SEPARATION OF TRANSITION METAL IONS
BY HPLC, USING UV-VIS DETECTION

THESIS

Presented to the Graduate Council of the
North Texas State University in Partial
Fulfillment of the Requirements

For the Degree of

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by

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Denton, Texas
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HPLC has been used and can quickly determine several ions simultaneously. The method of determination described for transition metals [Cr(III), Fe(III), Ni(II), Co(II), Cu(II), Zn(II), Cd(II), Mn(II)] and [Ca(II), Pb(II)] using HPLC with UV-VIS detection is better than the PAR complexation method commonly used.

The effects of both eluent pH and detector wavelength were investigated. Results from using different pHs and wavelengths, optional analytical conditions for the separation of [Ni(II), Co(II), Cu(II)], [Cr(III), Fe(III), Ca(II), Ni(II), Cu(II)], and [Ca(II), Zn(II), Pb(II)] in one injection, respectively, are described. The influence of adding different concentrations of Na$_2$EDTA solvent to the sample is shown. Detection limits, linear range, and the comparisons between this study and a post-column PAR method are given.
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CHAPTER I

INTRODUCTION

In the past, the separation of metal ions involved the use of simple ion-exchange columns which were based on gravity flow using a high-capacity resin. This method differs from modern chromatography in the way that eluent and the sample are delivered to the column, the type of resin and column used, and the methods of detection. Modern ion-exchange chromatography is faster, more convenient, and has greater separating ability than classical methods. Small, Stevens, and Bauman (1) invented a dual column method using a combination of resins, and have succeeded in neutralizing or suppressing eluent background prior to conductivity detection for all ionic species. Since a suppressor column is used which accumulates ions from the eluent, it is necessary to regenerate the suppressor column periodically to remove the unwanted ions. Also, the scope of this technique is limited because the hydroxide-form suppressor column would precipitate most polyvalent metal cations.

In 1979 and 1980, Gjerde, Schmuckler, and Fritz (2,3) used a special anion-exchange resin of very low capacity and a low-conductivity eluent to eliminate the need for the suppressor column. Another study investigating the
separation and detection of both cations and anions in a single injection has been reported by Tarter and Jones (4).

Recently, HPLC (High Performance Liquid Chromatography) with different detectors has been widely used because it is quick and can determine several ions simultaneously. HPLC, derived from classical column chromatography, has become one of the most important modern instrumental analytical techniques. Although some other instrumental analysis methods, such as AA (Atomic Absorption) (5) and Flame Emission Spectroscopic (6) are relatively easy to apply to transition metals, these methods suffer from interferences and can not distinguish different valences of metal ions.

In comparison with conventional wet chemical techniques such as gravimetry, turbidimetry, and colorimetry, HPLC has the advantages of speed, sensitivity, selectivity, and simultaneous analysis (7).

According to Majors and Barth's report (8) approximately 70% of all known compounds are nonvolatile and as more research and applications are conducted in environmental, material, clinical, agricultural, and pharmaceutical sciences, HPLC will continue to be a dominant technique. The various types of detectors that have been used in HPLC analysis of metal ions will now be discussed.
Detector:

Some of the detection systems that have been used for the HPLC of metal ions include AA (Atomic Absorption), UV-VIS (Ultraviolet-Visible), Fluorescence Emission, ICP (Inductively Coupled Plasma), Emission Spectrometry, Electrochemical Electrode, and Radioactivity Monitoring (9,10). Detectors for monitoring radioactive eluates are available but suffer from a poor sensitivity and a narrow range of applications. Fluorescence detectors could be used in combination with a post-column reactions, but would be susceptible to the quenching effects of many heavy-metal ions. Conductivity detectors are used extensively for the detection of anions (11,12). For cations, various mobile phases were studied by Shintani (13) and a high sensitivity was attained by using a UV-VIS detector. While all of these detection systems can provide useful information, the most promising systems for routine work are UV-VIS and coulometric detectors (14). One of the main disadvantages of coulometric detection for HPLC is the large electrode surface area required for complete conversion of the electroactive species (15); this leads to a large cell volume and it appears that the larger electrode surface area of coulometric systems results in corresponding larger background currents.
The determination of transition metal ions is suited to spectrophotometric detection using post-column reaction with a reagent such as PAR [4-(2-Pyridylazo) resorcinol], arsenazo (III), and dithizone, or to using an eluent that can complex with the metal ions, and then have a strong absorbance in UV-VIS spectrophotometric detection.

Elchuk and Cassidy (16) used a post-column reaction after the usual HPLC separation column but before the detector. In this study 4-(2-pyridylazo) resorcinol monosodium salt (PAR) was used as the color-forming reagent, and with spectrophotometric detection at 540 nm most metal ions could be determined in the low ppm ranges. A schematic diagram of this system is shown in Figure 1.

When using the PAR for detection of transition metals, nitrogen should always be used as the pressurizing gas since PAR is readily oxidized by oxygen. When stored under nitrogen, the PAR reagent solution has a shelf-life of approximately two months. PAR will usually tend to increase background noise. In order to prevent PAR contamination of the detector flow cell, it is necessary to rinse the flow cell with deionized water after each use (17).

In UV-VIS detection, a complexing eluent disodium EDTA was used to achieve selective chromatographic separations of metal ions. Disodium EDTA was added to the samples
SCHEMATIC DIAGRAM OF POST-COLUMN REACTOR SYSTEM

Eluent

Pump 1

Injection Valve

To Waste

Analytical Column

Color-Forming Reagent

Pump 2

Tank

Mixing Chamber

Spectrophotometric Detector

To Waste

Recorder
to increase the peak height and eliminate the post-column reactor described in the early work (16).

There are two common types of UV-VIS detectors, the fixed wavelength detector and the variable wavelength detector. In order to increase sensitivity for particular solute types by choosing different wavelengths, the variable wavelength detector was used in my research. Since the wavelength is chosen for a specific solute, only those compounds having absorbance in this region of the spectrum will be selectively detected.

**Ion Exchange:**

Ion exchange has been one of the most common separation techniques used in analytical procedures for metal ions and has found widespread use in HPLC.

Ion-exchange materials are created by chemically introducing suitable functional groups into polymer beads. Most cation exchangers are produced by sulfonation of the benzene rings of styrene-DVB (divinylbenzene) copolymers. The sulfonic acid groups are the active resin sites used for exchange. The $\text{-SO}_3^-$ anionic group is chemically bound to the resin and its movement thus is severely restricted. However, the $\text{H}^+$ counter ion is free to move about and can be exchanged for another cation. One example is shown as follows: $\text{Res-SO}_3^- \text{H}^+ + M^+ \rightarrow \text{Res-SO}_3^- M^+ + \text{H}^+$. 
Anion-exchange resins are made by chloromethylating the benzene rings in a styrene-divinylbenzene copolymer and then alkylation by reaction with an aliphatic amine. The most common type of anion-exchange resin contains a quaternary ammonium functional group, obtained by alkylation with trimethylamine.

\[
\text{Res} \xrightarrow{\text{ClCH}_2\text{OCH}_3\text{ZnCl}_2} \text{Res} \xrightarrow{\text{CH}_2\text{Cl} \text{N(CH}_3)_3^+} \text{Cl}^- 
\]

In these resins only the anion is mobile and can be exchanged for another anion.

Conventional ion-exchange resins are reacted completely to produce a high-capacity exchanger, and the speed of a separation can be limited by the diffusion of an ion into and out of the resin particle and by the exchange reactions in the resin phase. Because of the slow mass transfer and zone dispersion, an overall lack of efficiency is observed in some columns. An increase in both the speed of a separation and in column efficiency is obtained by increasing the transfer rates (18). There are two easy methods for increasing the transfer rates: (1) increase flow rate, (2) increase eluent concentration.
The purpose of this research was to develop a procedure for the determination of transition metals [Cr(III), Fe(III), Ni(II), Co(II), Cu(II), Zn(II), Cd(II), Mn(II)] and [Ca(II), Pb(II)] using HPLC with UV-VIS detection that would be better than the PAR complexation method that has been traditionally used.

Several parameters were investigated, including eluent pH and detector wavelength, in order to optimize the detection limit for each of the transition metals studied and the separation of the various metal ions in one injection. The addition of the complexing agent disodium EDTA to the sample was examined in an attempt to improve separation and detection. The experimented methods used will be discussed in Chapter II.
CHAPTER BIBLIOGRAPHY

1. Small, Hamish; Stevens, Timothy S.; Bauman, William C. Analytical Chemistry, 1975, 47, 1801.


CHAPTER II

APPARATUS AND EXPERIMENTAL METHOD

(1). **Apparatus:**

The HPLC equipment in Figure 2 consisted of a Kratos Spectroflow 783 variable-wavelength detector, a Kratos Specflow 400 pump, a Kratos Spectroflow 480 injection valve (80 l loop), and a Spectra-Physics Model SP 4290 computing integrator. All fittings in contact with the eluent were either teflon or stainless steel. All chromatograms were obtained at room temperature.

The column used in this study was a VYDAC Anion Exchanger. Col. Type: 302 I.C., 4.6 mm x 25 cm, which is packed with a low-capacity anion-exchanger. The efficiency of this column is 4,447 plates/col on NO$_3^-$.

(2). **Eluents:**

The eluent was prepared by placing 1-L of distilled water in a 2-L beaker; while continuously stirring this solution with a magnetic stirrer and a teflon-covered stirring bar, 7.4448 g of reagent grade disodium EDTA was added and was diluted to 2-L 0.01 M, and then the disodium EDTA solution was diluted to different eluent concentrations,
using distilled water. The pH of each eluent was adjusted with either CH₃COOH or NaOH, depending on the pH desired.

3. **Samples:**

Samples were prepared by using appropriate amounts of reagent grade of FeCl₃, Cr₂(SO₄)₃·15H₂O, NiSO₄·6H₂O, CoCl₂·6H₂O, Cu(CH₃COO)₂·H₂O, Zn(NO₃)₂·6H₂O, MnSO₄·H₂O, Cd(CH₃COO)₂·2H₂O, CaCl₂·2H₂O, Pb(N)_3Cl₂, and diluting with 0.01 M disodium EDTA (pH:4.3) to a 1000 or 500 ppm Stock Solution of each metal. The Stock Solution was then diluted by using 0.01 M disodium EDTA (pH:5.7) to provide 100, 50, 25, 10, and 1 ppm, etc. The pH of disodium EDTA solutions were adjusted by NaOH. The usable pH range was about 4.3 to 6.0. At higher pH values some metals are significantly complexed and are precipitated, such as Fe(OH)₃. At lower pH values the EDTA will precipitate. The solution can be made basic to redissolve the precipitated EDTA and then reacidified without affecting analytical results.

4. **Experimental Method:**

Sample solutions containing metal ions of different concentrations in 0.01 M disodium EDTA (pH:5.7) were injected into the HPLC system. Analytical conditions differed in the pH of the eluent and the detection
wavelength used. The best analytical conditions for separation of Ni(II), Co(II), and Cu(II) are shown in Table I, and this HPLC system using UV-VIS detector is shown in Figure 2.

In order to prevent contaminants from entering the HPLC system, all samples and eluents were prepared in clean glassware thoroughly rinsed with distilled water.

Peaks were manually collected and verified by I.C.P. No metal was present in system peaks.
Table I
Optimal Analytical Conditions for Separation of Ni(II), Co(II), Cu(II) Ions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
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<tr>
<td>Eluent</td>
<td>0.001M Na$_2$EDTA (pH=3.7)</td>
</tr>
<tr>
<td>Flow-rate:</td>
<td>2.0 ml/min</td>
</tr>
<tr>
<td>Chart Speed:</td>
<td>0.25 cm/min</td>
</tr>
<tr>
<td>Separation Column:</td>
<td>4.6 mm x 25 cm VYDAC Anion Separator Column</td>
</tr>
<tr>
<td>Attenuator:</td>
<td>2</td>
</tr>
<tr>
<td>Wavelength:</td>
<td>220 nm</td>
</tr>
<tr>
<td>Injection Volume of Sample Solution:</td>
<td>80 µL</td>
</tr>
<tr>
<td>Range:</td>
<td>0.08 AUFS</td>
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THE SCHEMATIC DIAGRAM OF HPLC USING UV-VIS DETECTOR
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CHAPTER III

RESULTS AND DISCUSSION

(1). **Effect of Wavelengths:**

Since the detection is made by UV-VIS detection, only those compounds having absorbance in this region of the spectrum will be detected; UV-VIS absorption detectors respond only to those substances that absorb UV-VIS light at the wavelength of the source light.

The relationship between the intensity of UV-VIS light transmitted through the cell and solute concentration is given by Beers' law:

\[ I_t = I_o e^{-k l c} \]

or \[ \ln \frac{I_t}{I_o} = -k l c \] \[\text{------------------ (a)},\]

where \( I_o \) is the intensity of the light entering the cell,
\( I_t \) is the light transmitted through the cell, 
\( l \) is the path length of the cell, 
\( c \) is the concentration of solute in the cell, 
\( k \) is the molar absorption coefficient of the solute for the specific wavelength of the UV light.

If equation (a) is put in the form \( I_t = I_o 10^{-k' l c} \),
then $k'$ is termed the molar extinction coefficient, and then $\log \frac{I_o}{I_t} = k'l_c = A$, and $\Delta A = K'l_ac$, where $A$ is the absorbance and $\Delta A$ is commonly employed to define the detection sensitivity (1).

To increase sensitivity for a particular sample, it is necessary to choose a suitable wavelength to get the largest absorbance. Figure 3 and 4 show the variation of peak heights of five metal ions at different wavelengths. From these figures, we can conclude that the best wavelength for separation of Cr(III), Fe(III), Ni(II), Co(II), and Cu(II) is 220 nm, and this wavelength is also best for Ca(II), Pb(II), Mn(II), Zn(II), and Cd(II). Although these wavelengths under 220 nm and above 180 nm can provide good results, increased noise that occurs in this region limits the suitability of these wavelengths for the transition metals studied.

Using the pH 3.7 eluent, Cr(III) and Fe(III) could not be separated because the peaks overlapped. However, Ni(II), Co(II), and Cu(II) could be separated. In contrast, in an eluent having pH of 4.3, Cr(III), Fe(III), Ni(II), and Cu(II) could be separated, but Ni(II), Co(II), and Cu(II) could not be separated.

Figure 5a and 5b show a separation of 1 ppm Ni(II), Co(II), and Cu(II) at wavelength = 220 and 240 nm respectively. From the comparison of these two figures,
Figure 3

Peak Heights of 10 ppm Cr(III), Fe(III), Ni(II), Co(II), Cu(II) at Different Wavelengths and the Following Conditions: (Eluent: 0.001 M, pH:3.7 Na₂EDTA, Flow-Rate: 2.5 ml/min, Range: 0.08 AUFS)
Peak Heights (cm) of 1 ppm Fe(III), Cr(III) at Different Wavelengths and Following Conditions: (Eluent: 0.002 M, pH:4.3 Na₂EDTA, Flow-Rate: 3.5 ml/min, At: 2, Range: 0.08 AUFS)
A Separation of 1 ppm Ni(II), Co(II), Cu(II) at Wavelength: 220 nm and 240 nm respectively for 5a and 5b and the Following Conditions: (Eluent: 0.001 M, pH:3.7, Na₂EDTA, Flow-Rate: 2.5 ml/min, Range: 0.08 AUFS, At: 2)
A Separation of 1 ppm Cr(III) and Fe(III) at Wavelength = 220 nm for 6a and Wavelength = 240 nm for 6b and the Following Conditions:

(Eluent: 0.002 M, pH=4.3 Na₂EDTA, Flow-Rate: 3.5 ml/min, Range: 0.08 AUFS, At: 2)
it appears that a wavelength of 220 nm is better than 240 nm for detection of Ni(II), Co(II), and Cu(II).

A separation of 1 ppm Cr(III) and Fe(III) at wavelength = 220 and 240 nm is shown in Figure 6a and 6b. Based on these figures, choosing a wavelength of 220 nm would appear better than 240 nm for the detection of Cr(III) and Fe(III).

(2). Effect of pH:

The successive macroscopic acid dissociation constants of EDTA, $H_4Y$, at 20° C and an ionic strength of 0.1, are $pK_1 = 2.0$, $pK_2 = 2.67$, $pK_3 = 6.16$, $pK_4 = 10.26$, as discussed by Laitinen and Harris (2). The fraction of EDTA as $H_4Y$, $H_3Y^-$, $H_2Y^{2-}$, $HY^{3-}$, $Y^{4-}$ can be calculated at any pH value by the following equations:

\[
\alpha_0 = \frac{[H_4Y]}{C_{H_4Y}} = \frac{[H^+]^4}{[H^+]^4 + K_1[H^+]^3 + K_1K_2[H^+]^2 + K_1K_2K_3[H^+] + K_1K_2K_3K_4}
\]

\[
\alpha_1 = \frac{[H_3Y^-]}{C_{H_4Y}} = \frac{K_1[H^+]^3}{[H^+]^4 + K_1[H^+]^3 + K_1K_2[H^+]^2 + K_1K_2K_3[H^+] + K_1K_2K_3K_4}
\]

\[
\alpha_2 = \frac{[H_2Y^{2-}]}{C_{H_4Y}} = \frac{K_1K_2[H^+]^2}{[H^+]^4 + K_1[H^+]^3 + K_1K_2[H^+]^2 + K_1K_2K_3[H^+] + K_1K_2K_3K_4}
\]

\[
\alpha_3 = \frac{[HY^{3-}]}{C_{H_4Y}} = \frac{K_1K_2K_3[H^+]}{[H^+]^4 + K_1[H^+]^3 + K_1K_2[H^+]^2 + K_1K_2K_3[H^+] + K_1K_2K_3K_4}
\]

\[
\alpha_4 = \frac{[Y^{4-}]}{C_{H_4Y}} = \frac{K_1K_2K_3K_4}{[H^+]^4 + K_1[H^+]^3 + K_1K_2[H^+]^2 + K_1K_2K_3[H^+] + K_1K_2K_3K_4}
\]
where $K_1$, $K_2$, $K_3$, $K_4$ are stepwise dissociation constants.
The fractions present as the anions $Y^4-$, $HY^3-$, $H_2Y^2-$, $H_3Y^-$ are very important in equilibrium calculations.
Values for $a_4$, $a_3$, $a_2$, $a_1$, are given in Table II, and formation constants $K_f$ and $Kfa_4$ (at pH = 3.7 and 4.3) of EDTA complexes of various samples are listed in Table III.

In terms of the major species present, the formation of metal-EDTA complexes for divalent and trivalent metals may be represented by the following equations:

$$M^{2+} + H_2Y^2- \rightarrow MY^2- + 2H^+$$
$$M^{3+} + H_2Y^2- \rightarrow MY^{-1} + 2H^+ \text{ (for pH=4 to 5), and}$$
$$M^{2+} + HY^3- \rightarrow MY^2- + H^+$$
$$M^{3+} + HY^3- \rightarrow MY^{-1} + H^+ \text{ (for pH=7 to 9).}$$

The elution behavior with EDTA as eluent can be analyzed as follows. The anion-exchange equilibrium is represented by

$$xS^{Y-} Ry + yE^{X-} \rightarrow yE^{X-} Rx + xS^{Y-} \text{ ----------- (b),}$$

where $E$ represents the eluent anion (EDTA), $S^{Y-}$ the sample anion, and $Ry$, $Rx$ the exchange sites on the resin used by the ions. The selectively coefficient, $K_s^E$ for the reaction is

$$K_s^E = \frac{[E^{X-}Rx]^y[S^{Y-}]^x}{[E^{X-}]^y[S^{Y-}Ry]^x}.$$
Table II
Fraction of EDTA as $Y^{4-}$, $HY^{3-}$, $H_{2}Y^{2-}$, $H_{3}Y^{-}$

<table>
<thead>
<tr>
<th>pH</th>
<th>$a_4$</th>
<th>$a_3$</th>
<th>$a_2$</th>
<th>$a_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.0</td>
<td>3.4</td>
<td>3.7</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>$2.5 \times 10^{-11}$</td>
<td>$2.01 \times 10^{-10}$</td>
<td>$8.69 \times 10^{-10}$</td>
<td>$3.6 \times 10^{-9}$</td>
</tr>
<tr>
<td></td>
<td>$4.57 \times 10^{-4}$</td>
<td>$1.46 \times 10^{-3}$</td>
<td>$3.16 \times 10^{-3}$</td>
<td>$6.56 \times 10^{-3}$</td>
</tr>
<tr>
<td></td>
<td>$6.6 \times 10^{-1}$</td>
<td>$8.36 \times 10^{-1}$</td>
<td>$9.1 \times 10^{-1}$</td>
<td>$9.49 \times 10^{-1}$</td>
</tr>
<tr>
<td></td>
<td>$3.1 \times 10^{-1}$</td>
<td>$1.56 \times 10^{-1}$</td>
<td>$8.5 \times 10^{-2}$</td>
<td>$4.4 \times 10^{-2}$</td>
</tr>
</tbody>
</table>
### Table III

Formation Constants Log $K_f$ and $K_{fa4}$ of EDTA Complexes at 20° C, Ionic Strength 0.1

<table>
<thead>
<tr>
<th>Metal Ion</th>
<th>Log $K_{MY}$</th>
<th>$K_{fa4}$ at pH 3.7</th>
<th>$K_{fa4}$ at pH 4.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr (III)</td>
<td>23</td>
<td>8.69x10^{13}</td>
<td>1.46x10^{15}</td>
</tr>
<tr>
<td>Fe (III)</td>
<td>25.1</td>
<td>1.09x10^{16}</td>
<td>1.84x10^{17}</td>
</tr>
<tr>
<td>Ca (II)</td>
<td>10.7</td>
<td>4.36x10^{1}</td>
<td>7.32x10^{1}</td>
</tr>
<tr>
<td>Cd (II)</td>
<td>16.5</td>
<td>2.75x10^{7}</td>
<td>4.62x10^{8}</td>
</tr>
<tr>
<td>Ni (II)</td>
<td>18.6</td>
<td>3.46x10^{9}</td>
<td>5.81x10^{10}</td>
</tr>
<tr>
<td>Zn (II)</td>
<td>16.5</td>
<td>2.75x10^{7}</td>
<td>4.62x10^{8}</td>
</tr>
<tr>
<td>Mn (II)</td>
<td>14.0</td>
<td>8.69x10^{4}</td>
<td>1.46x10^{6}</td>
</tr>
<tr>
<td>Co (II)</td>
<td>16.3</td>
<td>1.73x10^{7}</td>
<td>2.91x10^{8}</td>
</tr>
<tr>
<td>Pb (II)</td>
<td>18.0</td>
<td>8.69x10^{8}</td>
<td>1.46x10^{10}</td>
</tr>
</tbody>
</table>
At low loading of sample ion, the resin capacity $\text{[Cap]}$, is approximately $[E^{x^-}R_x^y]$, and the capacity factor $K = \frac{[S^yR_y]}{[S^y^-]}$, and then $K_{E} = \frac{[\text{Cap}]^y}{[E^{x^-}]^yR_x^y}$.

The adjusted retention time, $t$, for an eluted peak is equal to $t_0K$, where $t_0$ is the retention time of an unretained substance. Substituting $t/t_0$ for $K$ and taking logarithms gives
\[
\log t = \frac{y}{x} \log \text{[Cap]} - \frac{y}{x} \log [E^{x^-}] + \log t_0 - \frac{1}{x} \log K_{E}
\]
or
\[
\log t = -\frac{y}{x} \log [E^{x^-}] - \text{constant}.
\]

It has been indicated that the charge on the eluent anion is -3.0 by linear plots of $\log t$ versus $\log [\text{EDTA}]$ (3). Thus eqn. (b) for divalent metal complex anions can be written as
\[
3 \text{MEDTA}^2^- R_2 + 2 \text{HEDTA}^3^- 2 \text{HEDTA}^3^- R_3 + 3 \text{MEDTA},
\]
and for trivalent metal complex anions the eqn. (b) can be written as
\[
3 \text{MEDTA}^{-1} R + \text{HEDTA}^3^- \text{HEDTA}^3^- R_3 + 3 \text{MEDTA}^{-1}
\]
The retention times decreased with increasing pH owing to greater complexing ability of eluent at higher pH, which increases the percentage of HEDTA$^3^-$, and as the pH was lowered, the retention time became longer. Table IV, V, and VI list the retention times for five ions using different pH eluents.

From Table V, the retention times decreased as eluent pH decreased after eluent pH lower than 3.75.
Table IV
Retention Times (Min) of Three 10 ppm Metal Ions Using Different pH Eluents and the Following Conditions: 
(Eluent: 0.001M Na₂EDTA, λ: 420 nm, Range: 0.08 AUFS, Flow-Rate: 2.5 ml/min, At: 2)

<table>
<thead>
<tr>
<th>pH Ion</th>
<th>4.3</th>
<th>4.0</th>
<th>3.8</th>
<th>3.6</th>
<th>3.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni (II)</td>
<td>16.8</td>
<td>20.1</td>
<td>20.2</td>
<td>19.2</td>
<td>18.4</td>
</tr>
<tr>
<td>Co (II)</td>
<td></td>
<td>21.8</td>
<td>22.0</td>
<td>21.1</td>
<td>19.1</td>
</tr>
<tr>
<td>Cu (II)</td>
<td>18.2</td>
<td>23.1</td>
<td>23.6</td>
<td>23.2</td>
<td>20.1</td>
</tr>
</tbody>
</table>
Table V
Retention Times (Min) of Five 1 ppm Metal Ions
Using Different pH and the Following Conditions:
(Eluent: 0.001 M Na₂EDTA, λ: 220 nm, FR: 2.5 ml/min,
Range: 0.08 AUFS).

<table>
<thead>
<tr>
<th>Ion</th>
<th>pH 3.8</th>
<th>pH 3.75</th>
<th>pH 3.65</th>
<th>pH 3.56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr (III)</td>
<td>5.2</td>
<td>4.8</td>
<td>4.4</td>
<td>4.2</td>
</tr>
<tr>
<td>Fe (III)</td>
<td>5.2</td>
<td>4.8</td>
<td>4.4</td>
<td>4.2</td>
</tr>
<tr>
<td>Ni (II)</td>
<td>20.8</td>
<td>20.9</td>
<td>20.7</td>
<td>20.4</td>
</tr>
<tr>
<td>Co (II)</td>
<td>23.1</td>
<td>23.1</td>
<td>22.9</td>
<td>22.8</td>
</tr>
<tr>
<td>Cu (II)</td>
<td>24.4</td>
<td>24.6</td>
<td>24.5</td>
<td>24.1</td>
</tr>
</tbody>
</table>
Table VI

Retention Time (Min) of Five 1 ppm Metal Ions Using Different pH and the Following Conditions:
(Eluent: 0.0015M, Na₂EDTA, λ: 220 nm, Range: 0.08 AUFS, Flow-Rate: 2.0 ml/min, At:2)

<table>
<thead>
<tr>
<th>pH</th>
<th>Cr (III)</th>
<th>Fe (III)</th>
<th>Ni (II)</th>
<th>Co (II)</th>
<th>Cu (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.57</td>
<td>4.8</td>
<td>4.6</td>
<td>18.3</td>
<td>20.2</td>
<td>21.7</td>
</tr>
<tr>
<td>3.47</td>
<td>4.2</td>
<td>4.4</td>
<td>18.0</td>
<td>19.9</td>
<td>21.3</td>
</tr>
<tr>
<td>3.44</td>
<td>4.6</td>
<td>4.4</td>
<td>17.8</td>
<td>19.7</td>
<td>21.1</td>
</tr>
<tr>
<td>3.36</td>
<td>4.2</td>
<td>4.4</td>
<td>17.4</td>
<td>19.2</td>
<td>20.3</td>
</tr>
<tr>
<td>3.27</td>
<td>4.6</td>
<td>4.6</td>
<td>17.0</td>
<td>19.2</td>
<td>19.8</td>
</tr>
</tbody>
</table>
This means that considerable dissociation of \( \text{MEDTA}^{2-} \) (\( \text{MEDTA}^{2-} + \text{M}^{2+} + \text{EDTA}^{4-} \)) occurred below pH 3.75. These transition metals with stability constants greater than 10.0 are stable at pH 4.5 \( (3) \).

From Table IV, V, and VI, Ni(II), Co(II), and Cu(II) can be separated under conditions of either 0.001 M disodium EDTA, pH about 3.8 to 3.6 eluent or 0.0015 M disodium EDTA, pH about 3.6 to 3.4 eluent. However, Fe(III) and Cr(III) could not be separated under these conditions.

Figure 7a and 7b show the separation of Ni(II), Co(II), and Cu(II) with eluents at different pH values (3.75 and 3.47) and the following conditions:

(Eluent: 0.0015 M disodium EDTA, wavelength: 220 nm, Range: 0.08 AUFS, Flow-Rate: 2.0 ml/min, AT: 2).

Figure 7c shows that Ni(II), Co(II), and Cu(II) could not be separated by using 0.0015 M disodium EDTA at pH of 3.27 using a flow-rate of 2.0 ml/min. There is a possibility of achieving more rapid separation by using a higher eluent concentration and a larger column \( (4) \).

Figure 9 shows that Cr(III) and Fe(III) have nearly the same retention time and could not be separated by using 0.0015 M disodium EDTA, pH: 3.37 eluent, and flow-rate: 1.0 ml/min.
Figure 7

The Separation of Ni(II), Co(II), and Cu(II) with 0.0015 M Na₂EDTA Eluent at Different pHs [7a:3.75, 7b:3.47, and 7c:3.27] and the Following Conditions:
(Wavelength: 220 nm, Range: 0.08 AUFS, Flow-Rate: 2.0 ml/min, At: 2)
The separation of 2 ppm Ni(II) and 1 ppm Co(II), Cu(II) at the following conditions: (Eluent: 0.0015 M Na$_2$EDTA, pH: 3.37, wavelength: 220 nm, Flow-Rate: 1 ml/min, At: 2)
The Separation of 10 ppm Fe(III), Cr(III), and 1 ppm Ni(II), Co(II), and Cu(II) at the Following Conditions:
(Eluent: 0.0015 M, pH: 3.37 Na₂EDTA, Wavelength: 220 nm, Flow-Rate: 1 ml/min, At: 2)
(3). **Detection Limits and Retention Times for the Eight Transition Metals and Two Metals Studied:**

Many metal EDTA complexes are colorless, such as \( \text{ZnEDTA}^2- \), \( \text{PbEDTA}^2- \), \( \text{CaEDTA}^2- \), \( \text{MnEDTA}^2- \), and \( \text{CdEDTA}^2- \), which have been used in this experiment. However, each exhibited strong absorption in the UV-VIS region of the spectrum in this experiment. The disodium EDTA used results in a much weaker absorbance than the metal EDTA-complexes, so that a stable baseline could be established. Some inorganic anions such as \( \text{SO}_4^{2-} \) appear as negative peaks owing to their lower absorbance relative to the disodium EDTA eluent. Other anions such as nitrate ion appear as positive peaks, with shorter retention times than the metal ion peaks.

Table VII compares the retention times and detection limits for the ten ions with the UV-VIS detection. The detection limits are defined as signal = three times the baseline noise. The elution of all metal cations listed was verified by collecting the separated fraction and analyzing the ionic complex with I.C.P. (Perkin-Elmer ICP/5500).

From Table VII, it is possible to separate Fe(III), Cr(III), Ni(II), Ca(II), Cu(II) and to separate Ca(II), Zn(II), Pb(II) at the following conditions: [Eluent: 0.0015 M disodium EDTA (pH:4.3), Flow-rate: 2.0 ml/min, AT: 2, Range: 0.08 AUFS, Wavelength: 220 nm].
Table VII

Retention Times and Detection Limits with UV-VIS Detector at Following Conditions: (Eluent: 0.0015 M, pH: 4.3, Na$_2$EDTA, Flow-Rate: 2.0 ml/min, At: 2, Range: 0.08 AFS, $\lambda$: 220 nm)

<table>
<thead>
<tr>
<th>Ion</th>
<th>Retention Time (Min)</th>
<th>Detection Limit (ppm)</th>
<th>Detection Limit (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr (III)</td>
<td>4.1</td>
<td>0.1</td>
<td>8</td>
</tr>
<tr>
<td>Fe (III)</td>
<td>6.5</td>
<td>0.07</td>
<td>5.6</td>
</tr>
<tr>
<td>Ca (II)</td>
<td>10.5</td>
<td>4</td>
<td>320</td>
</tr>
<tr>
<td>Cd (II)</td>
<td>15.2</td>
<td>30</td>
<td>2400</td>
</tr>
<tr>
<td>Ni (II)</td>
<td>15.4</td>
<td>0.2</td>
<td>16</td>
</tr>
<tr>
<td>Zn (II)</td>
<td>15.5</td>
<td>10</td>
<td>800</td>
</tr>
<tr>
<td>Mn (II)</td>
<td>16.0</td>
<td>0.75</td>
<td>60</td>
</tr>
<tr>
<td>Co (II)</td>
<td>16.9</td>
<td>0.3</td>
<td>24</td>
</tr>
<tr>
<td>Pb (II)</td>
<td>17.5</td>
<td>0.75</td>
<td>60</td>
</tr>
<tr>
<td>Cu (II)</td>
<td>17.6</td>
<td>0.2</td>
<td>8</td>
</tr>
</tbody>
</table>
Figures 10 and 11 show the separation of Fe(III), Cr(III), Ca(II), Ni(II), Cu(II) and the separation of Ca(II), Zn(II), Pb(II). The elution sequence is dependent on the ionic charge and ionic radius of the metal complex ions. In general, monovalent ions are eluted before divalent ions. Fe(III) and Cr(III) form metal complexes $\text{MEDTA}^{-1}$, while divalent metal ions form $\text{MEDTA}^{-2}$ complexes. Consequently, Fe(III) and Cr(III) are eluted before divalent metal ions because they are less charged than the divalent complexes.

The relationship between Cr(III) concentrations and (peak heights) x (ranges) of the Cr-EDTA complexes is shown in Figure 12. The linear range of chromium is 0.1 to 200 ppm. Since the different ranges are used, it is necessary to modify the peak heights by the peak height x range.

Because the reaction between metal and 0.0015 M disodium EDTA eluent results in small peaks that are difficult to quantify, adding high concentrations of disodium EDTA to the sample before injection will increase peak heights. These results are shown in Table VIII and Figure 13.

The precision in Ni based upon the injection of five samples of Ni is 1%.
Chromatogram Obtained for 4 ppm Fe(III), Cr(III), Ni(II), Co(II), Cu(II) and 20 ppm Ca(II) at the Following Conditions: (Eluent: 0.0015 M, pH: 4.3 Na$_2$EDTA, Flow-Rate: 2 ml/min, Wavelength: 220 nm, At: 2)
Figure 11

Chromatogram Obtained for 20 ppm Ca(II), Pb(II), and 100 ppm Zn(II) at the Following Conditions: (Eluent: 0.0015 M Na₂EDTA (pH:4.3), Flow-Rate: 2 ml/min, Wavelength = 220 nm, At: 2)
Figure 12

Range X Peak Heights (cm) of Different Concentration (ppm) Cr(III) and the Following Conditions: (Eluent: 0.0015 M, pH: 4.3, Na$_2$EDTA, Flow-Rate: 2.0 ml/min, At: 2, Wavelength = 220 nm)
Table VIII

Peak Heights (cm) of 20 ppm Cr(III) Prepared at Different Concentrations Na₂EDTA Solvent and the Following Conditions: [Eluent: 0.0015M, Na₂EDTA (pH: 4.3), Flow-Rate: 2.0 ml/min, At: 2, λ: 220 nm]

<table>
<thead>
<tr>
<th>Range</th>
<th>0.5</th>
<th>0.5</th>
<th>0.5</th>
<th>0.1</th>
<th>0.1</th>
<th>0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Height (cm)</td>
<td>7.1</td>
<td>2.15</td>
<td>1.92</td>
<td>3.35</td>
<td>2.45</td>
<td>0.9</td>
</tr>
<tr>
<td>Range x Peak Height</td>
<td>3.55</td>
<td>1.075</td>
<td>0.96</td>
<td>0.335</td>
<td>0.245</td>
<td>0.09</td>
</tr>
<tr>
<td>Na₂EDTA Concentration</td>
<td>0.05M</td>
<td>0.01M</td>
<td>0.005M</td>
<td>0.001M</td>
<td>0.0005M</td>
<td>0.0001M</td>
</tr>
</tbody>
</table>
Figure 13

Peak Heights (cm) of 20 ppm Cr(III) Prepared at different Concentrations 
Na$_2$EDTA Solvent and the Following Conditions: (Eluent: 0.0015 M 
Na$_2$EDTA (pH:4.3), Flow-Rate: 2.0 ml/min, At: 2, Wavelength = 220 nm)
CHAPTER BIBLIOGRAPHY


CHAPTER IV

CONCLUSION

In comparison to a conductivity detector, UV-VIS detection in HPLC analysis of metal ions using a low-capacity anion exchange column and a disodium EDTA solution as the eluent was shown to improve peak identification. In contrast to the post-column PAR method, this method has the following advantages: it is inexpensive, it is simple, and it can be applied to a wide variety of metals. This method also offers a wide range of sensitivities for the determination of the transition metal ions, but the retention time of the PAR complex method is shorter than the method used in this study because different eluents were used.

The effect of several parameters was investigated, including eluent pH and detector wavelength, in order to optimize the detection limit for each of the transition metals studied, as well as to optimize the separation of the various metal ions. Results demonstrated that the detection limits could be as low as the nanogram level at wavelength = 220 nm. Cr(III), Fe(III), Ca(II), Ni(II), and Cu(II) could be separated in one injection, as could
Ca(II), Zn(II), and Pb(II), using 0.0015 M eluent at a pH of 4.3. If the eluent was changed to 0.001 M disodium EDTA at a pH of 3.7, then Ni(II), Co(II), and Cu(II) could be separated in one injection at wavelength = 220 nm.

At the pH of 3.7 the percentages of each anion of the EDTA species are $8.69 \times 10^{-8}\%$ EDTA$^{4-}$, $3.156 \times 10^{-1}\%$ HEDTA$^{3-}$, $91\%$ H$_2$EDTA$^{2-}$, and $8.5\%$ H$_3$EDTA$^{-1}$. While at the pH of 4.3, the corresponding percentages are $1.46 \times 10^{-6}\%$, $1.331\%$, $96.4\%$, and $2.298\%$ respectively. The results indicate that the retention times increased with decreasing pH, which decreases the percentage of HEDTA$^{3-}$ and EDTA$^{4-}$, and therefore increases the separation of Ni(II), Co(II), and Cu(II) until pH about 3.56 for 0.001 M disodium EDTA and until pH about 3.27 for 0.0015 M disodium EDTA. The ratio of the adjusted retention times shows that divalent analyte anions are more influenced by an eluent pH change than are monovalent analyte anions.

To further increase the sensitivity and separation ability of this method, a higher concentration of eluent and longer column might be used. Furthermore, decreasing the particle size of packing material of the column, using preconcentration techniques, or adding a high concentration of disodium EDTA to the sample before injection may also improve this method.
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