Experiment 21: Ion Pair Determinations of Lead (HPLC)

SYNOPSIS:

Lead is chelated to ethylenediaminetetraacetic acid (EDTA) to form a dianion, which, when paired with an alkylated cation is partitioned from an aqueous phase onto an organic phase (reverse phase) from a column. The complex is eluted from the column and monitored at 254 nm where the complex but not the uncomplexed reagent absorbs. The uncomplexed reagent absorbs at lower wavelengths. The shift to 254 nm is presumably due to perturbation of the electrons on the nitrogen.

READINGS Read Critical Review: pH effects (pages 273-274); EDTA chemistry (pages 279-285); and Separations chemistry (pages 331-334). Attached are two articles. The first details a similar method using a ligand somewhat like dithizone (Exp. 13). The second uses a chelate similar to EDTA (NTA) to achieve resolution using capillary electrophoresis.

INSTRUMENTS

column:	ZORBAX ODS 4.6 mmx15 cm
mobile phase	.01 EDTA, 0.02 tetrraethyl ammonium bromide, 80/20 H_2O/MeOH, pH 3.75 and 5.22 (mM phosphate buffer).
Flowrate:	1.0 ml/min (950 psi), 0.6 ml/min (800 psi)
detector:	UV at 254 nm

solutions

mobile phase:	Water with EDTA (sufficient for 0.01 M) and Tetraethyl ammonium bromide (TEAB) sufficient for 0.02 M, stirred until EDTA goes into solution. Methanol added to achieve 20/80 mix. Stir ½ hour more. Add Na phosphite to pH ~4, conc. Phosphoric acid to adjust pH down to 3.75. Let sit overnight. Use same procedure for the 5.22 pH mobile pahse.
Stock solutions	$Pb(NO_3)_2$, $Fe(SO_4)$, Hg_2Cl_2.
Cocktails	Ranges of metals in mobile phase from 50-100 ppm.
Standards	Range of Pb in mobile phase from 50-100 ppm.

Procedure

1. Specific instructions will be found with the instrument.
2. Note the gases used for purging, note the length of the column, and the internal and external diamters of the column, as well as the packing material.
3. At 1.0 ml/min for the standard in the pH 3.75 buffer, inject the lowest standard for lead and obtain a chromatogram. Note the retention time required, the peak height, peak area, and the marker peak retention time. Also note the base width of the triangulated peak.

4.   Repeat for each of the standards at 1.0 ml/min and pH 3.75 buffer.
5.   For one of the lead standards, repeat the chromatogram several times, varying the flow rate for the maximum ranges of your instrument.
6.   Obtain a second standard curve with the pH buffered at pH 5.22 at 1.0 ml/min.
7.   From the two possibilities above (pH 3.75 vs 5.22) select the best standard curve (you will need to plot this before moving on.)
8.   Using the conditions you have selected make three separate injections of the lowest concentration of lead standard.
9.   Using the conditions you have selected, inject one standard iron solution. Note the retention time.
10.   Using the conditions you have selected, inject one standard mercury solution. Note the retention time.
11   Inject a cocktail (or mixture) of the three metals. Note the retention times, the peak heights, peak widths, and triangulated baseline widths of all of the peaks. Label and identify the peaks and calculate the resolution of the metals.

REPORT: In addition to materials, methods, and results your report should include the following information.

1.   Report the relative standard deviation of the multiple standard lead injections.
2.   What is the LOD, LOQ, linear range and r value of your standard curve for this method? How does your LOD compare with the expected value?
3.   Convert the ppm of your LOD to ppm in your sample (soil, paint, etc.)
4.   What are the main background peaks in this measurement?
5.   Why or why not do you get Gaussian shaped peaks?
6.   Why is the uncomplexed EDTA in the sample not a problem in detection?
7.   Write out all of the chemical reactions that make this method work.
8.   Based on seven, what might be interferences.
9.   Plot the H vs flow rate obtained for the lead in step 5. What is the best flow rate?
10.   What is "H" and what information does it give you?
11.   Does the limit of detection for this method compare with others?
12.   Was the lead resolved from mercury? From iron? What is the mathematical resolution?
13.   What are the capacity factors for the peaks?
14.   What is the number of theoretical plates?
15.   What was the estimated time for turn around in samples?
16.   Are there any problems with disposal of hazardous materials?
17.   How easy would it be to instruct a technician on this method?
18.   How easy would it be to construct a paper trail for this method?