DETERMINATION OF TITANIUM CONCENTRATION IN BIOLOGICAL SAMPLES

Purpose and scope

To determine Titanium dioxide concentration as inert marker in biological samples.

Procedure

Chemicals/Reagents required:

1	Concentrated H_2SO_4 (95.0-98.0%)	Sigma-Aldrich No. 258105- 500mL
2	H_2O_2 (30 wt.% in H_2O)	Sigma-Aldrich No. 216763- 500mL
3	Titanium (IV) oxide (technical, ≥99%)	Sigma-Aldrich No. 14021 or 14027
4	Cuvettes	Fisherbrand: FB55147

Solution required:

1. Preparing the stock solution (standard titanium solution; 0.5 mg/mL or 150 ppm)
    i. Dissolve 250mg TiO_2 in 100mL concentrated H_2SO_4 in a heat proof beaker.
    ii. Heat the mixture just below boiling.
    iii. Carefully add with rinsing to approximately 200mL distilled water in a volumetric flask (500mL).
    iv. Add 100mL concentrated H_2SO_4 to the mixture.
    v. Dilute the mixture with distilled water to 500mL.

Note: The volumes required for each solution can be adjusted according to the total volume desired.

2. Preparing 1L of sulfuric acid (7.4M) solution.
    i. Slowly add 400 mL of concentrated H_2SO_4 to approximately 400 mL distilled water in a 1000 mL volumetric flask.
    ii. Dilute this mixture with distilled water to 1000mL.

Note: This is an extremely vigorous exothermic reaction; the flask will be hot. Follow the steps carefully.

NEVER ADD WATER TO ACID

3. Preparing diluent solution (300mL total volume)
    i. Add 30mL H_2SO_4 (7.4 M) solution to 60mL H_2O_2 in a 500 mL volumetric flask.
    ii. Dilute the mixture to 300mL with distilled water.

Note: The total volume can be adjusted to any volume desired but the ratio of 7:2:1 for distilled water, H_2O_2, and 7.4M H_2SO_4 should be maintained. Again, add the dilute acid to H_2O_2 and not the other way round.

4. Preparing the standard solutions for the calibration curve:

To prepare the concentration curve (Titanium concentration: 0, 12, 24, 36, 48, 60, 72 ppm), pipette into 7 individual 50 mL volumetric flasks using the chart below:

Making standard solutions.

Flask No.	Ti concentration (ppm)	Dilution factor	Stock solution (mL)	Diluent (mL)	Total std solution (mL)
1	0	0.00	0.0	50.0	50
2	12	0.08	4.0	46.0	50
3	24	0.16	8.0	42.0	50
4	36	0.24	12.0	38.0	50
5	48	0.32	16.0	34.0	50
6	60	0.40	20.0	30.0	50
7	72	0.48	24.0	26.0	50

Note: The sample without TiO2 (blank) is used to calibrate spectrophotometer reading to zero. Measure absorbance at 410 nm (minimum of 2 readings per sample), may use the template reading table below.

From the Ti concentration and an average of absorbance values, generate a linear regression equation of the type Y =a + bX where Y are the average absorbance readings and X are the Ti concentrations. The regression coefficient should not be less than 0.98.

Sample table:

Serial No.	Sample ID	Beaker No.	Sample Wt. (g)	Abs 1	Abs 2	Average Abs	% Ti

5. Preparation of biological samples (diets, excreta, feces, digesta):
    i. Ash at least duplicate aliquots (0.50 or 1g for feed samples and 0.25g for digesta or excreta samples) in porcelain crucibles in muffle furnace overnight at 580˚C.
    ii. After cooling, add 10mL 7.4M H_2SO_4 to each crucible.
    iii. Gently boil all the samples on hot plate for approximately 60 mins until completely dissolved.
    iv. After cooling, pour the solution quantitatively with rinsing into a beaker containing 25mL distilled water.
    v. Pour the contents of the beakers into 100mL volumetric flasks through filter paper (Whatman 541 or 542).
    vi. Add 20mL H2O2 (30%) to each flask.

vii. Dilute the content to 100mL with distilled water.
viii. Mix thoroughly by inverting the flasks.
ix. Measure the absorbance of aliquots of the resulting solution in a spectrophotometer at 410 nm.
x. Use the equation to calculate Ti in your biological sample:

$$\frac{((\boldsymbol{Av.absorbance - Intercept})/\boldsymbol{Slope}) \times \frac{\mathbf{100}}{\boldsymbol{Sample\ wt.}}}{\mathbf{10000}}$$

Where:

— Average absorbance is the average reading from the spectrophotometer.
— Intercept is the value for slope obtained from the regression analysis of the standard absorbance readings.
— Sample wt. of the biological sample used prior to ashing.

Safety and disposal:

i. While making up the stock solution and the H_2SO_4 (7.4M) solution, use gloves and a facemask and work in the fume cupboard.
ii. Wear gloves at times while handling the reagents.
iii. All glassware used in the assay should be rinsed well in tap water down the sink.
iv. All reagents to be disposed of down the sink, rinsing with copious amounts of water.