Cobalt Analysis

Procedure for Preparation of CoEDTA for Use as an Indigestible Marker

Materials:

Large beakers, 4 liters
Graduated cylinders, 1,000 ml
Stirring-hotplate
Large Buchner funnel
Whatman filter paper (fast) or white coffee filters

Reagents:

Cobalt II acetate$\cdot 4H_2O$ (Mallinckrodt Cat # 04519, FW 249.10, CAS # 6147-53-1 or Baker Cat # 1658-01, FW 249.10, CAS # 6147-53-1)

Ethylenediaminetetraacetic acid (acid form) (Sigma-Aldrich Cat # ED-1KG, FW 292.24, CAS # 60-00-4)

Lithium hydroxide ($LiOH\cdot H_2O$) (Fisher Cat # L127-500, FW 41.96, CAS # 1310-66-3)

30% Hydrogen peroxide solution (VWR Cat # JT2190-3, FW 34.02, CAS # 7722-84-1)

95% Ethanol (CAS # 64-17-5)

80% Ethanol, dilute 800 ml 95% ethanol (CAS # 64-17-5) to 1 liter with water

Procedure:

The following amounts of reagents represent a triple batch of CoEDTA and should be weighed into a 4 liter beaker.

1. Weigh 75 g cobalt II acetate, 87.6 g EDTA, and 12.9 g lithium hydroxide$\cdot H_2O$ (7.35 g anhydrous form) into a 4 liter beaker.
2. Add 600 ml dd water and dissolve with heating if necessary.
3. Cool and add 60 ml of 30% hydrogen peroxide solution.
4. Allow to stand 2-3 hours or overnight.
5. Add 900 ml of 95% ethanol and refrigerate overnight (loosely covered).
6. Filter with vacuum and wash crystals with about 3 liters of 80% ethanol.
7. Dry crystals in 60 °C oven and store dry crystals in a brown glass jar until ready to re-suspend.
8. Re-suspend crystals in dd water (2 g per 100 ml dd water) and measure cobalt concentration on an atomic absorption spectrophotometer.

Procedure for Extracting and Measuring Cobalt in Feces, Rumen Fluid or Dose

Materials:

30-50 ml screw cap tubes
Whatman #1 filter paper
Centrifuge
Pipettes
Atomic Absorption Spectrophotometer
Shaker

Reagents:

0.05 M EDTA (ethylenediaminetetraacetic acid) solution:

Weigh 58.4 g EDTA (Sigma-Aldrich Cat # ED-1KG, FW 292.24, CAS # 60-00-4) and 15.3 g KCl (potassium chloride) (Mallinckrodt Cat #6838-05, FW 74.55, CAS # 7447-40-7 or EM Cat # PX1405-5, FW 74.55, CAS # 7447-40-7); place both in a 4 liter volumetric flask. Add about 3,900 ml dd water, stirring to dissolve reagents, and adjust pH to 6.5 with concentrated reagent grade ammonium hydroxide (Fisher Cat # A470-500, FW 35.05, CAS # 1336-21-6). Add water to a total volume of 4 liters.

Cobalt Standards (Perkin Elmer Cat #N9303766)

18 MOhm water

Extraction of Cobalt in Feces:

1. Weigh out 0.2 g dried, ground (2 mm screen) fecal sample in duplicate and place in 30-50 ml screw cap tube.
2. Add 20 ml of the 0.05 M EDTA solution.
3. Cap the tubes and shake for 30 minutes.
4. Filter sample through a Whatman #1 filter paper, twice.
5. Read concentration and/or absorbency of cobalt on the atomic absorption spectrophotometer.

Cobalt Measurement of Rumen Fluid or Dose Sample:

1. Thaw rumen fluid in a plastic container.
2. Centrifuge rumen fluid at 16,000 to 18,000 x g.
3. Dilute supernatant to an appropriate range for measurement by atomic absorption spectrophotometer.

Example:

Post-dosing Hours	Rumen Fluid, ml	H_2O, ml	Dilution factor
1-8	1	4	5
8-14	2.5	2.5	2
15+	5	0	1

4. The AA spec is maximized with a 5 ppm standard, wavelength of 240.7, and slit of 0.2 nm. The working standard curve used is 0.5, 1, 2, 3, 4, 5 ppm and is diluted in 18 MOhm water.

Reference

E. C. Prigge and G. Varga, West Virginia, University, Uden et al. (1980) J. Sci. Food Agr. 31:625.

Hart, S. P. and C. E. Polan. 1984. Simultaneous extraction and determination of ytterbium and cobalt ethylenediaminetetraacetate complex in feces. J. Dairy Sci. 67:888-892.