

On-Line Electrochemical Redox Derivatization for Enhancement of Separation Selectivity of Liquid Chromatography

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An on-line electrochemical redox derivatization technique for enhancement of separation selectivity of HPLC using an electrolytic flow cell is presented. This HPLC system consists of two separation columns and a small electrolytic flow cell placed between them. A redox derivatization reaction proceeds in the electrolytic flow cell so that an analyte compound migrates in its original form in the first column, while it moves in its oxidized or reduced form in the second column. The selective separation of an analyte compound has been achieved, because redox reactions specific for the analyte in the electrochemical derivatization unit can be controlled by alteration of potential applied to the cell. A successful application of this method to the separation and determination of cobalt in a stainless steel is demonstrated.

High-performance liquid chromatography (HPLC) is one of the most powerful separation methods and has been widely used for separating a variety of chemical substances. However, it has also been recognized that the differences in retention of sample components in their native forms are not always sufficient for complete separation by conventional HPLC. Use of a chemical reaction specific for target compounds as a pre-column or on-column derivatization is an efficient way for enhancing the separation selectivity of HPLC.[1–4] Pre-column derivatization is usually carried out in a batchwise operation prior to injection into the column, whereas on-column derivatization is performed by direct injection of the analyte solution into the mobile phase containing a reagent that reacts with the analyte.

On the other hand, we have recently presented an on-line derivatization technique using double separation columns and one derivatization unit for enhancement of separation selectivity of HPLC.[5] This on-line derivatization HPLC system consists of two separation columns and one derivatization unit placed between them. The derivatization reaction proceeds in the derivatization unit so that an analyte compound migrates in its original form in the first column, while it migrates in its converted form in the second column. When the two separation columns are identical with each other except for the column length, the retention of the analyte is expected to be controlled by the lengths of the two columns in this system.[5]

So far, we have shown that porous graphitic carbon (PGC) packings display catalytic redox activity, which can be modified by treating them with a solution containing a suitable oxidizing or reducing agent.[5–7] We have thus adopted a small column packed with PGC as a redox derivatization unit and have investigated the on-line redox derivatization efficiency of PGC using ethylenediaminetetraacetate (edta) complexes of Co^{II} and Co^{III}.[5] PGC treated with hydrogen peroxide oxidizes Co^{II}–edta to Co^{III}–edta, while the other metal–edta complexes elute in their original oxidation states from the PGC column. The oxidation reaction proceeds rapidly and completely in the derivatization unit so that the Co^{II}–edta complex injected migrates in its original form in the first column, while it migrates in its oxidized form (Co^{III}–edta) in the second column. Co^{II}–edta thus elutes between the trivalent and divalent metal complexes and can be selectively separated from the other metal complexes.

PGC can also be reductive, if it has been treated with a suitable reducing agent such as sodium sulfite. However, the reduction of Co^{III}–edta by PGC does not go to completion, that is, only part of Co^{III}–edta injected is reduced to Co^{II}–edta.[1] Additionally, the PGC gradually loses its reduction activity with an increase in the volume of the mobile phase passing through it. These phenomena may be ascribed to the redox potential of PGC[7] and/or oxidation of PGC by oxygen slightly dissolved in the mobile phase.[5]

We have demonstrated that the on-line redox derivatization HPLC system with a column packed with a solid redox agent, such as PGC, enables one to accomplish highly selective separations of analyte compounds that can undergo redox reactions.[5] However, the use of chemical redox derivatization with a solid redox agent is not versatile because the redox activity is limited by its inherent redox potential. In the present paper, we adopted a small electrolytic flow cell as a redox derivatization unit in order to overcome this limitation. In recent years, some chromatographic approaches utilizing electrolytic flow cells have been presented. However, electrolytic cells are used exclusively for improvement of sensitivity or selectivity of the detectors[8–14] and for synthesis by electrochemical redox reactions.[15–17] To our knowledge, the present study is the first example of an electrolytic flow cell being applied to enhancement of separation selectivity of HPLC as an on-line derivatization unit. We evaluated the efficiency of the on-line electrochemical redox derivatization system using edta complexes of Co^{II} and Co^{III} as model compounds. It will be shown that the redox reactions of an analyte compound can easily be controlled by alterations in the potential applied to the electrolytic flow cell and the on-line electrochemical redox derivatization

Published on the web May 14, 2007; doi:10.1246/bcsj.80.951

HPLC is very useful for selective separation of oxidizable or reducible compounds. We also report the successful application of this method to the separation and determination of cobalt in stainless steel.

Experimental

Apparatus. The HPLC system consisted of an SI-1/2001 pump, an SI-1/2014 column oven and an SI-1/2002 UV–vis detector (all from Shiseido, Tokyo, Japan) or a Waters 996 photodiodearray detector (Waters, MA, U.S.A.). The mobile phase, which was maintained under a nitrogen atmosphere during use, was degassed through a DEGASYS DG-1310 on-line degassing system (UNIFLOWS, Tokyo, Japan). The electrolytic flow cell was a Model 5020 guard cell (ESA, MA, U.S.A.), which contained a porous graphite working electrode, a palladium counter electrode and a palladium–hydrogen reference electrode (Pd/H_2).[18,19] All potentials described in this study are given vs Pd/H_2. The column temperature was maintained at 20 °C. The injection was performed either using a metal-free manual injector (Shiseido, Tokyo, Japan) or an auto sampler DAS-80 (DIONEX, Osaka, Japan). The detection wavelength was set at 230 nm unless otherwise stated. Data analysis was carried out by means of CDS4 chromatographic software or CAC4 chromatographic software from Nihon Filcon (Tokyo, Japan). A UV–vis spectrophotometer, Model V-630 BIO (JASCO, Tokyo, Japan) was used for measurement of the inner volume of the electrolytic flow cell. Cyclic voltammetry was performed using an HSV-100 potentiostat (Hokuto Denko, Tokyo, Japan) and a VC-4 electrolytic cell (BAS, Tokyo, Japan). A three-electrode system used consisted of a PFCE-1 carbon working electrode, a platinum counter electrode, and an RE-2B Ag/AgCl reference electrode (all from BAS, Tokyo).

Reagents. All reagents used in this study were of analytical reagent grade unless otherwise stated. Disodium hydrogen ethylenediaminetetraacetate (Na_2H_2edta), Fe^{III}–edta, Co^{II}–edta, Ni^{II}–edta, Cu^{II}–edta and Bi^{III}–edta were obtained from Dojindo Laboratories (Kumamoto, Japan). Trimethylstearylammonium chloride (TMSA) and acetone were purchased from Kanto Chemicals (Tokyo, Japan). A standard solution of Co^{II} for atomic absorption use (1000 ppm) was obtained from Kanto Chemicals. Distilled and deionized water was further purified via passage through a Millipore Milli-Q purification system.

Co^{III}–edta complex was prepared as described previously.[5,20] A reference material of stainless steel, JSS 650-14 STAINLESS STEEL SUS 430, was obtained from the Japan Iron and Steel Federation.

Preparation of Stainless Steel Sample Solution. A sample of SUS 430 (0.5 g) was accurately weighed out into a beaker and was dissolved with a 1:1 (v/v) mixture of concentrated hydrochloric acid and 30% hydrogen peroxide (20 mL). The resulting solution was heated (100 °C) to decompose the excess hydrogen peroxide and then was filtered with a filter paper (Advantec No. 5C, Tokyo, Japan). The filtrate was poured into a separatory funnel, to which hydrochloric acid (20 mL, 9.5 M) and 4-methyl-2-pentanone (20 mL) were added. The mixture was shaken for 1 min. After complete phase separation occurred, the upper organic phase, into which iron was extracted, was removed; this step was repeated three times. The aqueous phase was evaporated to dryness by heating at 100 °C, and the residue was dissolved with hydrochloric acid (5 mL, 0.1 M). The resulting solution was diluted with water to 10 mL in a volumetric flask.

A 100 μL aliquot of the stock SUS 430 sample solution was added to a mixture of Na_2H_2edta solution (200 μL, 0.1 M) and an acetate buffer (1 mL, 1 M, pH 5.0). The solution was heated gently (70 °C) in a water bath in order to ensure complete complexation. The resulting solution was finally diluted with water to 10 mL in a volumetric flask. The concentrations of the metal ions, which were calculated from the recommended values, were 82.2 ppm for Cr, 2.43 ppm for Mn, 1.06 ppm for Ni, 238 ppb for Cu, 61 ppb for Mo, 10 ppb for Al, and 142 ppb for Co. The solutions of Co^{II}–edta used for constructing calibration curves were prepared from the standard cobalt solution in a similar manner as describe above.

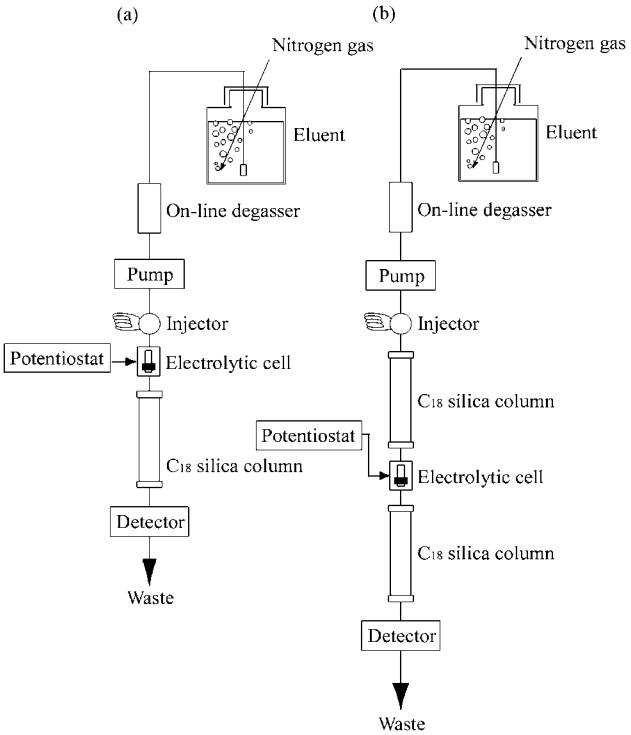

Fig. 1. Schematic diagram of the system used (a) for evaluation of the efficiency of the redox derivatization by the electrolytic flow cell, and (b) for selective separation of cobalt by on-line redox derivatization HPLC. See text for discussion.

Chromatographic Conditions. Figure 1 shows the three HPLC systems that were used in this study. Figure 1a represents the system that we used for evaluation of the efficiency of the redox derivatization by the electrolytic cell (system A). The on-line redox derivatization HPLC system (system B) is shown in Fig. 1b; the electrolytic flow cell is placed as an on-line redox derivatization unit between two C_{18} silica columns. The C_{18} silica columns were Capcell Pak C18 UG120 (3 μm, 1.5 × 150 mm) columns obtained from Shiseido. The mobile phase was 0.1 M acetate buffer with pH 5.0. The acetate buffer solution was prepared from acetic acid and sodium acetate. Metal–edta solutions to be injected were prepared by dissolving the metal–edta complexes in a solution, of which the composition was the same as that of the mobile phase. The mobile phase was delivered isocratically at a flow rate of 0.10 mL min^{-1} unless otherwise indicated. All solutions were filtered through a 0.45 μm membrane filter before use. The treatment of the C_{18} silica columns was performed by passing an adequate amount of the mobile phase solution containing 1 mM TMSA through the column in order to retain negatively charged metal–edta complexes on the column. TMSA was completely adsorbed

on the C_{18} silica under three experimental conditions unless the amount of TMSA did not exceed the adsorption capacity of the column.

Measurement of the Inner Volume of the Electrolytic Flow Cell. The electrolytic flow cell was filled with a 1 M acetone solution, and then the solution was washed out of the flow cell with about 9 mL of water. The effluent was collected in a 10 mL volumetric flask and was diluted to the mark with water. The concentration of acetone in the resulting solution was determined by UV absorptiometry. The inner volume of the electrolytic flow cell (V_{cell}) was calculated according to the following equation:

$$V_{call}(\mu L) = \frac{C_f}{C_i} \times 10^4, \quad (1)$$

where C_f and C_i are the concentrations of acetone in the final and initial solutions, respectively. We confirmed that acetone exhibited no adsorption on the electrodes and the inner surface of the cell.

Cyclic Voltammetry. Cyclic voltammograms were obtained in a 0.1 M acetate buffer solution (pH 5.0) containing 5 mM Co^{II}–edta under a nitrogen atmosphere at a scan rate of 50 mV s^{-1} from −1.2 to 1.5 V (vs Ag/AgCl, 1 M NaCl).

Results and Discussion

Redox Derivatization by an Electrolytic Cell. There are three requisites for the cell to be used for the on-line redox derivatization HPLC: (1) the cell should withstand high pressure; (2) the inner volume of the cell should be small; (3) the efficiency of electrolysis should be 100%. The maximum operating pressure of the cell shown by the manufacturer was 422 kgf cm^{-2}, and the inner volume of the cell we determined was 44 μL. The electrolytic cell that we used met the first two requisites. Therefore, we evaluated the electrolysis efficiency of the cell using Co^{II}–edta and Co^{III}–edta as model compounds.

Figure 2 shows the chromatograms obtained when a solution containing Co^{III}–edta was injected into the system A equipped with the electrolytic flow cell shown in Fig. 1a. As we have already shown,[5–7] C_{18} silica columns do not exhibit any effect on the equilibrium and kinetics of the redox reaction, the retention time of the Co^{II}–edta complex is much larger than that of the Co^{III}–edta complex on the C_{18} column treated with TMSA, and the molar absorption coefficient of the Co^{III}–edta complex is larger than that of the Co^{II}–edta complex by a factor of about 15 at the detection wavelength of 230 nm. As expected, the chromatographic profile varied with the potential applied to the cell. Co^{III}–edta was partially reduced, when the applied potential was −0.50 V, and was completely reduced to Co^{II}–edta at −0.70 V as shown in Fig. 2.

The inset in Fig. 2 shows the dependence of the peak area of Co^{III}–edta and Co^{II}–edta on the applied potential. The increase in peak area of Co^{II}–edta began at −0.4 V, and the area reached a maximum at −0.6 V or below. This result showed that Co^{III}–edta injected into the system was completely converted to Co^{II}–edta in the electrolytic flow cell at −0.6 V or below. We have reported that the PGC column cannot be used as a reduction derivatization unit owing to the limitation of reduction activity.[5] This means that electrochemical derivatization with an electrolytic flow cell may be superior to chemical redox derivatization with a solid redox agent with respect to the derivatization efficiency.

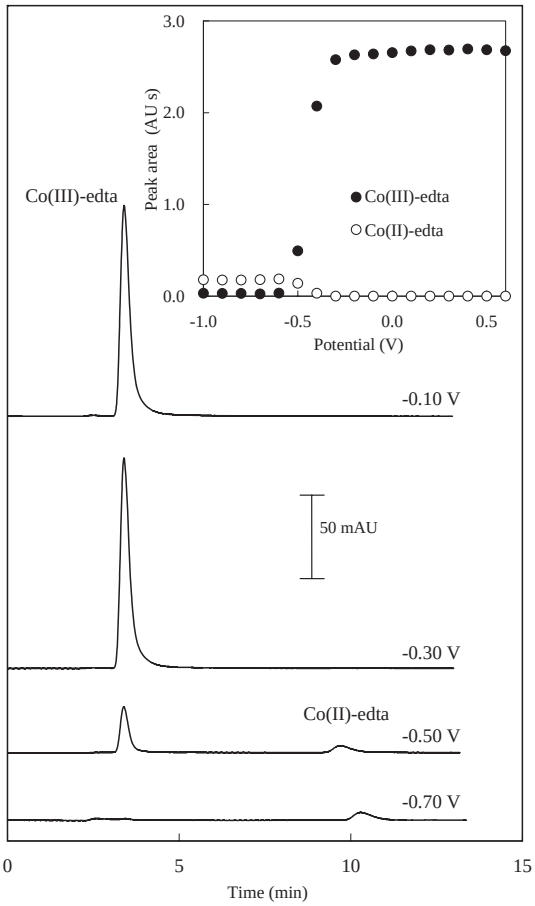

Fig. 2. Variation of chromatograms obtained for an injection of Co^{III}–edta with potential applied to the electrochemical flow cell in system A. Conditions: mobile phase, 100 mM acetate buffer solution (pH 5); flow rate, 0.10 mL min^{-1}; column temperature, 20 °C; amount of TMSA loaded, 4.5 μmol; sample volume injected, 4 μL; amount of sample injected, 0.3 nmol. See text for discussion. Inset: Dependence of peak areas of Co^{II}–edta and Co^{III}–edta on the applied potential.

Figure 3 shows the chromatograms obtained when a solution containing Co^{II}–edta was injected into the system A. Co^{II}–edta was oxidized to Co^{III}–edta by the electrolytic cell and the peak of Co^{II}–edta disappeared at 0.1 V or above. This oxidation behavior is similar to that observed when the PGC column oxidized with hydrogen peroxide is used as the derivatization unit. However, the oxidation of Co^{II}–edta by the electrolytic cell caused the formation of another species observed as a small peak denoted by "X" in Fig. 3, which eluted in front of the Co^{III}–edta peak.

The UV–vis spectrum obtained for the peak X is shown in Fig. 4 together with the spectrum for Co^{III}–edta; the spectrum for the peak X showed an absorption maximum in the visible region at 550 nm, while that for Co^{III}–edta had a maximum at 538 nm. Doi has reported that the oxidation of the Co^{II}–edta complex with permanganate ion rapidly proceeds to give $[Co^{III}(edta)(H_2O)]^-$, of which the wavelength of absorption maximum (λ_{max}) is 550 nm, and then the pentadentate edta in the complex slowly becomes hexadentate in the final prod-

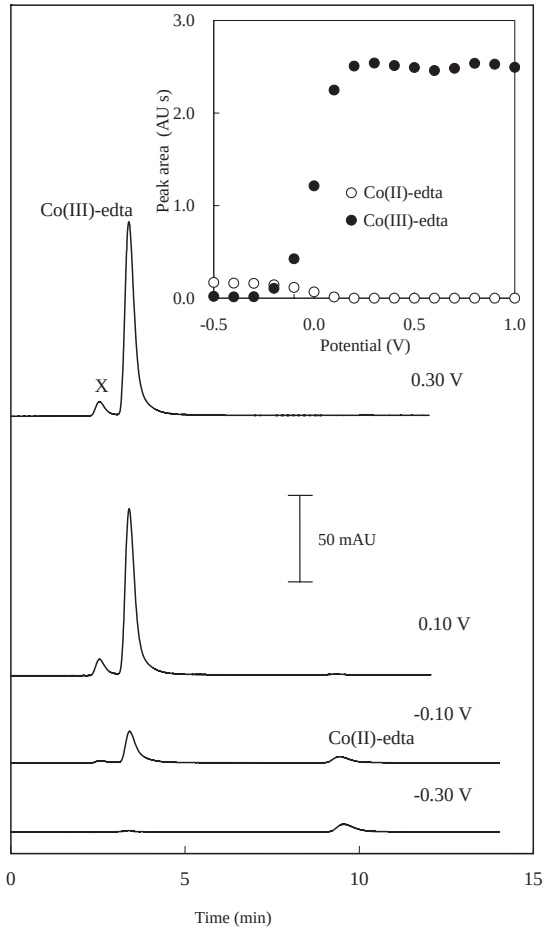

Fig. 3. Variation of chromatograms obtained for an injection of Co^{II}–edta with potential applied to the electrolytic flow cell in system A. Amount of sample injected, 0.3 nmol. For other experimental conditions, see Fig. 2. Inset: Dependence of peak areas of Co^{II}–edta and Co^{III}–edta on the applied potential.

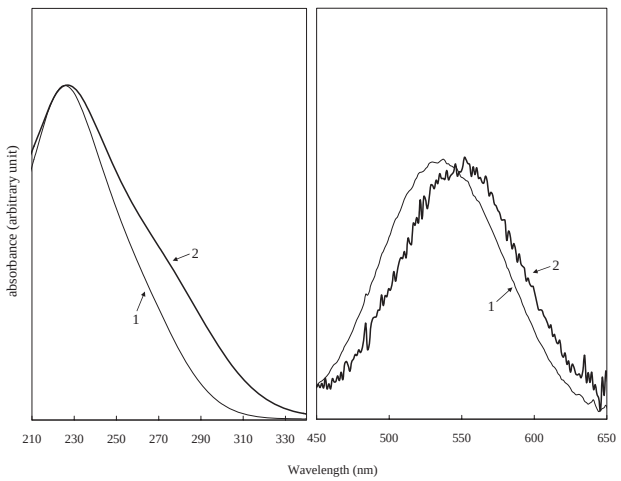

Fig. 4. UV–vis spectra obtained for the peak X shown in Fig. 3 and that for Co^{III}–edta. Conditions: applied potential, 0.30 V; amount of sample injected, 15 nmol. Spectrum: 1 = Co^{III}–edta, 2 = peak X. For other experimental conditions, see Fig. 2.

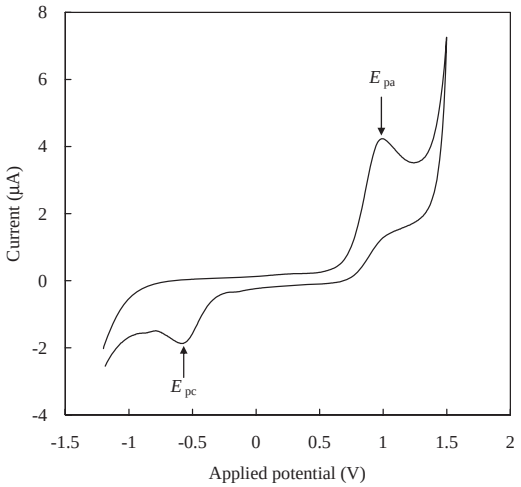

Fig. 5. Cyclic voltammogram of Co–edta in 0.1 M acetate buffer (pH 5). Conditions: sample concentration, 5 mM; scan rate, 50 mV s^{-1}. See text for discussion.

uct, $[Co^{III}(edta)]^-$ (λ_{max} = 538 nm).[21] As shown in Fig. 4, the visible spectrum for peak X corresponds to that of $[Co^{III}(edta)(H_2O)]^-$. Therefore, we concluded that the ring-closure reaction of $[Co^{III}(edta)(H_2O)]^-$ to $[Co^{III}(edta)]^-$ did not proceed completely in the cell so that two peaks were observed on the chromatogram. Further investigations on the oxidation reaction of Co^{II}–edta in an electrolytic cell are currently under way.

We evaluated the oxidation efficiency of Co^{II}–edta by the electrolytic cell using the sum of the peak area of $[Co^{III}(edta)(H_2O)]^-$ and that of $[Co^{III}(edta)]^-$, assuming that these two Co^{III}–edta species have the same molar absorptivity at 230 nm. The inset in Fig. 3 shows the dependence of the total peak area of the two Co^{III}–edta complexes and the peak area of Co^{II}–edta on the applied potential. The total peak area of Co^{III}–edta began to increase at −0.2 V, and then, it reached a maximum at 0.2 V. This result showed that Co^{II}–edta injected into the system was completely converted to two Co^{III}–edta species in the electrolytic flow cell at 0.2 V or above.

The electrochemical redox behavior of Co–edta was investigated by cyclic voltammetry, and the results are shown in Fig. 5. The composition of the solution used for this experiment was the same as that of the mobile phase solution. The cyclic voltammogram showed two distinct redox waves with anodic peak potential (E_{pa}) of 0.99 V and cathodic peak potential (E_{pc}) of −0.59 V (vs Ag/AgCl, 1 M NaCl), which may correspond to the oxidation of Co^{II}–edta and the reduction of Co^{III}–edta, respectively. The peak separation ($E_{pa} - E_{pc}$) was much larger than the difference between the potentials observed for the oxidation of Co^{II}–edta and that for the reduction of Co^{III}–edta by the electrolytic flow cell. This may be attributed to the difference in structure and surface area between the working electrodes of the two electrolytic cells.

We also investigated the dependence of redox efficiency of the electrolytic flow cell on the flow rate and found that the oxidation of Co^{II}–edta at 0.1 V and the reduction of Co^{III}–edta at −0.6 V were independent of the flow rate in the range of 0.02 to 0.10 mL min^{-1}. All of the results, described above, indicate that the electrolytic flow cell can be used as an effective on-

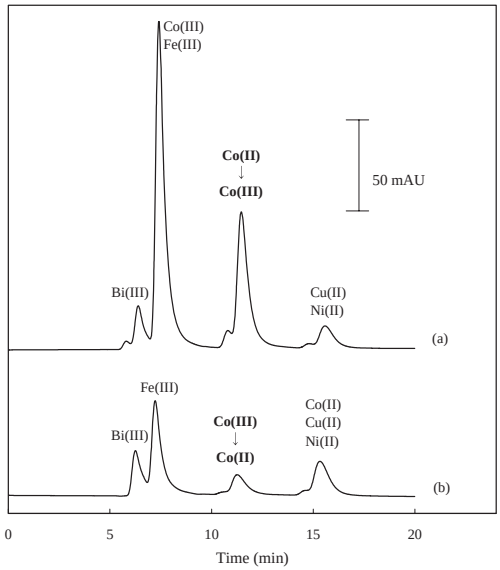

Fig. 6. Chromatograms of metal–edta complexes obtained by the on-line electrochemical redox derivatization HPLC. Conditions: flow rate, 0.07 mL min^{-1}; applied potential, (a) 0.30 V and (b) −0.60 V. For other experimental conditions, see Fig. 2.

line redox derivatization unit.

Selective Separation of Cobalt by On-Line Electrochemical Redox Derivatization HPLC. We have already shown that divalent metal–edta complexes can be completely separated from trivalent ones by reversed-phase ion-pair liquid chromatography, whereas the complexes of the same charges exhibit almost the same retention and are unable to be separated from one another.[5–7] However, selective separations of Co^{II}–edta and Co^{III}–edta from other metal–edta complexes are expected to be accomplished by the on-line electrochemical redox derivatization technique with the HPLC system C shown in Fig. 1b.

Figure 6 shows chromatograms obtained when a sample solution containing edta complexes of Co^{III} and Co^{II} as well as Bi^{III}, Fe^{III}, Ni^{II}, and Cu^{II} was injected into the on-line electrochemical redox derivatization HPLC system. From Fig. 6, the Co^{III}–edta eluted between the other trivalent and divalent metal–edta complexes when −0.6 V was applied to the cell, while applying 0.3 V changed the retention of Co^{II}–edta and made it possible to separate completely Co^{II}–edta from the trivalent and divalent metal–edta complexes. These results indicate that the Co^{III}–edta and Co^{II}–edta complexes injected migrate in their original forms in the first C_{18} column and in their reduced or oxidized form in the second C_{18} column. Since the retention in reversed-phase ion-pair liquid chromatography is mainly a result of ion-exchange interactions, the retention factor of the cobalt complex in this on-line derivatization HPLC system is represented by Eq. 2.

$$k = \frac{k_1 Q_1 + k_2 Q_2}{Q_1 + Q_2}, \tag{2}$$

where Q_1 and Q_2 denote the anion-exchange capacities of the first and the second separation columns (eq column^{-1}), respectively. Equation 2 indicates that the retention of the analyte showed be controlled by ion-exchange capacities of the two

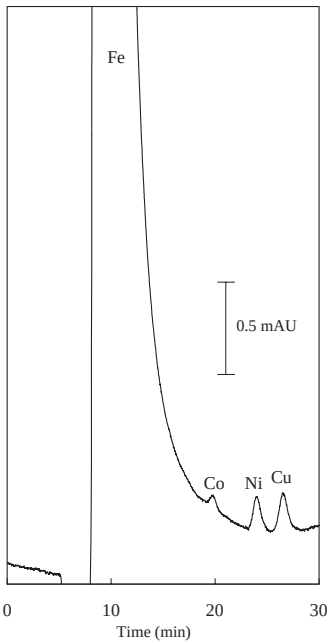

Fig. 7. Separation of trace amount of cobalt from large amount of iron using the on-line electrochemical derivatization HPLC. Conditions: concentrations of metal ions injected: 100 ppm for Fe^{III}, 200 ppb for Cu^{II}, 200 ppb for Ni^{II}, and 10 ppb for Co^{II}; applied potential, 0.30 V; flow rate, 0.07 mL min^{-1}; amount of TMSA loaded, 9.5 μmol for first separation column and 4.5 μmol for second separation column. For other experimental conditions, see Fig. 2.

separation columns in this system. Thus, the on-line electrochemical derivatization HPLC presented in this study makes it possible to accomplish a highly selective separation of analyte compounds that can undergo redox reactions.

We thus tried to separate a trace amount of Co from a large amount of Fe in order to apply this technique to the separation and determination of cobalt in a steel sample. Figure 7 shows a chromatogram obtained for a sample solution containing Fe^{III} (100 ppm), Cu^{II} (200 ppb), Ni^{II} (200 ppb), and Co^{II} (10 ppb). In order to separate Co from the large amount of Fe^{III}, we loaded 9.5 μmol of TMSA on the first separation column, which was about twice the amount of TMSA loaded on the second column, i.e., $Q_1 = 2Q_2$. From Fig. 7, Co could be separated from the other metal ions, although the large tailing of the peak for Fe interfered with complete separation and precise determination of Co. Therefore, we attempted to determine trace amount of cobalt in a stainless steel sample, after iron had been removed by solvent extraction. We chose the reference material SUS 430 as a target sample. SUS 430 is a stainless steel containing Fe and Cr in high concentrations as well as other minor metal elements including Co. Figure 8 shows a chromatogram obtained for a SUS 430 sample solution. The amounts of TMSA loaded on the two separation columns were adjusted so that a complete separation of Co from the other metal ions could be achieved. It can be seen from this figure that Co was completely separated from the other metal ions. The calibration plot of the peak area against the concentration for the standard cobalt solution showed good linearity ($r^2 = 0.999$)

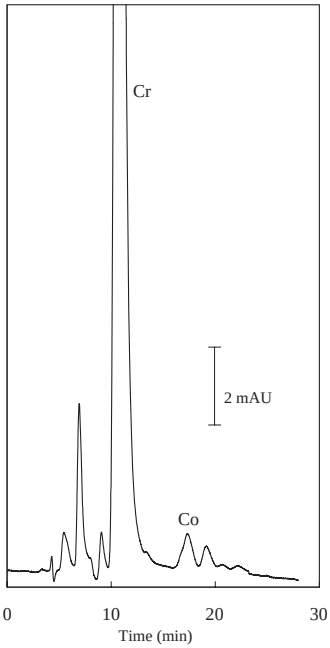

Fig. 8. Separation of cobalt in SUS 430 reference sample using the on-line electrochemical redox derivatization HPLC. Conditions: applied potential, 0.30 V; flow rate, 0.07 mL min^{-1}; amount of TMSA loaded, 6.0 μmol for first column and 3.0 μmol for second column. For other experimental conditions, see Fig. 2.

Table 1. Quantification Results of the Reference Stainless Steel Sample SUS 430 for Cobalt

Determined value/μg g^{-1}	Recommended value/μg g^{-1}
0.28 ± 0.0112[a] ($n = 4$)	0.28 ± 0.0106[b]

a) Values are mean ± S.D. b) Mean of eleven reported by five different laboratories. Analytical methods used for determination were atomic absorption spectrometry and absorption photometry.

over the range of 10 ppb–10 ppm. The results of the quantification of cobalt in a SUS 430 sample obtained by the present method are shown in Table 1 together with the recommended value. The cobalt concentration determined by the present method is in good agreement with the recommended value. This result demonstrates that the on-line redox derivatization technique with the electrolytic cell is very useful for selective separation and determination of compounds that can be converted to different species through redox reaction.

Conclusion

In this paper, we presented an on-line electrochemical redox derivatization technique for enhancing the separation selectivity of HPLC. As a redox derivatization unit, we used a small electrolytic cell that withstood high pressure and placed it between two separation columns. The separation efficiency of the on-line electrochemical redox derivatization HPLC was evaluated using edta complexes of Co^{II} and Co^{III} ions. The electrolytic cell enabled use to control redox reactions by changing the applied potential. Therefore, not only the complete oxidation of Co^{II}–edta but also the complete reduction of Co^{III}–edta occurred in contrast to a chemical redox derivatization unit, such as a column packed with porous graphitic carbon, which we have previously reported. Co^{III}–edta eluted between the other trivalent and divalent metal–edta complexes when −0.6 V was applied to the cell, while applying 0.3 V changed the retention time of Co^{II}–edta and enabled as to separate it completely from the other metal–edta complexes.

We also successfully applied this method to the separation and determination of cobalt in a stainless steel sample. In other words, on-line redox derivatization using an electrolytic flow cell is very useful for selective separation and determination of compounds that can undergo redox reactions to give different species.

This research was supported by a grant from High Technology Research Center, College of Industrial Technology, Nihon University.

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