
**Lignins — Determination of
carbohydrate composition in kraft
lignin, soda lignin and hydrolysis
lignin**

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 6, *Paper, board and pulps*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

There is a rapidly-growing worldwide interest in developing novel applications for lignin as a replacement for fossil-based raw materials in products including carbon fibre, adhesives, thermoplastics, resins, composites, and various chemicals. In addition, the use of lignin in these and other applications will offload recovery boilers in pulp mills, allowing more efficient recovery of pulping chemicals and increased pulp production. These benefits translate into reduced environmental impact and improved sustainability owing to the use of renewable materials.

In order to ensure harmonization of testing practices among lignin producers and to facilitate trade, the use of international standard methods is needed to characterize the lignin raw material for a wide range of properties such as general composition, functional groups, molecular weight distribution, particle size, structural features, and thermal behaviour and stability.

The carbohydrate composition - the contents of the five principal, neutral monosaccharides; arabinose, galactose, glucose, xylose and mannose - provides chemical information about the main polysaccharides in lignin. The total content and composition of carbohydrates also provide an indication of the purity of the lignin isolated from the kraft pulping process (kraft lignin) or the soda pulping process (soda lignin), or that obtained by hydrolysis of biomass (hydrolysis lignin).

The methods described in this document are based on those described in other publications^{[1][2]}. Although the principle is similar to that described in ISO 21437^[3] and other related methods^{[4][5][6]} for the determination of carbohydrates in pulp, the properties and end-use applications of lignin, as well as several steps in the testing procedure, including sampling, sample preparation, and others, are different from those of pulp.

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Lignins — Determination of carbohydrate composition in kraft lignin, soda lignin and hydrolysis lignin

1 Scope

This document describes a method for the determination of carbohydrate composition in kraft lignin, soda lignin and biorefinery lignin.

The method is applicable to lignin isolated from a kraft pulping process, a soda pulping process, or lignin obtained by hydrolysis of biomass.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1

lignins

class of complex organic macromolecules, containing aromatic sub-units, that play a key role in the formation of cell walls in wood and bark, conferring mechanical strength and rigidity to the cell walls and to plants as a whole

Note 1 to entry: Lignin is the main non-carbohydrate constituent of wood.

3.2

kraft lignin

depolymerized and chemically modified lignin isolated from a kraft pulping process, such as that originating from kraft black liquor

3.3

soda lignin

depolymerized and chemically modified lignin isolated from a soda pulping process, such as that originating from soda liquor

3.4

hydrolysis lignin

lignin produced for commercial applications by conversion of biomass, through enzymatic or acid hydrolysis, into sugars and lignin streams, followed by separation of the lignin fraction

3.5

biomass

biological material derived from living, or previously living organisms, such as wood, agricultural crops, and other plant-based biodegradable material

3.6

acid-insoluble lignin

Klason lignin

residue after treating wood or pulp with sulfuric acid in a two-step hydrolysis procedure to solubilize the carbohydrates into monosaccharides

3.7

acid-soluble lignin

portion of lignin that is soluble during the acid-insoluble lignin determination

3.8

carbohydrate composition

amounts of the five principal, neutral monosaccharides; arabinose, galactose, glucose, mannose and xylose, in a sample

4 Principle

A lignin sample is treated with sulfuric acid in a two-step (primary and secondary) hydrolysis process to dissolve the carbohydrates. The acid-insoluble lignin is filtered off. The filtrate consists of hydrolysed carbohydrates and a small amount of acid-soluble lignin. The amounts of the different monosaccharides in the filtrate are determined using either ion chromatography (IC) or gas chromatography (GC) in the presence of an internal standard to validate the results. If GC is used, the hydrolysed sample is reduced and acetylated, and the resulting alditol acetates of the monosaccharides are then separated and determined by GC.

5 Apparatus

5.1 Filtration equipment

5.1.1 Filtering flask, 250 ml.

5.1.2 Gooch filtering crucible, fritted glass, medium or fine porosity, 30 ml; adapter for the filtering crucible, siphon tube (optional).

NOTE 1 The choice of fritted glass porosity depends on the rate of filtration of the particular type of sample. For slow-filtering samples, the use of medium (M) porosity is preferable. Filtration can be facilitated by using a medium porosity crucible with a disc of fine porosity glass-fibre filter paper fitted over the sintered glass in the crucible.

NOTE 2 Other types of filtering crucibles, such as alundum or porous porcelain crucibles lined with a mat of fine fibres can also be used.

5.2 Constant temperature water bath, capable of maintaining a temperature of $(30 \pm 1)^\circ\text{C}$.

5.3 Autoclave, capable of maintaining a temperature of $(120 \pm 3)^\circ\text{C}$.

5.4 Drying oven, conduction type, maintained at $(105 \pm 2)^\circ\text{C}$.

A convection oven shall not be used, as this could lead to increased flare-ups and fire hazard, as well as loss of sample due to material being ejected from the crucible.

5.5 Analytical balance, accurate to 0,1 mg.

5.6 Equipment specific to the determination method

5.6.1 IC determination

Ion chromatograph (IC) with an anion-exchange column for monosaccharide determination and pulsed amperometric detector (PAD)

5.6.2 GC determination

5.6.2.1 Water bath, maintained at a temperature of $(40 \pm 0,5) ^\circ\text{C}$.

5.6.2.2 Gas chromatograph (GC) with a suitable column for monosaccharide determination and flame ionization detector (FID).

6 Reagents

All chemicals shall be of ACS or reagent grade.

6.1 Water, of high purity, distilled or deionized.

6.2 Monosaccharide standards

Monosaccharide standards, for calibration: arabinose, galactose, glucose, mannose and xylose. Prepare standard solutions of appropriate concentrations, each standard solution containing all five monosaccharides.

6.3 Sulfuric acid, 72 % (720 g/kg, specific gravity 1,633 8 at 20 °C). 72 % sulfuric acid is available commercially. It can also be prepared from concentrated sulfuric acid as follows:

Slowly add 650 ml of concentrated sulfuric acid (H_2SO_4 sp gr 1,84) to 400 ml of water, while cooling under a cold-water tap. When the temperature has reached equilibrium with the ambient temperature, adjust the specific gravity of the sulfuric acid solution to 1,633 8 with the use of a hydrometer by careful addition of concentrated sulfuric acid or water.

6.4 Petroleum ether.

6.5 Eluent solution (for IC determination)

The composition of this solution depends on the type of IC column to be used. Therefore, follow the recommendations given by the IC column supplier.

Reagents [6.6](#) to [6.13](#) are required only for GC determinations:

NOTE Alternative reagents (to those in [6.6](#) to [6.13](#)) and procedures (to those in [10.3.3.1](#), [10.3.3.2](#) and [10.3.3.3](#)) for the neutralization, reduction and derivatization steps, such as those described in TAPPI T249^[6] can also be used, provided that it is indicated in the report.

6.6 Ammonia, NH_3 conc. 25 % (g/100 g) 13 M (moles/l).

6.6.1 Ammonia, 12 M (moles/l). Mix 9 parts ammonia (25 %) with 1 part water.

6.7 Potassium hydroxide, KOH 7,5 M Weigh 105 g KOH pellets into a 250 ml beaker. Add approximately 150 ml water ([6.1](#)) while stirring. Transfer the solution into a 250 ml volumetric flask, using an additional 20 ml to 30 ml of water to rinse the beaker in order to complete the transfer. Allow the solution to cool to ambient temperature and dilute it to the mark with water.

6.8 Potassium borohydride, KBH_4 solution.

Dissolve 150 mg KBH_4 in 250 μl 13 M NH_3 and 750 μl distilled water in a septum vial (4 ml).

This solution shall be freshly prepared before use.

6.9 Acetic acid, CH_3COOH , concentrated.

6.10 Acetic acid anhydride, concentrated.

6.11 1-methylimidazole.

6.12 Ethanol, 95 ml/100 ml to 99 ml/100 ml (95 % to 99 %).

6.13 Sodium sulfate, Na_2SO_4 , water-free.

6.14 2-Deoxy-galactose.

6.14.1 Internal standard solution, 2-deoxy-galactose 20 mg/ml.

Weigh 1,00 g 2-deoxy-galactose to the nearest 0,1 mg, transfer it quantitatively to a 50 ml volumetric flask, and dilute to the mark with distilled water.

Other internal standards than 2-deoxy-galactose, such as fucose or myo-inositol, can also be used. However, this shall be specified in the report.

7 Sampling

Obtain a representative sample of lignin equivalent to about 2 g to 3 g on an air-dry basis. Report the origin of the sample and the sampling procedure. In particular, if the sample is first ground to ensure its homogeneity, and/or a sieving step is required in order to obtain samples with a uniform particle size or narrow particle size distribution, this shall be reported.

Lignin samples can contain a significant amount of resins. The resins shall be extracted with petroleum ether before testing. The percentage resin in the sample and the extraction method shall be included in the test report.

Extraction with petroleum ether should be carried out by a method similar to that described in ISO 14453^[7].

Although acetone is an effective solvent for extracting resin, it cannot be used here since it will also dissolve part of the lignin.

NOTE Resins, if not extracted from lignin samples prior to analysis, will remain insoluble in acid and be weighed as acid-insoluble lignin.

8 Drying

Prior to drying to complete dryness, lignin samples shall be air-dried to over 75 % (g/100 g) solids. This step is necessary in order to minimize the extent of lignin degradation reactions and the drying period.

Determine the dry matter content of the lignin by drying a 2 g to 3 g specimen in an oven (5.4) at $(105 \pm 2)^\circ\text{C}$ initially for 2 h, and then for additional 1-h periods up to a maximum of 7 h, until the difference in mass between two successive dryings in a desiccator, does not exceed 0,5 % mass fraction (g/100 g) of the test piece before drying.

9 Test Specimens

Weigh (5.5) a test specimen, equivalent to about 100 mg of oven-dried lignin, to the nearest 0,1 mg and transfer to a 100 ml beaker. Calculate and record the oven-dry mass W of the test portion, in grams.

10 Procedure

10.1 General

Carry out the entire procedure in duplicate.

10.2 Hydrolysis

Add 1,0 ml of 72 % sulfuric acid (6.3) to the test specimen in the beaker. Stir the contents of the beaker with a glass rod. To avoid losses, ensure that no material is sticking to the glass rod when it is removed. Place the beaker in a (30 ± 1) °C water bath (5.2) for 1 h. Stir occasionally.

Add 28 ml of water (6.1). Mix, cover the beaker with aluminium foil and place it in autoclave (5.3) at (120 ± 3) °C for 1 h. Remove the beaker from the autoclave and allow the beaker and its contents to cool to approximately 80 °C.

NOTE If the lignin is finely dispersed, it can require overnight or longer to settle.

10.3 Determination of monosaccharides

10.3.1 General

Carry out the determination according to either 10.3.2 or 10.3.3.

NOTE The determination of carbohydrate composition can also be carried out by HPLC (High Performance Liquid Chromatography), as mentioned in Reference [4] for example, provided that the results have been validated against those obtained with this document.

10.3.2 Determination using an IC instrument

10.3.2.1 Solution preparation

Without stirring the residue remaining after hydrolysis (from 10.2), which consists primarily of insoluble lignin, decant or siphon off the supernatant solution through a filtering crucible (5.1.2) into a 250 ml filtering flask (5.1.1). Transfer the filtrate to a 250 ml volumetric flask. Wash the precipitate on the filtering crucible with 2×30 ml warm water and add the washings to the volumetric flask. Rinse the filtering flask with a small amount of water and add the rinsings to the 250 volumetric flask. Add an appropriate volume of the internal standard 2-deoxy-galactose solution (6.14.1). Allow to cool to room temperature and fill up to the mark with water.

NOTE 1 The acid-insoluble lignin and acid-soluble lignin can be determined from the residue after hydrolysis, and from the filtrate in the volumetric flask, respectively, as described in ISO 24196[8].

NOTE 2 Transfer of the filtrate to a volumetric flask is not necessary if the determination of acid-soluble lignin is not required.

NOTE 3 Addition of the internal standard at this stage ensures that the ratio of monosaccharides to internal standard remains fixed throughout the entire procedure.

10.3.2.2 Calibration

Calibrate the IC instrument (5.6.1) using the monosaccharide standard solutions (6.2) containing the internal standard 2-deoxy-galactose (6.14.1). Use the conditions recommended by the manufacturer or