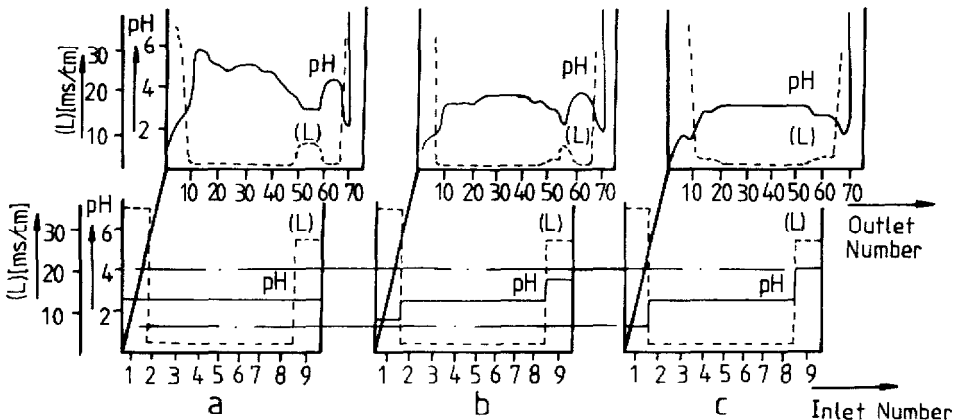

Fig. 6. Influence of the pH of initial solutions 1 and 9 on the stability of the pH curve. Initial solutions:
1. (a) + HCl to pH 2.5 + KCl to (L) = 35; (b) + HCl to pH 1.6 + KCl to (L) = 35; (c) + HCl to pH
1.2 + KCl to (L) = 35; 2 8, (a), (b), (c) + HCl to pH 2.5; 9, (a) + HCl to pH = 2.5 + KCl to (L) =
27.5; (b) + HCl to pH 3.7 + KCl to (L) = 27.5; (c) 0.05 M sodium acetate 0.05 M acetic acid + KCl
to (L) = 27.5. Voltage, 700 V; residence time, 135 sec.

moved towards the cathode. Lithium, sodium and potassium were focused at the fronts of the second, third and fourth steps, respectively.

Fig. 4 shows the separation of alkaline earth metals with CDTA as complexing agent. Sodium acetate–acetic acid buffer solution was used. The transition metals which have stability constants similar to or larger than that of manganese(II) were found in the vicinity of the latter. As can be seen, this system is suitable for group separations between alkali, alkaline earth and transition metal ions and for the separation of individual alkaline earth metals.

Fig. 5 illustrates several separations of transition elements with EGTA as complexing agent in the same electrolytic solution system.

The pH value in the electrophoretic zone should remain constant during the whole of the electrophoresis. To stabilize the pH course a very dilute buffer should be used, and the initial solutions 1 and 9 should be highly conductive to retain the H^+ and OH^- ions from the electrode chambers produced by electrophoresis. If a very low pH is required, such as in the separation of transition metal ions in Fig. 5, it is difficult to find a suitable buffer solution. In this event the pH in the electrophoretic zone may be controlled by adjusting the pH of solutions 1 and 9. Fig. 6
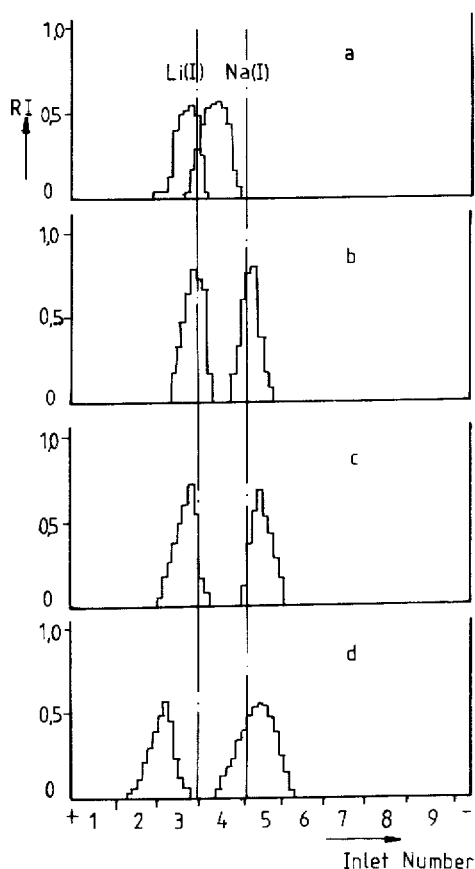

Fig. 7. Influence of residence time on separation and focusing affects. Initial solutions as in Fig. 4.

shows how the pH curve of the electrophoretic zone changed after electrophoresis as the pH of initial solutions 1 and 9 varied.

The electric field in the electrophoretic zone should be linear. This can be achieved by adjusting the conductivities of the electrolyte solutions.

The choice of the complexing agent depends on the elements to be separated. The greater the difference between the stabilities of the components to be separated the better. It is very important that the steps in the system should be kept unchanged during the whole of the electrophoresis. Hence the mobility of the chosen complexing agent must be small, much smaller than that of the metal ions to be separated. Preferably it is a high-molecular-weight polyvalent carboxylic acid.

The residence time of the electrolyte solutions in the electrophoresis chamber should be reasonable. If it is too short, separation will not be complete, and if it is too long bad focusing can occur. Fig. 7 shows how the separation and focusing depend on the residence time. A residence time of about 200 sec is optimal. After 290 sec the sample zones were broadened and the peak of lithium changed its position. These phenomena mean that the concentration steps were damaged to some extent. However, the peaks separated can retain their positions and remain compact for a relatively long time. Of course, the residence time should be adjusted with the voltage used.

In Fig. 5 there is a focusing effect for potassium. However, in fact, potassium could not find a step in this system where its velocity direction would change, because the rate of migration of the species at the front of the sample zone is lower than that at the back.

The experiments were designed simply to verify the separation method rather than to find optimal conditions and practical applications of the method. The main advantages are (1) the separation is rapid, (2) the sample zone is compact and (3) the separation performance is relatively high. The method is suitable for group separations and for the separation of individual components. The major requirement is to find a suitable complexing agent for the components to be separated. In addition, the determination of the concentration of the metal ions after separation is fairly easy.

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