CALCIUM LIGNOSULFONATE (40-65)

New specifications prepared at the 69th JECFA (2008), published in FAO JECFA Monographs 5 (2008). An ADI of 0-20 mg/kg bw was established at the 69th JECFA (2008).

SYNONYMS Lignosulfonic acid, calcium salt (40-65)

DEFINITION Calcium lignosulfonate (40-65) is an amorphous material obtained from the sulfite pulping of softwood. The lignin framework is a sulfonated random polymer of three aromatic alcohols: coniferyl alcohol, *p*-coumaryl alcohol, and sinapyl alcohol, of which coniferyl alcohol is the principle unit. After completion of the pulping, the water-soluble calcium lignosulfonate is separated from the cellulose, purified (ultrafiltration), and acidified. The recovered material is evaporated and spray dried. The commercial product has a weight-average molecular weight range of 40,000 to 65,000.

DESCRIPTION Light yellow-brown to brown powder

FUNCTIONAL USES Carrier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water. Practically insoluble in organic solvents.
IR spectrum (Vol. 4)	The infrared absorption spectrum of a potassium bromide pellet of dried sample exhibits characteristic absorptions at 1210-1220 cm^{-1}, 1037 cm^{-1}, and 655 cm^{-1}.
UV spectrum (Vol. 4)	A 0.05% sample solution is diluted 1:10 and adjusted to a pH of 2.0-2.2 by addition of 3 drops of 5 M hydrochloric acid. This solution exhibits an absorption maximum at 280 nm.
Weight-average molecular weight	Between 40,000 to 65,000 (>90% of the sample ranges from 1,000 to 250,000) See description under TESTS
pH (Vol. 4)	2.7 - 3.3 (10% solution)
Calcium (Vol. 4)	Passes test (“General Methods, Identification Tests,” Volume 4)
Degree of sulfonation	0.3 – 0.7, on the dried basis See description under TESTS

PURITY

Calcium	Not more than 5.0 %, on the dried basis See description under TESTS
Loss on drying (Vol. 4)	Not more than 8.0% (105°, 24 h)

Reducing sugars	Not more than 5.0%, on the dried basis See description under TESTS
Sulfite	Not more than 0.5%, on the dried basis See description under TESTS
Total Ash	Not more than 14.0%, on the dried basis See description under TESTS
Arsenic (Vol. 4)	Not more than 1 mg/kg Determine by the atomic absorption hydride technique. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under "General Methods, Metallic Impurities"). Alternatively, determine arsenic using Method II of the Arsenic Limit Test, taking 3 g of the sample rather than 1 g, following the procedure for organic compounds.
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under "General Methods, Metallic Impurities").

TESTS

IDENTIFICATION TESTS

Weight-average molecular weight

Principle

Size-exclusion chromatography is used to obtain the molecular-weight distribution profile of the sample.

Reagents

(NOTE: All solutions and dilutions are to be made using distilled, deionized water)

Dimethylsulfoxide (DMSO), HPLC grade.

Disodium hydrogen phosphate ($Na_2HPO_4 \cdot 7H_2O$), Reagent grade

50 % sodium hydroxide (NaOH), Reagent grade

Sodium dodecylsulfate (SDS), Gradient grade (ultra grade)

Equipment

Size-exclusion chromatograph (Agilent Technologies or equivalent) equipped with autosampler, HPLC-pump, degassing unit, UV-detector or RI-detector, MALLS (Multi-Angle Laser Light Scattering) detector (Wyatt Technology or equivalent) with interference filters.

Columns - Glucose-divinylbenzene (DVB), 10^4 Å pore size, 500x10 mm (Jordi Associates or equivalent) and TSK gel PWXL 6 mm x 4 cm guard column (TOSOH Bioscience or equivalent)

Syringe filter - 0.2 μm GHP (Pall Corp. or equivalent)

Filter paper - 0.22 μm Millipore GSWP (Millipore Corp. or equivalent)

Eluent:

Weigh 1600.0 g of water into a 2 litre flask. Add 161.8 g DMSO, mix, and add 21.44 g $Na_2HPO_4 \cdot 7H_2O$. Adjust the pH to 10.5 with NaOH, add 1.6 g of SDS, and filter the mixture through the GSWP filter paper.

Sample solution

Accurately weigh and transfer 20 mg of previously dried sample into a 10-ml volumetric flask and dilute to the mark with water. Using the syringe filter, filter the solution into a vial.

Procedure

Set the oven temperature of the chromatograph at 60°. Begin the flow of eluent (1.0 ml/min - the pressure should not exceed 1000 psi.) through the chromatography system. After at least one hour has elapsed, inject the Sample solution (20 μl) onto the column and record the chromatograph. Calculate the weight-average molecular weight from the chromatogram using suitably certified software.

Degree of sulfonation

Principle

The Degree of sulfonation is the ratio of Organic sulfur to the Methoxyl content of the sample. Organic sulfur is calculated as the difference between Total sulfur (determined by elemental analysis) and Inorganic sulfur (determined by ion chromatography).

Determination of Total sulfur

Equipment and Reagents

Elemental Analyser (Thermo Fisher Scientific or equivalent)
Analytical balance
Tin capsules
BBOT standard (2,5-(Bis(5-tert-butyl-2-benzo-oxazol-2-yl) thiophene))
Vanadium pentoxide

Analytical conditions

Carrier gas - Helium	120 ml/min
Combustion furnace temp.	1000°
Oven temp.	70°
Helium pressure	150 kPa
Oxygen pressure	150 kPa
Oxygen loop	5 ml
Run time	300 sec.

System checks

Vanadium pentoxide
Vanadium pentoxide and BBOT

Procedure

System checks: Introduce small amounts of the two System checks separately into two tin capsules (no need to weigh). Run the two System checks through the analyzer. Observation of a sulfur peak in the chromatogram confirms that the system is working properly.

Standards: Introduce approximately 0.2 mg of vanadium pentoxide into each of four tin capsules and weigh them. Accurately weigh 0.5, 1.0, 1.5 and 2.0 mg of BBOT standard into the four capsules. Run the four standards through the analyzer and construct a calibration curve. The calibration curve should be a straight line with a correlation coefficient of at least 0.999.

Sample: Introduce approximately 0.2 mg of vanadium pentoxide into

each of two tin capsules and weigh them. Accurately weigh 1-2 mg of sample, previously dried, into each capsule and run them through the analyzer. Run additional samples in duplicate. After every fourth sample, accurately weigh 0.5-2.0 mg of the BBOT standard into a tared tin capsule containg 0.2 mg of vanadium pentoxide to run as a control. (NOTE: The weight of BBOT taken is chosen to fall within the calibration curve.) The standard deviation of the control BBOT standard should be no more than 0.20. Obtain the weight (mg) of total sulfur for each sample (w) from the calibration curve and calculate the percent Total sulfur for each by dividing by the weight of the corresponding sample taken (W) using the formula:

$$\% \text{ Total sulfur} = 100 \times w/W$$

Compute the average % Total sulfur.

Determination of Inorganic sulfur

(NOTE: All solutions and dilutions to be made using distilled, deionized water)

Equipment

Ion Chromatograph (Dionex Corporation or equivalent) with conductivity detector and autosampler

Anion Self-Regenerating Suppressor (ASRS-Ultra 4 or equivalent)

Analytical Column - IonPac AS 11 (Dionex Corporation or equivalent)

Guard Column - IonPac AG 11 (Dionex Corporation or equivalent)

Syringe filter - 0.2 μm GHP (Pall Corp. or equivalent)

Reagents

0.1 M NaOH (sodium hydroxide): 5.265 ml 50% NaOH (Reagent grade), diluted to 1000 ml

1% NaOH (sodium hydroxide): 2 ml 50% NaOH (Reagent grade), diluted to 100 ml

3% H_2O_2 (hydrogen peroxide): 50 ml 30% H_2O_2 (Reagent grade), diluted to 500 ml

Eluent: 0.1 M NaOH/water (10/90)

Stock standard solution

1 mg/ml, prepared by dissolving 0.1479 g sodium sulfate in 100 ml of water

Standard sulfate solutions (2.0 mg/l, 5.0 mg/l, 20.0 mg/l, and 40.0 mg/l)

Pipet 0.1, 0.25, 1.0 and 2.0 ml of the Stock standard solution into separate 50-ml volumetric flasks. Add 1 ml of 3 % H_2O_2, dilute to volume with water, and mix.

Sample solution

Accurately weigh and transfer 30 mg of previously dried sample into a 50-ml volumetric flask and dissolve it in 10 ml of 1% NaOH. Add 5 ml of 3% H_2O_2 and allow to stand overnight. Then, dilute to volume with water.

Procedure

(NOTE: Filter all solutions through the syringe filter prior to injection into the ion chromatograph.) Set the eluant flow rate to 0.7 ml/min.

Separately inject 10 µl of the standard sulfate solutions and the Sample solution and record the chromatograms for a run time of 15 min. (NOTE: The sulfate retention time is 7 min.) Construct a calibration curve and determine the sulfate concentration of the Sample solution. Determine the weight (mg) of sulfate in the sample, w, and calculate the percentage of Inorganic sulfur in the sample using the following equation:

$$\% \text{ Inorganic sulfur} = 100 \times w \times 32/(W \times 96)$$

where

W is the weight (mg) of the sample taken
32 is the formula weight of sulfur
96 is the formula weight of sulfate

Determination of Organic sulfur

$$\% \text{ Organic sulfur} = (\% \text{ Total sulfur}) - (\% \text{ Inorganic sulfur})$$

Determination of Methoxyl (-OCH_3)

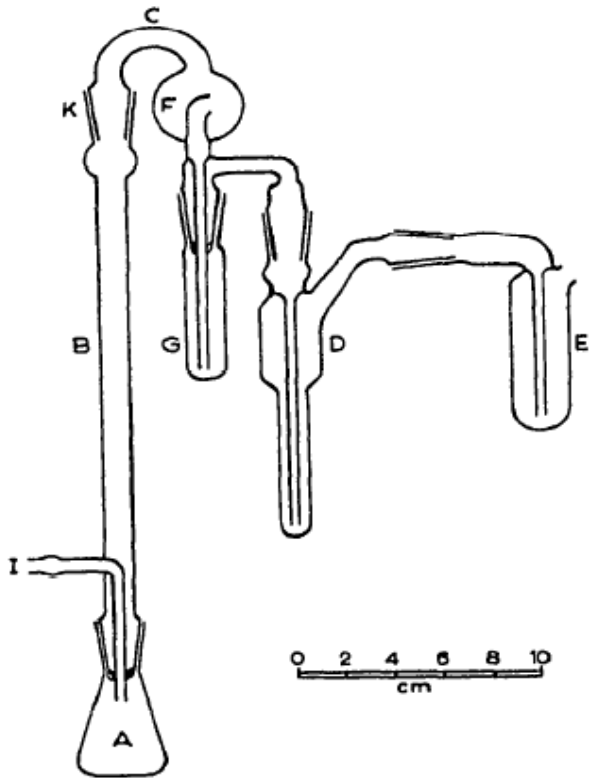
Principle

Heating with hydroiodic acid decomposes the sample to form methyl iodide which reacts to form iodine. The iodine is quantitatively determined by titration with sodium thiosulfate.

Reagents

Phenol, Reagent grade
Hydroiodic acid, HI, (min. 57%), Reagent grade
Red phosphorus
5% Cadmium sulfate ($CdSO_4$) solution
Bromine, Reagent grade
Formic acid (concentrated), Reagent Grade
1 M Sulfuric acid (H_2SO_4), Reagent grade
10% Potassium iodide solution (KI), Reagent grade
0.025 M Sodium thiosulfate ($Na_2S_2O_3$), Reagent grade
Acetic acid (glacial) saturated with Sodium acetate, Reagent grade
3 % Sodium carbonate (Na_2CO_3) solution

Equipment (Anal. Chem. Acta, vol. 15 (1956) p. 279-283)



Procedure

Accurately weigh 15-20 mg of previously dried sample on a small square of aluminium foil. Wrap the foil around the sample and put it into the reaction flask (A) to which 5 ml of hydroiodic acid, approx. 2 g of phenol, and a few glass beads have been added. Add 5 ml of 5% cadmium sulfate solution containing about 0.3 mg of red phosphorus into the washer (G). Add 10 ml of acetic acid (saturated with sodium acetate) and 10 droplets of bromine to the receiver (D). Finally, fill the U-trap (E) with sodium hydroxide or other suitable absorbant that will prevent bromine from leaving the system.

Pass nitrogen gas through a 3% Na_2CO_3 solution and into the system through the side arm (I) of the air condensor (B). Heat the reaction flask (A) to 140-145° for 1 hour in a glycerin bath. Wash the contents of the receiver (D) into a 250 ml Erlenmeyer flask containing 10 ml of acetic acid (saturated with sodium acetate). Rotate the flask and add formic acid dropwise until the colour disappears. Add 5 ml 10 % potassium iodide solution and mix. Then add 10 ml of 1 M sulfuric acid and let the flask stand for 3 minutes. Titrate the solution with 0.025 M $Na_2S_2O_3$ until the colour changes from yellowish to colourless. Calculate the percent methoxyl from the following equation:

$$\% \text{ Methoxyl} = V \times 0.025 \times 31 \times 100/(W \times 6 \times 1000)$$

where

V is the volume (ml) of sodium thiosulfate used in the titration
W is the weight (mg) of the sample taken
0.025 is the concentration of the sodium thiosulfate
31 is the formula weight of methoxyl

6 is stoichiometric conversion factor between the titrant and the methoxyl moiety

Degree of sulfonation

Calculation

(% Organic sulfur)/(% methoxyl)

PURITY TESTS

Calcium

Reagents

(NOTE: All solutions and dilutions to be made using distilled, deionized water)

Calcium reference standard, Certified 1000 ppm (Mallinckrodt or equivalent)

Nitric acid (65%), Reagent grade

Hydrogen peroxide (30%), Reagent grade

Cesium chloride, suprapur

Ionization buffer: 12.1 mg/ml of cesium chloride

Standard calcium solution

3 µg/ml, prepared by diluting with water 1.5 ml of the Calcium reference standard to 500 ml. Store in polyethylene bottles.

Sample solution

Accurately weigh 0.2 g of a previously dried sample into a graduated Pyrex flask. Add 5 ml of 65% nitric acid and 2 ml of 30% hydrogen peroxide. Boil the sample for 1 hour in a microwave oven. Dilute the sample stepwise and quantitatively to a suitable concentration level with purified water (< 0.00007 mS). A sample with 5% Calcium should be diluted by a factor of 5000 to give a final concentration of 2 µg/ml.

Procedure

Using a suitable atomic absorbtion spectrophotometer optimized according to the manufacturer's instructions, measure the absorbance of the Sample solution at 422.7 nm. By dilution of the working standard (manually or using the auto-diluter of the instrument) prepare solutions for constructing a 4-point calibration curve to correspond to a calcium content in the range 0 – 7.5 %, The sample and standard solutions and the Ionization buffer are mixed automatically by the sampling system of the instrument. Set the mixing ratio for standard/sample solutions to Ionization buffer at 3:1. Obtain the calcium concentration of the Sample solution from the calibration curve, determine the weight (g) of calcium in the sample, w, and calculate the percent of calcium in the previously dried sample from the equation:

$$\% \text{ Calcium} = 100 \times w/W$$

where W is the weight (g) of sample taken.

Reducing sugars

Principle

Reducing sugars react with p-hydroxybenzoichydrazide (PHBH) in alkaline environments. The substance formed absorbs yellow light at 410 nm. Calcium is used to enhance the colour.

Equipment

Flow Injection Analyser (O.I. Analytical or equivalent)
Cellulose membranes, Type C 25 MM (Astoria-Pacific or equivalent)

Reagents
Glucose, anhydrous quality for biochemistry analysis
Brij-35 ((Polyoxyethyleneglycol dodecyl ether), ultra grade (O.I. Analytical or equivalent)
Calcium Chloride, $CaCl_2$, Reagent grade
Citric Acid, Reagent grade
Hydrochloric Acid, HCl, Reagent grade
1 M Sodium Hydroxide, NaOH, Reagent grade
PHBH, p-Hydroxybenzoichydrazide (Sigma-Aldrich or equivalent)

Standard glucose solutions
100 mg/l, 1000 mg/l, and 2000 mg/l, prepared using deionized water

Sample solution
Accurately weigh 0.5 g of a previously dried sample into a 50-ml volumetric flask. Dissolve and dilute to volume with deionized water.

Procedure
(NOTE: Set the analyzer flow to the "low" position on both pumps and the temperature of the heater to 90°. The instrument should stabilize in about 15 minutes. The signal should be less than ±1000 micro-Absorbance Units before starting the analysis.) Introduce separately 100 µl of each of the Sample solution and Standard glucose solutions into the analyzer. For each analysis, air is introduced followed by addition of 0.2% Brij-35 at a continuous flow of 0.287 ml/min. The solutions are then dialyzed through a cellulose membrane. After dialysis, add 1M NaOH at 0.385 ml/min, $CaCl_2$ and PHBH, both at 0.074 ml/min, into the mixing chamber of the analyzer. The mixture then enters the heater (previously set at 90°) where bubbles are eliminated, after which it reaches the detector (set at 410 nm).

Run duplicate injections of every Sample solution. Construct a calibration curve from the Standard glucose solutions and obtain the concentration of reducing sugars in the Sample solution. Determine the weight (mg) of reducing sugars in the sample, w, and calculate the percentage of reducing sugars in the sample using the equation:

$$\% \text{ Reducing sugars} = 100 \times w/W$$

where
W is the weight (mg) of sample taken

Sulfite

Principle
Sulfite is stabilized in an aqueous solution with formaldehyde and subsequently separated from other anions utilizing an ion-exchange column.

Equipment
Ion Chromatograph ((Dionex Corporation or equivalent) with conductivity detector and autosampler
Anion Self-Regenerating Suppressor (ASRS-Ultra 4 or equivalent)
Analytical Column - IonPac AS 11 (Dionex Corporation or

equivalent)
Guard Column - IonPac AG 11 (Dionex Corporation or equivalent)
Syringe filter - 0.2 μm GHP (Pall Corp.or equivalent)

Reagents
(NOTE: All solutions and dilutions to be made using distilled, deionized water.)
Formaldehyde (37%), Reagent grade
Formaldehyde solution: 0.5 ml Formaldehyde (37%) diluted to 1000 ml (Prepare fresh on day of use.)
Sodium Sulfite (Na_2SO_3), Reagent grade.
0.1 M Sodium Hydroxide (NaOH), Reagent grade

Eluant
0.1 M NaOH/water (10/90)

Stock standard solution
1 mg/ml, prepared with 0.1574 g Na_2SO_3 in 100 ml of Formaldehyde solution.

Standard sulfite solutions
2.0 mg/l, 5.0 mg/l, 10.0 mg/l, and 20.0 mg/l, made with freshly prepared Formaldehyde solution

Sample solution
Accurately weigh and transfer about 0.15 g of sample, previously dried, into a 50-ml volumetric flask. Dilute to mark with Formaldehyde solution.

Procedure
(NOTE: Filter all solutions before injection into the Ion Chromatograph.) The chromatographic system is run isocratically with eluent flow rate of 0.7 ml/min. Separately inject 10 µl of the Standard sulfite solutions and the Sample solution and record the chromatograms for a run time of 15 min. The sulfite retention time is 6 min. Construct a calibration curve and determine the sulfite concentration of the Sample solution. Determine the weight (mg) of sulfite in the sample, w, and calculate the percentage of sulfite in the sample using the following equation:

$$\% \text{ Sulfite} = 100 \times w/W$$

where W is the weight (mg) of sample taken.

Total Ash

Accurately weigh 0.5 -1 g of a previously dried sample in a tared platinum crucible that has been cleaned with potassium bisulfate and dried at 105°. Heat the sample cautiously over a flame. Ignite at 550° for 1 hour, and then at 900° for at least 10 minutes, until all dark particles have disappeared and the ash is white. Allow the ash to cool in a desiccator and determine the weight (mg) of the residue (W_R).

$$\% \text{ Ash} = 100 \times W_R/W_S$$

where W_S (mg) is the weight of sample taken.