Experiment 13: Dithizone Extraction + UV-Vis

SYNOPSIS Lead is selectively removed from an aqueous phase into an organic phase by a chelating agent. The molecular electronic transition of the chelating reagent is perturbed by the presence of lead causing a UV-Vis absorption band which can be monitored. The selectivity of the process will be monitored by difference spectroscopy in the presence of Zn^{2+}.

READINGS Read pages 304-309 in the Critical Reviews. Review the section on soluble lead chemistry on pages 273-279. Attached is an article describing some of the chemistry of dithizone. The attached article is not as complete as the Critical Reviews discussion.

INSTRUMENT HP 8452A Diode Array.

GLASSWARE
Six 100 mL volumetrics
Separatory flask
Assorted Beakers
Micropipettes, 2 mL pipette

SOLUTIONS The dithizone must be made fresh on the day of use as it is unstable. *(How would you prove this to yourself?)*

Wash bottle of acetone
10 mg dithizone/L Methylene chloride, CH_2Cl_2
20 g of KCN diluted to 1 L in the buffer
Buffer: 225 mL of 0.2 M sodium carbonate, 25 mL of 0.2 M sodium bicarbonate, diluted to 1000 mL. (0.2 M anhydrous sodium carbonate from 21.2 g in 1000 mL; 0.2 M anhydrous sodium bicarbonate from 16.8g in 1000 mL) results in pH 11.
1000 ppm Pb stock solution.

CAUTION!!!!!
If you use gloves to protect your hands from the organic reagents the gloves should be free of powder as the powder will cause a false positive. This lab was a staple (standard method) for 40 years, but any lack of cleanliness will cause this method to fail.

PROCEDURE

A. Wavelength scanning.
1. Set the range (F2) to 400-700 nm. Run blank (methylene chloride) (F8).
2. Scan 2 mL of 0.001% dithizone in CH_2Cl_2 solution over 400nm to 700 nm.

Set the y scale to some preset value and print. Also, export data to a diskette for

further work in Excel.

Repeat this experiment with new additions of dithizone in CH_2Cl_2 three times.

WHY??

3. Add 5 ml of stock dithizone/CH_2Cl_2 solution to a separatory flask. Add 100 µL of concentrated NH_4OH (*What is the purpose of NH_4OH?*) and 100 µl of 1000 ppm stock Pb. Shake 15-20 seconds. **Measure the exact number of seconds. Do not estimate.** One student should be shaker and should use the same shaking procedure every time. The green dithizone solution should turn "carmine" red. Remove solution from bottom of separatory funnel and monitor it's absorbance from 400nm to 700 nm.

4. Superimpose the plots and note the region where the lead chelate absorbs as compared to the free dithizone.

5. Export and save data to an excel format.

B. Calibration curve. For this method you should be able to get a calibration curve linear from 0.1 to 4 ppm. **You will have to make these solutions up.**

1. Place 10 mL of lead standard in separatory funnel. Add 4 mL of dithizone solution and 10 ml of cyanide mixture. Shake well for 1 minute. **Measure this time exactly with a second hand watch.** Allow the phases to separate. Pull CH_2Cl_2 solution from bottom of separatory funnel scan between 400 and 700 nm. Export the data to a diskette.
2. Rinse the cuvette and funnel with MeOH and then acetone between samples.

C. Deconvolution and Calibration Curve

Use Excel to deconvolute the spectral data. Deconvolution instructions follow. Deconvolution is necessary in this experiment because there will be a large amount of unreacted dithizone present which will cause the baselines to vary substantially. If you measure the peak absorbance and plot it vs the added lead you will often get poor calibration curves because the peak absorbance contains absorbance from the unreacted dithizone.

Try reworking the shown example deconvolution before trying it out on your own data. Deconvolute all spectra to get good absorbances for the calibration curve.

From the calibration curve estimate the LOD. This can be done by calculating the regression curve for the calibration curve and obtaining the y-intercept “estimate” which is a measure of the anticipated uncertainity of the y-intercept.

D. Sample

The sample procedure is the same, except that it must be adjusted for the presence of the nitric acid. The extraction of lead into the organic phase by dithizone requires the dithizone to have lost some protons. The pH of your 5 mL of sample extract should be adjusted to pH 10.9 (use, a pH electrode).
If your sample absorbance is large (>2) dilute the sample. When diluting the sample, dilute into the buffer. Check the pH of the diluted sample and adjust to pH 10.9.

REPORT In addition to materials, methods, and results, report:

1. What is the LOD, LOQ, linear range, and r value of your standard curve for this method? How does the LOD compare with other methods you have tried thus far?
2. If you are using a soil or dust solution, convert the ppm of your LOD to ppm in the soil using the dilutions and measured quantities of soil used in the extraction procedure. How does the LOD compare with the cutoff between what is considered to be contaminated and un-contaminated soils?
3. What is the chemistry of the separation?
4. What is the purpose of adding citrate and cyanide?
5. Why does the pH have to be adjusted to 10.9?
6. What constitutes a blank in this procedure? What are the sources of error embodied in the standard deviation of the blank? How would you determine the source of error attributed solely to the UV-Vis spectrometer?
7. How does sample matrix affect your results, if at all?
8. What was the estimated time for turn around in samples?
9.. Are there any problems with disposal of hazardous materials?
10.. Identify the chromophore in dithizone. What electronic perturbation gives rise to the change in color when complexed with lead?
11. How does deconvoluting the data to remove background or blank absorbance compare to doing a baseline subtraction? Which do you prefer?
12. Why can you treat absorption bands as Gaussian curves?