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Modification of 4-(2-pyridylazo)-resorcinol postcolumn reagent selectivity through competitive equilibria with chelating ligands

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Abstract

Aminopolycarboxylate ligands were added to the 4-(2-pyridylazo)-resorcinol (PAR) postcolumn reagent to alter the reagent selectivity towards transition metals. Addition of ethylenediaminetetraacetic acid (EDTA) completely suppressed the reaction between PAR and the metal ions. Addition of 0.1 mM nitrilotriacetic acid (NTA) to 1 mM PAR lowered the response to specific transition metal ions, but completely suppressed the PAR response to the lanthanides. Increasing the NTA concentration to 8 mM resulted in complete suppression of the PAR response to all metal ions except Cu^{2+} and Co^{2+} , for which the detection limits were 3 and 1 ng, respectively. The observed selectivity results from the slow rate of conversion of metal ions from the $\text{M}(\text{NTA})_2^{4-}$ form to $\text{M}(\text{PAR})_2$.

Keywords: Derivatization, LC; Complexation; Pyridylazoresorcinol; Metal cations

1. Introduction

Postcolumn reaction detection is the most common means of detecting metal cations in ion chromatography [1–3]. In postcolumn reaction detection, analytes eluting from a chromatographic column are mixed with a flowing stream of reagent. The reaction product is then monitored by absorbance or fluorescence. An ideal postcolumn reagent will possess a number of characteristics. The reaction between the analyte and reagent should be rapid, so as to maximize the concentration of product and minimize extra-column band broadening. The product of the reaction should be strongly absorbing ($\epsilon_{\text{max}} > 10\,000$) at a wavelength at which the reagent does not absorb. For metal ion detection, this means that the reagent should undergo a strong bathochromic shift upon complexation with a metal ion. Finally, since metal separations in ion chromatography typically

use complexing eluents such as tartaric acid and α -hydroxyisobutyric acid (HIBA), the postcolumn reagent must form a very strong complex in order to displace the eluent ligand from the metal.

Of the many reagents [4–7] used for postcolumn reaction detection of metal ions, the most common is 4-(2-pyridylazo)-resorcinol (PAR). PAR is a broad spectrum reagent that responds to transition metals, lanthanides and actinides. The properties of PAR and its metal complexes have been discussed in detail in the literature [8,9]. Typical postcolumn reagent conditions are 0.2 mM PAR in 2 M ammonia and 1 M ammonium acetate. The use of lower pH has been noted to allow postcolumn reaction with metals which are subject to hydroxide formation at high pH [10]. The reasons for PAR's widespread use as a postcolumn reagent in ion chromatography are its sensitivity (typically 1–20 ng) and its versatility.

PAR forms complexes with Mn^{2+} , Fe^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Pb^{2+} , lanthanides and uranium, which absorb strongly in the 500–540

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nm range. However, the response of PAR can be quickly altered by the addition of secondary equilibria into the postcolumn chemistry. The most widely quoted example of such a system is the Zn–EDTA–PAR reagent [11,12]. In this reagent, the addition of equimolar concentrations of Zn^{2+} and EDTA to a solution containing PAR extends the applicability of the reagent to include the alkaline earth metals. Alternatively, the addition of complexing agents to a PAR postcolumn reagent can be used to selectively mask the PAR response to one or more metal ions. Cassidy and Elchuk used this procedure to monitor trace metal impurities in UO_2 by masking the PAR response to UO_2^{2+} with carbonate [13].

The present work discusses the addition of aminopolycarboxylate ligands as masking agents to PAR solutions, to alter the selectivity of PAR postcolumn reagents. Specifically, the addition of ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetic acid (NTA) to postcolumn reagents containing PAR will be examined.

2. Experimental

2.1. Apparatus

The HPLC system consisted of a Waters metal-free delivery system (Model 625, Waters Associates, Milford, MA, USA), a Rheodyne injection valve (Model 9125, Rheodyne, Berkeley, CA, USA) fit with a 50- μ l loop and a fixed-wavelength (546 nm) absorbance detector (Model 440, Waters). The analytical column was a 150 mm \times 4.6 mm I.D. reversed-phase column (5 μ m; Prodigy, Phenomenex). Data acquisition was performed with PeakSimple III (SRI) on a 386-based microcomputer.

The postcolumn reagent was delivered by constant pressure pumping supplied by application of helium pressure (24 p.s.i.) to a multireagent cylinder [14,15] fit with a six-port low-pressure switching valve (Model 5011, Rheodyne) to allow selection between up to six reagents (1 p.s.i.=6894.76 Pa). Low-pressure PTFE tubing and fittings (Alltech) connected the postcolumn reagent delivery system to the column effluent stream. The carrier stream from the HPLC pump (1.0 ml/min) merged with the postcolumn reagent (0.2–0.3 ml/min, measured for each experi-

ment) at the mixing tee (P/N P-712, Upchurch) and then flowed through 45 cm \times 0.010 in. I.D. polyether ether ketone (PEEK) tubing to the detector flow cell (1 in.=2.54 cm). All postcolumn reactions were performed at room temperature ($\sim 22^\circ\text{C}$).

2.2. Reagents and standards

All reagent solutions were prepared using distilled deionized water (Nanopure Water System, Barnsted). Analytical grade reagents were used throughout. Postcolumn reagents were prepared by dissolving PAR (Janssen Chimica) or PAR and NTA (Eastman) in 2 M ammonium hydroxide (BDH) buffer solution. Ammonium hydroxide and acetic acid (BDH) were used to adjust the pH of the postcolumn reagents. All PAR and PAR–NTA solutions were stored in plastic bottles and in the dark [12].

Five lanthanides (Dy^{3+} , Er^{3+} , Gd^{3+} , Lu^{3+} , Sm^{3+}) and five transition metals (Cd^{2+} , Co^{2+} , Cu^{2+} , Mn^{2+} , Ni^{2+}) were tested. Stock metal solutions ($1\cdot 10^{-5}$ M) were prepared using analytical grade salts. Lower concentrations were achieved by dilution in volumetric Nalgeware. Standard solutions were stored in Nalgene bottles.

Eluent was prepared by dissolving tartaric acid (BDH) in deionized water and adjusting the pH to 3.4 with NaOH. 1-Octanesulfonic acid ($C_8SO_3^-$) eluent was prepared by dissolving its salt (Sigma) in deionized water. All eluents and standards were vacuum filtered through 0.45 μ m nylon filters just prior to use.

2.3. Procedures

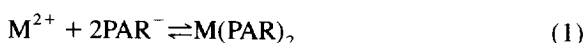
Metal ions were separated using a reversed-phase column converted into a dynamic ion-exchange column by inclusion of 2.6 mM $C_8SO_3^-$ in the 0.11 M tartaric acid eluent (pH 3.4) [16]. The column was equilibrated with eluent for at least 20 min prior to sample injection. The concentration of PAR in the postcolumn reagent was constant at 1.0 mM, while the NTA concentration ranged from 0.1 to 16.0 mM. The pH of the postcolumn reagent was adjusted from 9.0 to 11.7.

Kinetic measurements were performed on a Hewlett-Packard Model 8452A photodiode array UV–

visible spectrophotometer using the Kinetic mode of the HP 89532A control software. The NTA–PAR reagent was rapidly added (mixing time ~2 s) to the metal sample within a 1.0 cm cuvette, and then data acquisition was initiated. Data acquisition rates from 0.17 to 1 Hz were used, depending on the metal.

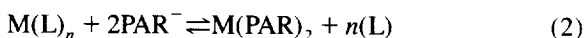
3. Results

PAR can form either 1:1 or 1:2 complexes with metal ions. Based on literature stability constants [8], the transition metal ions were fully in the 1:2 complex form under the conditions used herein:



It is the 1:2 complex which causes the red or red–purple color which is observed. While stability constants are not available for the lanthanides, Sandell and Onishi do indicate that 1:2 lanthanide:PAR complexes are formed [9]. Thus, it is reasonable to assume that Eq. (1) illustrates the reaction in a typical PAR postcolumn reaction system.

Addition of a chelating ligand (L) to the postcolumn reagent introduces a competitive equilibria, such that the overall equilibrium is:

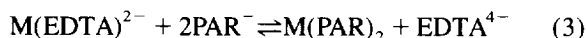


The selectivity of the postcolumn reagent now depends on the relative stabilities of the metal ions with the ligand and with PAR. Those metals for which the ligand forms a more stable complex will be masked, i.e., not detected. As stated by Perrin [17], “the best ligands to use as masking agents would be those which form strong, colorless complexes with the ions to be masked.” In this work, ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetic acid (NTA) were tested as masking agents in PAR postcolumn reagents.

3.1. EDTA

When EDTA was used as the competitive chelating ligand in the PAR postcolumn reagent, no response was observed for any of the transition

metals (Cd^{2+} , Co^{2+} , Cu^{2+} , Mn^{2+} , Ni^{2+}) or lanthanides studied. These results were somewhat surprising. The equilibrium for the distribution of the metal ions between PAR and EDTA in the postcolumn reagent is:



The overall conditional equilibrium constant for Eq. (3) is significant for each of the transition metal ions, as can be seen in Table 2. As such, the equilibrium should favor formation of the metal–PAR complex, and so a response should be observed. The reason for the lack of response in this case will be discussed in Section 4.

3.2. NTA

A classical colorimetric procedure using NTA with PAR was observed to respond to a number of metal ions [18]. Thus NTA should be suitable for modifying the selectivity of the PAR postcolumn reagent. Table 1 shows the response for six metal ions to the addition of zero to 16 mM NTA to 1 mM PAR in 2 M ammonia adjusted to pH 10.0. Samarium, and all other lanthanide metal ions tested, was completely suppressed by addition of as little as 0.1 mM NTA. The PAR response to Ni^{2+} , Cd^{2+} and Mn^{2+} also decreases significantly upon addition of 0.1 mM NTA, although 8 mM NTA was required to fully suppress their response. Co^{2+} and Cu^{2+} show distinctly different behavior than the other metals studied. Their response remains almost constant upon addition of NTA to the PAR reagent.

Fig. 1 presents the effect of pH on the response of Cu^{2+} and Co^{2+} to a postcolumn reagent of 1 mM PAR with 8 mM NTA in 2 M ammonia. The behavior observed for these metal ions mirrors that observed for the PAR reagent alone.

3.3. Application

The NTA–PAR postcolumn reagent can be used to suppress the response of metal ions which might overlap with the analytes of interest. Fig. 2 compares the response observed for a mixture of metal ions using the PAR and the NTA–PAR postcolumn reagents. When PAR is used as the postcolumn

Table 1
Effect of added NTA on the response of the PAR postcolumn reagent^a

Concentration (mM) NTA with 1 mM PAR	Peak area					
	Cd ²⁺	Co ²⁺	Cu ²⁺	Mn ²⁺	Ni ²⁺	Sm ³⁺
0	296	592	431	460	252	136
0.1	211	771	499	311	114	13
0.5	85	769	446	129	34	3
1.0	33	713	365	67	18	-7
2.0	19	596	353	36	29	-2
5.0	12	480	303	23	31	6

^a Conditions: eluent, 1.0 ml/min 0.11 M tartaric acid at pH 3.4 and 0.0026 M 1-octanesulfonic acid; reagent, 0.2 ml/min 1 mM PAR and indicated concentration of NTA in 2 M ammonia adjusted to pH 10.0; sample, $1 \cdot 10^{-5}$ M of each metal.

reagent, the response due to lanthanides interferes with determination of early eluting metal ions such as Cu²⁺. Addition of NTA to the postcolumn reagent eliminates this interference.

Calibration curves were generated for $1.0 \cdot 10^{-6}$ to $1.0 \cdot 10^{-5}$ M Cu²⁺ and Co²⁺ using the conditions shown in Fig. 2. The calibrations were rectilinear (Cu²⁺: slope = $4.6 \pm 0.4 \cdot 10^7$; intercept = 3 ± 23 ; $r = 0.99$. Co²⁺: slope = $7.2 \pm 0.3 \cdot 10^7$; intercept = 91 ± 17 ; $r = 0.998$). Detection limits were 3 ng for Cu²⁺ and 1 ng for Co²⁺. These are comparable to those achieved using PAR alone.

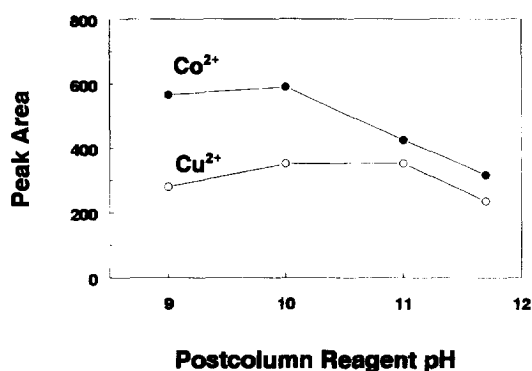


Fig. 1. Effect of pH on the response of the NTA–PAR postcolumn reagent. Conditions: eluent, 1.0 ml/min 0.11 M tartaric acid at pH 3.4 and 0.0026 M 1-octanesulfonic acid; reagent, 0.2 ml/min 1 mM PAR and 8 mM NTA in 2 M ammonia adjusted to pH indicated; sample, $1 \cdot 10^{-5}$ M of each metal.

4. Discussion

The complexation kinetics for NTA with a metal ion are much more rapid than between PAR and the metal ion [19]. Thus, upon addition of the NTA–PAR postcolumn reagent, the metal ions will form a metal–NTA complex. Under the reaction conditions used in Table 1, the NTA complexes would be predominantly in the $M(NTA)_2^{4-}$ form rather than the $M(NTA)^-$ form. Therefore the competitive equilibria can be written as:

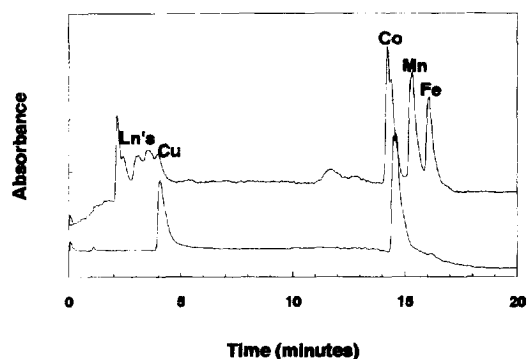


Fig. 2. Response of PAR (upper trace) and NTA–PAR (lower trace) postcolumn reagents to metal ions. Conditions: eluent, 1.0 ml/min 0.11 M tartaric acid at pH 3.4 and 0.0026 M 1-octanesulfonic acid; reagent, 0.2 ml/min 1 mM PAR or 1 mM PAR and 8 mM NTA in 2 M ammonia adjusted to pH 10; sample, $1 \cdot 10^{-5}$ M each of Lu³⁺, Er³⁺, Dy³⁺, Gd³⁺, Sm³⁺, Cu²⁺, Ni²⁺, Co²⁺, Mn²⁺, Cd²⁺ and Fe²⁺.

The overall conditional stability constant for equilibria 3 is given by:

$$K' = \frac{\beta_{2,M(PAR)_2} \alpha_{PAR^-}^2}{\alpha_{M^{2+}}} \frac{\alpha_{M^{2+}}}{\beta_{2,M(NTA)_2} \alpha_{NTA^{3-}}^2} = \frac{\beta_{2,M(PAR)_2} \alpha_{PAR^-}^2}{\beta_{2,M(NTA)_2} \alpha_{NTA^{3-}}^2} \quad (5)$$

where β_2 are the cumulative formation constants (i.e., $K_{f,1} K_{f,2}$) for the metal–PAR and metal–NTA complexes, α_{PAR^-} and $\alpha_{NTA^{3-}}$ are the fraction of PAR and NTA in the specific ionic form indicated based on literature acid dissociation constants [8,22] (at pH 10 $\alpha_{PAR^-} = 1.00$ and $\alpha_{NTA^{3-}} = 0.69$), and $\alpha_{M^{2+}}$ is the fraction of metal ion present in the free form. The fraction of metal ion in the free form factors out of Eq. (4), and so is not considered further. Values for the overall conditional equilibrium constant for the NTA–PAR postcolumn reagent were calculated for the conditions used in Fig. 1. The results of these calculations are presented in the second column of Table 2. No cumulative stability constants were available for the lanthanide–PAR complexes. Therefore the lanthanides are omitted from Table 2.

For each of the transition metal ions, the competitive equilibria with NTA is thermodynamically favored to produce $M(PAR)_2$. That is, all of the transition metal ions should respond to the NTA–PAR reagent. Thus, as with the EDTA–PAR reagent, fundamental equilibrium calculations fail to explain the selectivity observed. This failure is consistent with Perrin's [17] statement that such quantitative

treatments are hampered by many of the equilibrium constants not being known with sufficient accuracy, particularly under the relevant ionic strength conditions. Perrin [17] also notes that mixed ligand–complexes may form. However, the only literature reference to such a mixed ligand complex [nickel(ethylenediaminediacetic acid)(PAR)] noted that the complex containing one PAR molecule possessed a molar absorptivity equal to half that of the $M(PAR)_2$ complex [20]. Thus, formation of a mixed ligand complex could be expected to half the observed response, but cannot not completely suppress the response.

The cause of the unexpected selectivity for EDTA and NTA may lie not in the thermodynamics or problems with the thermodynamic data, but rather in the kinetics of the ligand exchange reaction. The kinetics of exchange of a metal ion between two chelating ligands has been extensively studied [21]. Under favorable conditions, ligand–ligand exchange rates can be rapid. However, previously reported rate constants for the exchange of Ni^{2+} between NTA and PAR were much slower than required in post-column reaction detection [20].

To determine if slow ligand–ligand exchange kinetics was the cause of the observed selectivity (Table 1), kinetic studies were conducted using a spectrophotometer. It is estimated that 2 s were required to mix the reagents and initiate data collection. Thus, the most rapid reaction which might be monitored would have a half-life on the order of a second. The initial half-lives observed for conditions comparable to the use of a postcolumn reagent of 1

Table 2

Thermodynamic and kinetic characteristics associated with modification of the selectivity of PAR postcolumn reagents by addition of chelating agents^a

Metal ion	$\log K' \text{ (EDTA)}^b$ (Eq. (3))	$K' \text{ (NTA)}^b$ (Eq. (4))	Half-life of response ^c (NTA)
Cu ²⁺	5.0	6.3	<1 s
Cd ²⁺	5.6	7.5	25 000 s
Co ²⁺	1.2	3.1	<1 s
Mn ²⁺	5.5	8.3	8100 s
Ni ²⁺	7.4	10.0	60 s

^a Experimental conditions: $8.3 \cdot 10^{-6}$ M metal ion; $1.7 \cdot 10^{-4}$ M PAR; $1.3 \cdot 10^{-3}$ M NTA; 0.33 M ammonia; pH 10.

^b Stability constants for PAR are from Ref. [8] and those for the aminopolycarboxylates are from Ref. [22].

^c The reactions did not follow any simple order kinetics. Therefore, only the initial half-life for the reaction is reported.

mM PAR and 8 mM NTA are presented in Table 2. Co^{2+} and Cu^{2+} displayed rapid reactions in the presence of NTA, and were essentially complete before kinetic measurements could be initiated (i.e., half-life <1 s). Given a reaction time of approximately 1.1 s in our postcolumn system, this rate is sufficient to ensure that the reaction proceeds almost to completion prior to detection.

For the rest of the metal ions reported in Table 2, the rates are far too slow to yield a response in a postcolumn reaction detector. Thus, the selectivity observed for the NTA–PAR reagent results from the kinetics of the ligand–ligand exchange. Increasing the concentration of NTA in the postcolumn reagent ensures a greater fraction of the metal is initially present as the $\text{M}(\text{NTA})^-$ complex, as opposed to directly forming the $\text{M}(\text{PAR})_2$ complex.

Often the kinetics of postcolumn reactions is enhanced by heating the postcolumn reactor. However, the kinetics of exchange of Ni^{2+} between NTA and PAR was only doubled by a 10°C increase in temperature [20]. Thus increasing the temperature cannot enhance the exchange kinetics sufficiently to alter the observed selectivities.

For the lanthanides, the lack of stability constant data for the PAR complexes makes it more difficult to say whether the suppression by NTA results from thermodynamic or kinetic control. However, no response was observed for Gd^{3+} after 20 h reaction. It would seem therefore that the lanthanides are thermodynamically disfavored from reacting with the NTA–PAR reagent. The question then arose, would we achieve a transition metal specific response if PAR and then NTA were added sequentially? That is, if PAR was added to the effluent to produce the $\text{M}(\text{PAR})_2$ complexes, and then NTA was added to mask the lanthanides. Unfortunately, kinetic studies indicate that the reverse reaction (NTA displacing PAR) is also too slow to be analytically useful.

Acknowledgments

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