

LEAD

PRINCIPLE

Organic matter in the sample is destroyed by ignition in the presence of sulfuric acid. The residue is dissolved in dilute acid, and the lead is complexed with dithizone. The colored complex is extracted with chloroform and the concentration measured spectrophotometrically (Note 1).

SCOPE

The procedure is applicable to commercial starches and their modifications.

SPECIAL APPARATUS

1. Spectrophotometer: An instrument having a continuously-variable wavelength control in the visible spectrum and equipped with matching 1.0 cm cuvettes provided with covers is recommended.
2. Muffle Furnace: Equipped with pyrometer and capable of operating at controlled temperatures up to 500 °C
3. Evaporating Dishes: Fused silica, VYCOR or platinum, 100 mL capacity

SAFETY NOTE

This procedure requires the use of several extremely hazardous chemicals. Thorough knowledge of the dangers and safety procedures is required. Proper protective equipment is also required.

REAGENTS

1. Chloroform: Reagent Grade
2. Dithizone (Diphenylthiocarbazone) Solution, 0.1%: Dissolve 100 mg of dithizone in 100 mL of chloroform. Store in amber (low actinic glass) bottle in refrigerator (Note 2).

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3. Ammoniacal Dithizone Solution: Shake 6 mL of dithizone chloroform solution with 10 mL of 0.5 *N* ammonium hydroxide in a separatory funnel for 2 mins., allow the phases to separate and discard the chloroform layer. Prepare fresh daily (Note 3).
4. Ammonium Hydroxide Solution, 0.5 *N*: Add 33 mL of concentrated ammonium hydroxide (28% NH_4OH , sp g 0.90) to 500 mL of purified water and dilute to 1 L volume.
5. Hydrochloric Acid Solution, 1 *N*: Add 86 mL of concentrated hydrochloric acid (37% HCl , sp g 1.19) to 500 mL of purified water and dilute to 1 L volume.
6. Hydroxylamine Hydrochloride Solution: Dissolve 20 g of hydroxylamine hydrochloride ($\text{NH}_2\text{OH} \cdot \text{HCl}$) in 100 mL of purified water (Note 4).
7. Ammonium Citrate Solution: Dissolve 50 g of dibasic ammonium citrate [$(\text{NH}_4)_2 \text{HC}_6\text{H}_5\text{O}_7$] in 100 mL of purified water (Note 5).
8. Sodium Sulfite Solution, 2%: Dissolve 2 g of anhydrous sodium sulfite (Na_2SO_3) in 98 mL of purified water.
9. Potassium Cyanide Solution, 10%: Dissolve 10 g of potassium cyanide (KCN) in 90 mL of purified water.

POTASSIUM CYANIDE IS EXTREMELY POISONOUS; CYANIDE IN CONTACT WITH ACID FORMS DEADLY PRUSSIC ACID. NEVER PIPET BY MOUTH. DO NOT INHALE THE VAPORS.

10. Ammonia-Cyanide-Sulfite Reagent: To 605 mL of purified water add: 340 mL of concentrated ammonium hydroxide (28% NH_4OH , sp g 0.90), 75 mL of 2% sodium sulfite solution and 30 mL of 10% potassium cyanide solution (CAUTION) and mix (Note 6).

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11. Sulfuric Acid Solution, (25% by volume): Cautiously add 100 mL of concentrated sulfuric acid (96% H₂SO₄, sp g 1.84) to 300 mL of purified water with constant stirring while cooling in an ice bath.
12. Ammonium Hydroxide Solution, 1 N: Add 66 mL of concentrated ammonium hydroxide (28% NH₄OH, sp g 0.90) to 500 mL of purified water and dilute to 1 L volume.
13. Standard Lead Solution, 2.5 µg lead per mL:

Stock Solution: Dissolve 0.160 g of reagent grade, anhydrous lead nitrate [Pb(NO₃)₂] (which has been dried overnight in a desiccator) in purified water, acidify with 1 mL of concentrated nitric acid (70%, w/w or by weight, density 1.42), and dilute to volume with purified water in a 1 L volumetric flask.

Standard Solution: Pipet 25.0 mL of stock solution into a 1 L volumetric flask and dilute to volume with purified water. Prepare fresh daily.

PROCEDURE

Standardization: Pipet 2.0, 4.0 and 6.0 mL of standard lead solution (respectively 5.0, 10.0 and 15.0 µg of lead) into separate 125 mL separatory funnels (Note 7); add purified water to bring the total volume in each funnel to 10.0 mL. To each of three additional separatory funnels add 10.0 mL of purified water to provide triplicate reagent blanks (Note 8). To each separatory funnel add 2.0 mL of 1 N hydrochloric acid solution, 1.0 mL of hydroxylamine hydrochloride solution, 2.0 mL of ammonium citrate solution and mix.

The following operations must be performed without interruption, each sample being processed separately (Note 9). Add 30.0 mL of ammonia-cyanide-sulfite reagent, 10.0 mL of chloroform and 0.5 mL of ammoniacal dithizone solution. Shake the separatory funnel for 1 min. and allow the phases to separate. Insert a cotton plug in the stem of the funnel and drain the chloroform layer slowly into a 1 cm cuvette. Without delay, determine transmittance (% T) at 515 nm, using chloroform as reference (100% T). Convert the percentage transmittance values for each blank and standard to absorbance. Plot the net absorbance (standard

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absorbance minus average blank absorbance) for each standard against the respective μg lead concentration.

Analysis: Weigh accurately 10.00 ± 0.01 g of sample or an amount containing not more than $20 \mu\text{g}$ of lead in an evaporating dish, add 5 mL of sulfuric acid solution distributing it uniformly through the sample. Place the dish on a steam bath to evaporate most of the water. Transfer the dish to a heat resistant surface, place an infrared lamp above the dish to char and dehydrate the sample. Place the dish in a muffle furnace at 500°C until the residue is free from carbon (Note 10). Run a duplicate blank on 5.0 mL of sulfuric acid solution. Cool and cautiously wash down the inside of each dish with purified water, and then add 5.0 mL of 1 *N* hydrochloric acid solution. Place the dish on a steam bath and evaporate to dryness. Add 10.0 mL of 1 *N* hydrochloric acid solution and heat briefly, while stirring, on a steam bath. Transfer quantitatively to a 125 mL separatory funnel with a minimum amount of purified water. Add 8.0 mL of 1 *N* ammonium hydroxide solution to produce an acidity equivalent to 2.0 mL of 1 *N* hydrochloric acid solution. Prepare each blank with 10 mL of 1 *N* hydrochloric acid and 8 mL of 1 *N* ammonium hydroxide solutions.

To each sample solution or blank prepared as outlined above, add 1.0 mL of hydroxylamine hydrochloride solution, 2.0 mL of ammonium citrate solution and mix. Proceed as directed under Standardization treating each sample or blank independently and without interruption. Convert the percent transmittance values for each blank and sample to absorbance. Compute the net sample absorbance (sample absorbance minus average blank absorbance) and determine the μg of lead contained in the sample by reference to the standardization curve.

CALCULATION

$$\text{Lead, } \mu\text{g/g} = \frac{\text{Micrograms Lead (From Graph)}}{\text{Sample Wt., g}}$$

NOTES AND PRECAUTIONS

1. The spectrophotometric determination is made in the presence of an excess of dithizone at 515 nm, where the specific absorbance of lead dithizone exceeds that of dithizone.

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2. Chloroform dithizone solution is reported to be stable for a period of 5 mos. if refrigerated in the dark. It decomposes rapidly when exposed to light at higher temperatures.
3. Ammoniacal dithizone solution is stable for only 6 hrs.
4. Hydroxylamine reduces oxidizing materials (e.g., Fe^{III} and Cu^{II}) which oxidize dithizone.
5. Ammonium citrate prevents the precipitation of metallic hydroxides, and also delays the precipitation of alkaline earth phosphates in alkaline medium, which occlude lead.
6. Addition of ammonia-cyanide-sulfite reagent is essential because it complexes interfering metals (e.g., Fe^{II}, Zn, Mn, Cu^{II}).
7. The standardization curve is linear and reproducible when the same reagents are used. The standards should be analyzed daily or when any of the reagents are freshly prepared. For optimum precision the standard should be analyzed in duplicate.
8. Reagent blank absorbance (corresponding to 75% to 95% T) is caused by dithizone absorption and also by lead impurities in reagents. For this reason accurate measurement of reagent volumes is essential.
9. Dithizone and its complexes are photosensitive; therefore, the spectrophotometric measurement must be performed immediately after extraction with chloroform.
10. Addition of sulfuric acid prevents volatilization of lead during ignition. Temperatures above 550 °C cause partial or complete loss of lead.