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Journal of Chromatography A, 671 (1994) 95–99

JOURNAL OF
CHROMATOGRAPHY A

Detection of transition metals during their separation in an isoconductive pH gradient

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Abstract

The possibility of the conductimetric detection of transition metals following separation with a linear pH gradient was demonstrated. Such detection is possible owing to the constant conductivity of the polyampholyte eluent over a wide pH range due to the use of a starting buffer with the same conductivity as the polyampholyte eluent. The separation of metals was performed on a column packed with tetraethylenepentamine-bonded silica.

1. Introduction

Chromatofocusing is a widely used ion-exchange chromatographic technique applied to the separation and purification of biological macromolecules owing to the difference in their isoelectric points [1]. The high resolving ability of the method is connected with the property of primary and secondary amino groups at the surface of a polybuffer ion exchanger (PBE) to form a linear pH gradient in the chromatographic column. Recently, it was shown that the principle of chromatofocusing can be modified and used for the concentration and high-performance separation of transition metals [2,3]. Such an ability is based on the property of functional amino groups at the surface of the PBE to form complexes of transition metals at a high pH of the eluent and their consequent destruction with a decrease in the pH gradient. Good separation was obtained with tetraethylenepentamino-bonded silica and a commercially available polysaccharide-based ion exchanger, PBE-94 [3].

A problem with the reliable detection of the separated metals arises in the separation of transition metals with an induced pH gradient. Spectrophotometric detection in a flow of effluent with postcolumn reaction with 4-(2-pyridylazo)resorcinol (PAR), as usually used, does not provide the reliable results, for the following reasons: (i) the difficulty of maintaining the optimum pH in the postcolumn reactor for quantitative complexation of metals with PAR during the pH gradient; the buffer capacity for polyampholyte eluents used in chromatofocusing is 75 μmol/pH unit/ml, which produces a significant decrease in the pH of the mixture by 1.0–1.5 after mixing the column effluent with the postcolumn reagent; (ii) the appearance of a

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SSDI 0021-9673(94)00131-R

ghost peak at the end of the pH gradient due to the shift in the acid–base equilibrium of PAR (Fig. 1); and (iii) the significant complexing ability of the polyampholyte eluent used in chromatofocusing [4].

The determination of the separated metals in each fraction may solve the above problem, but it significantly complicates the detection procedure and affects the resulting profile of the chromatogram.

Another possible solution to the problem is to use conductivity detection. This is connected with the property of polyampholyte eluents to provide a constant conductivity in solutions with varying pH. The aim of this work was to show the potential of conductimetric detection for this chromatographic technique.

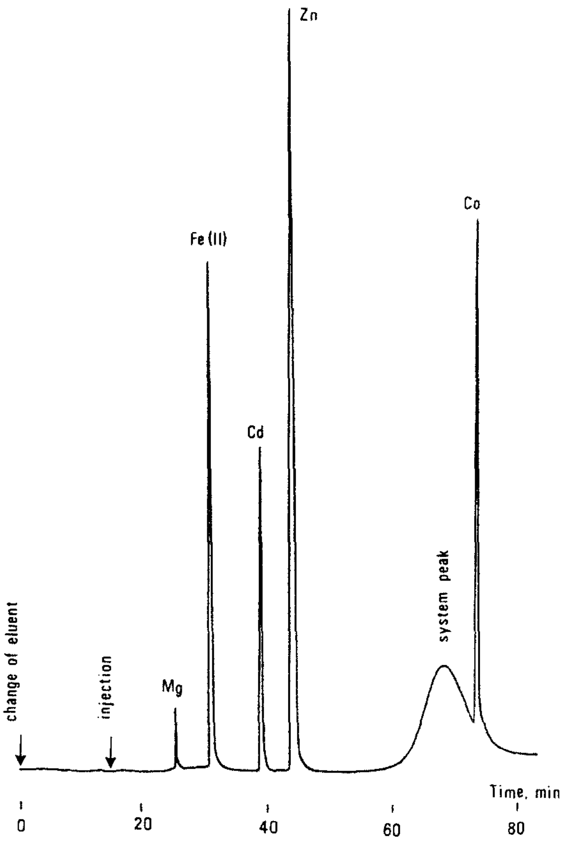

Fig. 1. Chromatogram of model mixture of metals. Column, 250 × 4.6 mm I.D. Tetren-SiO$_2$, particle size 10 μm; capacity, 0.28 mmol/g; starting buffer, 0.01 M Tris–HCl (pH 7.5); eluent, 1:20 Polybuffer 74 (pH 3.4); flow-rate, 1 ml/min; spectrophotometric detection at 540 nm with postcolumn reaction with PAR.

2. Experimental

2.1. Apparatus

An isocratic chromatographic system, consisting of a Beckman (Berkeley, CA, USA) Model 114M high-pressure pump, a Rheodyne (Cotati, CA, USA) Model 7125 injection valve, a Conductolyzer 5300B conductimeter (LKB, Bromma, Sweden), a Model 204 variable-wavelength spectrophotometric detector (Linear, Reno, NV, USA), a DP-700 integrator–recorder (Spectra-Physics, San Jose, CA, USA) and a RediRac 2112 fraction collector (LKB), was used. The stainless-steel chromatographic column was slurry packed with the silica-based polybuffer ion exchanger Tetren-SiO$_2$ from 2-propanol–water (1:3). The measurement of conductivity and conductimetric titration of the starting buffers and polyampholyte eluents were performed with a Model MM-34-04 conductimeter (Moscow, Russian Federation) equipped with smoothed platinum electrodes. The concentration of transition metals in the fractions was determined spectrophotometrically by reaction with PAR as described [5].

2.2. Reagents

The polybuffer ion exchanger Tetren-SiO$_2$ was prepared by modification of silica (Silasorb Si300, 10 μm; Lachema, Brno, Czech Republic) surface with 3-glycidoxypropyltriethoxysilane followed by treatment with tetraethylenepentamine (both reagents from Reachim, Moscow, Russian Federation) according to slightly modified procedure [6]. Aqueous solutions of Tris, glycylglycine and L-histidine (Serva, Heidelberg, Germany) were used as starting buffers. Dilute solutions of Polybuffer 74 (Pharmacia, Uppsala, Sweden) were used as polyampholyte eluents. The required pH of the eluents was adjusted with 0.1 M HCl. Stock solutions of transition metals were prepared from the corresponding nitrates [sulphate for iron(II)].

3. Results and discussion

A number of papers have reported applications of different zwitterionic eluents such as N-substituted-aminoalkanesulphonic acids and aminocarboxylic acids for the determination of anions by ion chromatography [7–10]. The main advantage of these eluents in their low conductivity, providing sensitive detection in the "nonsupressed" mode. Another advantage is the simplicity of suppression of the conductivity of zwitterionic eluents for their concentration gradient required for the elution of strongly retained solutes.

It is also known that carrier ampholytes used in isoelectrofocusing and the polyampholyte eluent Polybuffer 74 used in chromatofocusing are zwitterionic substances with higher molecular masses. They both have the following main properties [1,11,12]: background conductivity as low as 2–10 μS/cm; constant value of conductivity over a wide pH range; and good and constant buffer capacity in the same pH range.

The first step, conductimetric titration of Polybuffer 74 diluted 1:8 with 0.025 *M* histidine was performed to ensure the above-mentioned characteristics of Polybuffer 74. It was found that the conductivity of the 1:8 diluted solution of Polybuffer 74 and the buffer capacity are virtually constant over the pH range 3.9–7.1. The conductivity of the solution was 8–10 μS/cm. It should be noted that the same interval of pH covers the range of the pH gradient formed in the column.

The properties of Polybuffer 74 provide the possibility of forming an isoconductive linear pH gradient in the chromatographic column and consequently of performing conductimetric detection. Evidently, in this instance the Tetren-SiO_2 ion exchanger acts as a solid-phase suppressor with limited capacity. The initial chromatogram of a model mixture of transition metals obtained with a pH gradient is shown in Fig. 2. A good separation of manganese(II), cadmium(II), zinc(II) and cobalt(II) was obtained. The chromatographic peaks of the metals were detected by on-line measurement of the conductivity of the effluent and also by the spectrophotometric determination in the collected fractions by reaction with PAR. The sensitivity of detection in both instances is virtually identical. However, the background conductivity of the effluent during a chromatographic run is decreased corresponding to the pH profile. This is connected with the difference in the conductivity values of the starting buffer and the polyampholyte eluent. This difference can be minimized by optimization of the nature and concentration of the starting buffer and the polyampholyte

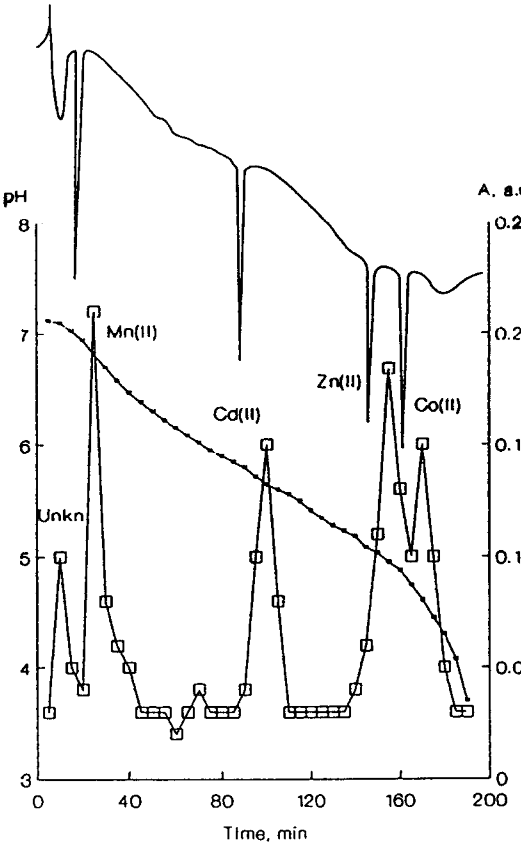

Fig. 2. Separation of standard mixture of metals. Column as in Fig. 1. Starting buffer, 0.025 *M* histidine–HCl (pH 7.5); eluent, 1:8 Polybuffer 74 (pH. 3.4); flow-rate, 1 ml/min; conductimetric and spectrophotometric detection at 540 nm with PAR in collected fractions ($V = 5$ ml).

eluent. The relationship between the conductivity of various starting buffers and the polyampholyte eluent and their concentration was investigated for this purpose (Fig. 3). The dilution of the starting buffer made on the basis of Tris, glycylglycine or histidine produces a greater decrease in conductivity in comparison with the dilution of Polybuffer 74. Hence during the optimum dilution good correspondence of the conductivities was achieved for the pair 0.004 *M* histidine–1:50 Polybuffer 74. As a result, no significant disturbance of the baseline was observed for this system of eluents during a blank chromatofocusing run.

The chromatogram of the model mixture of metals obtained under the optimum conditions (Fig. 4) demonstrates the straight baseline and the suitability of conductimetric detection of transition metals with the use of a pH gradient.

There are no data available on the chelating properties of Polybuffer 74. However, assuming complexation for the polyampholyte eluent used and taking into account its low background conductivity, one can suggest a direct detection mode of the separated metals in the form of complexes with aminopolycarboxylic fragments of the zwitterionic molecules of polyampholyte eluent. In comparison with the results of the determination of the negatively charged complexes of transition metals with EDTA by "non-supressed" IC at a fixed pH of the eluent with conductivity detection [13,14], the sensitivity of detection was the same. The detection limits of metal ions with the isoconductive pH gradient system using 0.004 *M* histidine–1:50 Polybuffer 74 as the eluent were Fe(II) 10, Co(II) 10, Mn(II)15, Zn(II) 7 and Cd(II) 5 μg/l. Moreover, the sensitivity of the proposed chromatographic method can be simply improved by increasing the sample volume with preconcentration of transition metals on the same column.

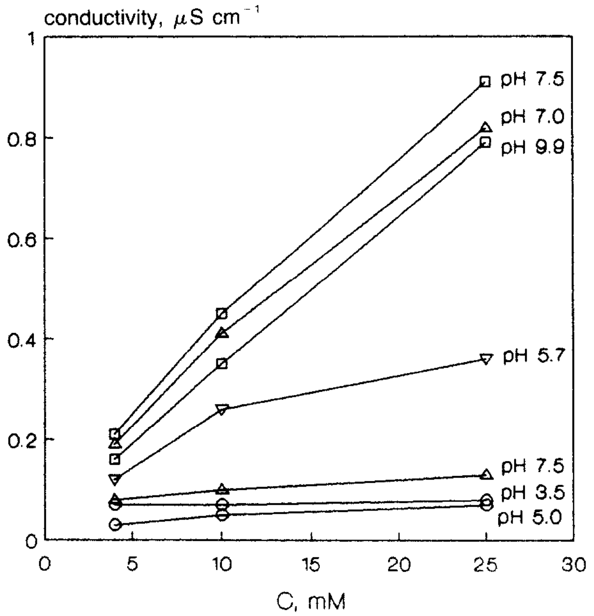

Fig. 3. Relationship between the conductivity of the starting buffers and of the polyampholyte eluent and the concentration and pH. □ = Tris; △ = histidine; ▽ = glycylglycine; ○ = Polybuffer 74.

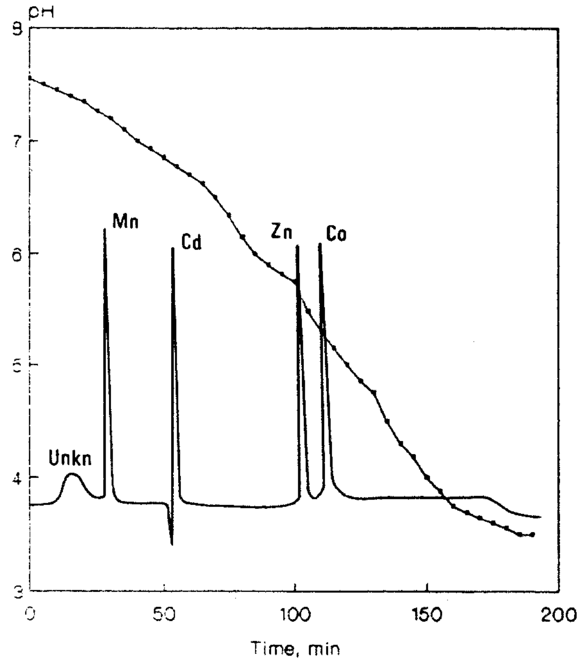

Fig. 4. Chromatogram of model mixture of metals using an isoconductive pH gradient. Column as in Fig. 1. Starting buffer, 0.004 *M* histidine (pH 7.6); eluent, 1:50 Polybuffer 74 (pH 3.4); flow-rate, 1 ml/min; conductimetric detection.

4. Conclusions

The properties of different eluents used in chromatofocusing were investigated. An isoconductive pH gradient system consisting of 0.004 *M* histidine (pH 7.5) starting buffer and 1:50 Polybuffer 74 (pH 3.4) polyampholyte eluent is proposed for the separation of transition metals on a column of tetraethylenepentamine-bonded silica. The possibility of conductimetric detection of separated metals without "suppression" was demonstrated for a linear gradient from pH 7.5 to 3.5 formed during the separation.

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