5550 TANNIN AND LIGNIN*

5550 A. Introduction

Lignin is a plant constituent that often is discharged as a waste during the manufacture of paper pulp. Another plant constituent, tannin, may enter the water supply through the process of vegetable matter degradation or through the wastes of the tanning industry. Tannin also is applied in the so-called internal treatment of boiler waters, where it reduces scale formation by causing the production of a more easily handled sludge.

* Approved by Standard Methods Committee, 2010.

5550 B. Colorimetric Method

1. General Discussion

a. Principle: Both lignin and tannin contain aromatic hydroxyl groups that react with Folin phenol reagent (tungstophosphoric and molybdophosphoric acids) to form a blue color suitable for estimation of concentrations up to at least 9 mg/L. However, the reaction is not specific for lignin or tannin, nor for compounds containing aromatic hydroxyl groups, inasmuch as many other reducing materials, both organic and inorganic, respond similarly.

b. Applicability: This method is generally suitable for the analysis of any organic chemical that will react with Folin phenol reagent to form measurable blue color at the concentration of interest. However, many compounds are reactive (see ¶ *c* below) and each yields a different molar extinction coefficient (color intensity). Hence, the analyst must demonstrate conclusively the absence of interfering substances.

c. Interferences: Any substance able to reduce Folin phenol reagent will produce a false positive response. Organic chemicals known to interfere include hydroxylated aromatics, proteins, humic substances, nucleic acid bases, fructose, and amines. Inorganic substances known to interfere include iron (II), manganese (II), nitrite, cyanide, bisulfite, sulfite, sulfide, hydrazine, and hydroxylamine hydrochloride. Both 2 mg ferrous iron/L and 125 mg sodium sulfite/L individually produce a color equivalent to 1 mg tannic acid/L.

d. Minimum detectable concentrations: Approximately 0.025 mg/L for phenol and tannic acid and 0.1 mg/L for lignin with a 1-cm-path-length spectrophotometer.

e. Quality control (QC): The QC practices considered to be an integral part of each method are summarized in Table 5020:I.

2. Apparatus

Colorimetric equipment: One of the following is required:

a. Spectrophotometer, for use at 700 nm. A light path of 1 cm or longer yields satisfactory results.

b. Filter photometer, provided with a red filter exhibiting maximum transmittance in the wavelength range of 600 to 700 nm. Sensitivity improves with increasing wavelength. A light path of 1 cm or longer yields satisfactory results.

c. Nessler tubes, matched, 100-mL, tall form, marked at 50-mL volume.

3. Reagents

a. Folin phenol reagent: Transfer 100 g sodium tungstate, $Na_2WO_4 \cdot 2H_2O$, and 25 g sodium molybdate, $Na_2MoO_4 \cdot 2H_2O$, together with 700 mL distilled water, to a 2000-mL flat-bottom boiling flask. Add 50 mL 85% H_3PO_4 and 100 mL conc HCl. Connect to a reflux condenser and boil gently for 10 h. Add 150 g Li_2SO_4, 50 mL distilled water, and a few drops of liquid bromine. Boil without condenser for 15 min to remove excess bromine. Cool to 25°C, dilute to 1 L, and filter. Store finished reagent, which should have no greenish tint, in a tightly stoppered bottle to protect against reduction by air-borne dust and organic materials.

Alternatively, purchase commercially prepared Folin phenol reagent and use before the recommended expiration date.

b. Carbonate-tartrate reagent: Dissolve 200 g Na_2CO_3 and 12 g sodium tartrate, $Na_2C_4H_4O_6 \cdot 2H_2O$, in 750 mL hot distilled water, cool to 20°C, and dilute to 1 L.

c. Stock solution: The nature of the substance present in the sample dictates the choice of chemical used to prepare the standard, because each substance produces a different color intensity. Weigh 1.000 g tannic acid, tannin, lignin, or other compound being used for boiler water treatment or known to be a contaminant of the water sample. Dissolve in distilled water and dilute to 1000 mL. If the identity of the compound in the water sample is not known, use phenol and report results as "substances reducing Folin phenol reagent" in mg phenol/L. Interpret such results with caution.

Note that tannin and lignin are not individual chemical species of known molecular weight and structure; rather, they are substances containing a spectrum of chemicals of different molecular weights. Their chemical properties depend on source and method of isolation. If a particular substance is being added to the water, use it to prepare the stock solution.

d. Standard solution: Dilute stock solution with distilled water to desired range. Prepare a minimum of 3 standards bracketing expected sample concentration range.

4. Procedure

Bring 50-mL portions of clear sample and standards to a temperature above 20°C and maintain within a ±2°C range. Add in rapid succession 1 mL Folin phenol reagent and 10 mL carbonate-tartrate reagent. Allow 30 min for color development. Compare visually against simultaneously prepared standards in matched Nessler tubes or make photometric readings against a reagent blank prepared at the same time. When using this method for regulatory compliance, use photometric detection. Use the following guide for instrumental measurement at a wavelength of 700 nm:

Tannic Acid in 61-mL Final Volume *μg*	Lignin in 61-mL Final Volume *μg*	Light Path *cm*
50–600	100–1500	1
10–150	30–400	5

Report results in mg/L of the compound known to be present or as "substances reducing Folin phenol reagent" in mg phenol/L.

5. Precision and Bias

In a single laboratory analyzing seven replicates for phenol at 0.1 mg/L the precision was ±7% and recovery was 107%.

6. Bibliography

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