Short communication

Removal of metallic impurities from mobile phases in reversed-phase high-performance liquid chromatography by the use of an in-line chelation column

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Abstract

An in-line stripper column packed with iminodiacetate chelation resin is placed between the pump and injection valve and shown to remove metallic impurities from an HPLC system. We used a test procedure based on assessing the relative peak asymmetries of 2,2′-bipyridyl, a chelating analyte and 4,4′-bipyridyl, a non-chelating analyte. Results from use of polyether ether ketone (PEEK) and stainless steel pumps are evaluated. Analytical columns with titanium/PEEK and stainless steel frits are used to assess the role of frits in metallic contamination. We demonstrated that although metal-free pumping systems contribute significantly lower metallic impurities than stainless steel systems, metal is nevertheless present in the mobile phase and the chelating stripper columns were found useful in protecting the analytical columns from contamination. The stainless steel frits were not found to be significant contributors to the contamination. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Chelation; Mobile phase composition; Metals; Bipyridyls

1. Introduction

The metal content of silica-based bonded phases is often used as one parameter for assessing the phase quality. Most column vendors report the metal content of their bonded phases, with the most significant metals usually being iron and aluminum. The presence of metals is thought to contribute to unwanted ion-exchange retention and poor chromatography of chelators. It is apparent from chromatographic performance however that as a column is

used, the metal content increases. Ideally, HPLC system hardware should not contribute to changes in column properties or performance.

Iminodiacetate resin provides a convenient method for removing transition metals [1–4] from a variety of matrices. These metals include Ti(IV), V(IV and V), In(III), Y(III), Cd(II), Mn(II), Fe(II and III), Co(II), Pb(II), Ni(II), Al(III), Cu(II), Zn(II), Ag(I) and lanthanides. The pH constraints are that the metals are retained on the resin at pH greater than 4 and eluted from the resin at pH less than 2. In the present work, we remove metals from the mobile phase by placing a column packed with a highly crosslinked iminodiacetate resin between the pump and the polyether ether ketone (PEEK) injection valve. This resin has a higher affinity for calcium, magnesium and transition metals than for sodium

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[5]. The high crosslinking minimizes swelling due to changes in counter-ion or from solvents.

Engelhardt and Lobert proposed a test procedure for assessing the metal content of reversed-phase columns using 2,2′-bipyridyl as a chelating analyte and 4,4′-bipyridyl as a non-chelating analyte [6]. We use this method to assess the presence of metals derived from various system components.

The aim of this work is to assess the sources of metal contamination and provide a convenient way to minimize the metal content that enters the analytical column.

2. Experimental

2.1. Chromatographic conditions

An HPLC system was assembled that included either an all PEEK pump (GP50, Dionex, Sunnyvale, CA, USA) or a stainless steel pump (Dionex), an all PEEK Rheodyne injection valve and a photodiode array detector (PDA-100, Dionex). A thermostatic column compartment (LC-30, Dionex) was also used. The eluent lines were Teflon and the reservoirs were glass (Schott Duran). The methanol was Fisher Optima brand and the deionized water came from a Millipore at-site Milli-Q Plus ultra-pure water system. Data was handled by PeakNet 6.1 software (Dionex) and Excel (Microsoft).

The in-line metal stripper column was MetPac CC-1 (50×4 mm I.D., Dionex). The MetPac CC-1 column contains a highly crosslinked, macroporous vinylbenzyl–divinylbenzene resin that is grafted with iminodiacetate functional groups in the ammonium-form. The particle size is 17 μm. This column has a capacity of 0.4 mequiv./column and binds many transition metal ions at pH>4. The column was regenerated with 100 mM nitric acid followed by buffer at pH>4.

An experimental 5-μm ODS phase was packed into 250×4.6 mm I.D. stainless steel column hardware. The ODS had a metal content of 50 ng/ml iron and 70 ng/ml aluminum. The bed supports were either stainless steel (Upchurch, Oak Harbor, WA, USA or Alltech, Deerfield, IL, USA) or titanium/PEEK (Upchurch). The iron and aluminum content

of the silica was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES; Thermo-Jarrell-Ash AtomScan 25) after the silica was dissolved using (50:50) hydrofluoric acid–nitric acid. The acids were trace metal grade.

The mobile phases were prepared by separately adding water and methanol to the reservoir. When buffers were used as the aqueous phase, the pH of the buffer was adjusted before addition of the organic phase. The mobile phase for the bipyridyls test was unbuffered 50% (v/v) methanol.

2.2. Testing protocol

The mobile phase of 50% (v/v) aqueous methanol was pumped at 1.0 ml/min. Detection was at 254 nm and the temperature of the column compartment was controlled at 35°C. The sample loop size was 10 μl. The test sample contained 250 ng/μl 4,4′-bipyridyl and 1000 ng/μl 2,2′-bipyridyl as used in Ref. [6]. A sample was injected every 20 min. and the peak asymmetries were calculated based on the EP standard $A = (RW5\% + LW5\%)/2 \cdot LW5\%)$ present in the PeakNet 6.1 software. The peak areas were monitored since the peak asymmetry value for the 2,2′-bipyridyl peak is sensitive to the mass injected.

The MetPac CC-1 column was placed in-line between the pump and injection valve as indicated in the experiments.

3. Results and discussion

3.1. The basic experiment — PEEK pump

In the first experiment, the ratio of peak asymmetry values for 4,4′-bipyridyl to 2,2′-bipyridyl was evaluated from an all-PEEK chromatograph without use and then with use of an in-line chelator column (Table 1). The chelator column and the ODS column contained titanium/PEEK frits. Asymmetry values increase for both analytes although the increase for the chelator, 2,2′-bipyridyl, is greater. After 100 ml of mobile phase the peak asymmetry for 4,4′-bipyridyl increased 19.6% and the peak asymmetry for 2,2′-bipyridyl increased 32.6% This indicates that there is metal in the mobile phase. The

Table 1
Peak asymmetry values: PEEK Pump without and with MetPac in-line[a]

Volume of mobile phase pumped (ml)	Peak asymmetry 2,2′-bipyridyl (chelator)	Peak asymmetry 4,4′-bipyridyl (non-chelator)	Ratio (2,2′/4,4′)
20	1.38	1.43	0.96
40	1.51	1.61	0.94
60	1.65	1.64	1.00
80	1.71	1.65	1.03
100	1.83	1.71	1.07
MetPac in-line	1.65	1.57	1.05
140	1.69	1.62	1.04
160	1.60	1.54	1.04
180	1.56	1.57	0.99
200	1.56	1.53	1.02
220	1.54	1.51	1.02
240	1.51	1.52	0.99
260	1.51	1.50	1.01

[a] Conditions given in Section 2.2.

increase in asymmetry for the 4,4′-bipyridyl may indicate an increase in acidity of the silanol sites when metal is present in the system.

As a second part of this same experiment, a MetPac CC-1 column was added between the pump and the injector. High asymmetry values continued for a short period of time as the system continued to pump mobile phase and then decreased 4.5% for 4,4′-bipyridyl and 8.5% for 2,2′-bipyridyl, during a total volume flow of 100 ml of mobile phase. The conclusion is that metal present in the mobile phase chelates with the 2,2′-bipyridyl which causes the peak tailing and does not irreversibly bind to the silica packing material. The MetPac column is effective at in-line removal of metal contamination from a mobile phase within the pH constraints of the iminodiacetate functional groups.

3.2. Stainless steel pump

The first experiment was repeated using a stainless steel pump instead of the PEEK pump with all other details remaining the same. The ratio of peak asymmetry values showed higher metal contamination when a MetPac was not in-line (Table 2). The values decreased to starting levels when MetPac was placed in-line. Since the only difference between these two systems is the pump, the conclusion is that the stainless steel pump contributes metal above the

levels contributed by the mobile phase reagents but that the MetPac is effective in removing the metal.

3.3. Stainless steel frits

Table 3 shows the peak asymmetry values for the two analytes using the stainless steel pump with a MetPac column between the pump and the injector and stainless frits in the analytical column. The peak asymmetry values for 2,2′-bipyridyl and 4,4′-bipyridyl increased equally indicating that metal contamination is not increasing in the analytical system. No increase in the ratio of peak asymmetry for 2,2′-biyridyl/4,4′-bipyridyl was observed. This experiment shows the lack of contribution of the stainless steel frits to the metal contamination in the system. With the MetPac inline, mobile phase and pump contributions are eliminated. Any increase in peak asymmetry would have been from the inlet bed support.

3.4. Other mobile phases

The MetPac system was also tested with a mobile phase containing acetonitrile–25 mM sodium phosphate pH 7 (50:50). Fe (1 µg/ml) was added to the mobile phase. One hundred milliliters of mobile phase were then pumped through a MetPac column and the effluent was tested by ICP–AES. The post-

Table 2
Peak asymmetry values: stainless pump without and with MetPac in-line[a]

Volume of mobile phase pumped (ml)	Peak asymmetry 2,2′-bipyridyl	Peak asymmetry 4,4′-bipyridyl	Ratio (2,2′/4,4′)
20	1.67	1.72	0.97
40	1.87	2.01	0.93
60	1.91	1.97	0.97
80	2.10	2.20	0.95
100	2.15	2.14	1.00
120	2.22	2.16	1.03
140	2.38	2.15	1.11
160	2.43	2.19	1.11
180	2.65	2.28	1.16
200	2.76	2.29	1.20
MetPac in-line	2.80	2.20	1.27
220	2.40	2.16	1.11
240	1.64	1.75	0.94
260	1.62	1.77	0.92

[a] Conditions given in Section 2.2.

Table 3
Peak asymmetry values: stainless steel pump; analytical column with stainless steel frits[a]

Volume of mobile phase pumped (mls)	2,2′-Bipyridyl (chelator)	4,4′-Bipyridyl (non-chelator)	Ratio (2,2′/4,4′)
20	1.44	1.22	1.18
60	1.43	1.20	1.19
80	1.42	1.22	1.16
100	1.51	1.18	1.28
120	1.39	1.16	1.20
140	1.44	1.17	1.23
MetPac in-line	1.39	1.18	1.18
180	1.49	1.24	1.20
200	1.62	1.36	1.19
220	1.88	1.41	1.33
240	1.79	1.53	1.17
260	1.77	1.52	1.16
280	1.84	1.54	1.19

[a] MetPac column added in-line as noted; conditions given in Section 2.2.

MetPac mobile phase had an Fe level below the detection limit of 10 ng/μl. This result indicates that the MetPac resin can remove iron in the presence of sodium-based buffers. MetPac CC-1 does not function with mobile phases at pH less than 4.

In summary, stainless steel pumps and mobile phases can reversibly contaminate analytical columns with metals. Analytical columns can be protected from these metals by using an in-line column packed with a highly crosslinked iminodiacetate resin.

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