

10.12 Determination of zinc – Colorimetric method

Apparatus

- Colorimeter – A photo electric colorimeter or suitable spectrophotometer capable of measuring optical density at a wavelength of 540 nm.

Reagents

1. Copper sulphate solution – Prepared by dissolving 8.0 g copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in distilled water and diluted to one litre.
2. Ammonium citrate solution – Dissolve 225 g ammonium citrate ($(\text{NH}_4)_3\text{C}_6\text{H}_5\text{O}_7$) in distilled water, make alkaline to phenol red ($\text{pH} \pm 7.4$) with concentrated ammonium hydroxide (25 per cent) and add a further 75 ml. Dilute to 2 litre. Before use, purify by adding a slight excess of dithizone solution and extract with successive portions of carbon tetrachloride until the solvent layer has a clear bright green colour. Remove the dithizone remaining in the solution by means of successive extractions with chloroform followed by a final extraction with carbon tetrachloride (The dithizone shall be entirely removed to prevent loss of zinc during the removal of cobalt).
3. α -Nitroso- β -Naphthol solution – Dissolve 0.25 g α -Nitroso- β -Naphthol in chloroform and make the volume up to 500 ml with chloroform.
4. Chloroform – redistilled. Store in an amber bottle.
5. Alizarin indicator solution – Dissolve 0.02 g of sodium alizarin sulphonate in water and make up the volume to 100 ml.
6. Dilute hydrochloric acid – 0.05 N.
7. Buffer solution – Dissolve 0.1 g of hydroxylamine.
8. Standard zinc solution – Dissolve 0.500 g of pure granulated zinc in a slight excess of hydrochloric acid. Dilute to 1 litre with double distilled water. One millilitre of this solution contains 0.5 microgram of zinc.
9. Standard zinc working solution – Dilute 10 ml of standard zinc solution with 0.04 N hydrochloric acid to make 1 litre. One millilitre of this solution contains 5 mg of zinc.
10. Ammonium hydroxide solution – An aqueous solution of ammonia containing approximately 5 per cent ammonia (m/m).
11. Hydrochloric acid solution – Concentrated hydrochloric acid diluted with distilled water in the proportion 1:6.
12. Bromine water – A saturated solution of bromine in water.
13. Hydrogen sulphite
14. Methyl red indicator solution – Dissolve 25 mg methyl red in 100 ml of 60 per cent ethyl alcohol.
15. Phenol red indicator solution – Dissolve 100 mg phenol red sodium salt in 100 ml distilled water.

Procedure

1. Dilute 10 ml of the test solution to about 40 ml. Add 2 drops of methyl red indicator and 1 ml copper sulphate solution and neutralize with ammonia. Add sufficient hydrochloric acid solution to bring the concentration of this acid to 0.15 N. The pH value of the solution should now be between 1.9 and 2.1. Pass a stream of hydrogen sulphite through the solution until precipitation is complete. Filter through a fine filter paper (previously washed with hydrochloric acid solution and with water) into a 250 ml beaker. Wash the precipitate and filter paper with three or four small portions of water, adding the washings to the filtrate. Boil the solution until all trace of hydrogen sulphite has been removed, add 5 ml of bromine water and continue boiling until

free from bromine. Cool, neutralize to phenol red with ammonium hydroxide solution and add 0.6 ml hydrochloric acid solution. Make up to a suitable volume and take an aliquot containing 4 to 20 microgram of zinc for the determination. Adjust the volume of the aliquot to about 20 ml by the addition of distilled water and transfer to a 125 ml separating funnel. Add 5 ml ammonium citrate solution and 10 ml α -nitroso- β -naphthol solution. Shake for 2 minutes, allow the phases to separate and discard the solvent layer.

2. Wash the aqueous layer with small portions of chloroform to remove residual α -nitroso- β -naphthol. If necessary, adjust the pH value of the aqueous solution to 8.0 to 8.2 by the addition of ammonium hydroxide or hydrochloric acid solutions and add 2 ml dithizone solution and 10 ml carbon tetrachloride. Shake for 2 minutes, allow the phases to separate and, using a pipette, withdraw the aqueous phase as completely as possible and discard it. Wash down the sides of the separating funnel with 25 ml distilled water and again withdraw the aqueous phase and discard it. Add 25 ml of 0.04 N hydrochloric acid to the content of the separating funnel, shake for 1 minute to transfer zinc to the aqueous phase, allow the phases to separate and discard the solvent layer. To the aqueous solution remaining in the separating funnel add 5 ml ammonium citrate solution and adjust the pH value, if necessary, to 8.8 to 9.0. Add 10.0 ml carbon tetrachloride accurately measured. Determine the quantity of dithizone solution to be added.
3. To a separating funnel containing 4.0 ml of the standard zinc working solution (20 microgram zinc) made up to 25 ml with 0.04 N hydrochloric acid add 5.0 ml ammonium citrate solution and 10 ml carbon tetrachloride; then add dithizone solution from a burette in 0.1 ml increments, shaking after each addition, until a faint yellow color in the aqueous phase indicates a slight excess of reagent. Multiply the volume of dithizone solution used by 1.5 and add this quantity to the test solution. Shake for 2 minutes and allow the phases to separate. Pipette 5 ml of the solvent phase into a test tube, dilute with 10 ml carbon tetrachloride and use for the determination of the absorption. Prepare a series of standards containing 5, 10, 15 and 20 microgram zinc diluted in each case to 25 ml with 0.04 N hydrochloric acid solutions and treated in the same manner as the test solution. Prepare a blank simultaneously by using the same quantities of reagents as were used in the digestion of the test sample and in the subsequent procedure, making up to 200 ml and developing the color in the same size aliquot and in the same manner as in the actual determination. Measure the absorptions of the standard and test solutions at 540 nm in the colorimeter. From the absorptions of the standard solutions prepare a graph by plotting absorptions against concentrations and from it determine the concentration of zinc in the test solution. Express the result as percentage of zinc in the test sample.

Reference: IS:7874 (part-II) – 1975. Methods of tests for animal feeds and feeding stuffs. Part – II, Minerals and Trace elements.