GLYCEROL ESTER of WOOD ROSIN

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 37th JECFA (1988), published in FNP 38 (1988) and in FNP 5 (1992). Metals and arsenic specifications revised at the 55th JECFA (2000). An ADI of 0- 25 mg/kg bw was established at 46th JECFA (1996)

SYNONYMS Ester Gum; INS No. 445

DEFINITION A complex mixture of tri- and diglycerol esters of resin acids from wood rosin obtained by the solvent extraction of aged pine stumps followed by a liquid-liquid solvent refining process. Excluded from these specifications are substances derived from gum rosin, and exudate of living pine trees, and substances derived from tall oil rosin, a by-product of kraft (paper) pulp processing. The final product is composed of approximately 90% resin acids and 10% neutrals (non-acidic compounds). The resin acid fraction is a complex mixture of isomeric diterpenoid monocarboxylic acids having the typical empirical formula of $C_{20}H_{30}O_2$, of which the main component is abietic acid. The substance is purified by steam stripping or by countercurrent steam distillation.

C.A.S. number 8050-30-4

DESCRIPTION Hard, yellow to pale amber-coloured solid

FUNCTIONAL USES Chewing gum base component, emulsifier and stabilizer/density adjustment agent for flavouring oils in beverages

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water, soluble in acetone

Infrared absorption The infrared spectrum of a thin film of the sample deposited on a potassium bromide plate corresponds with the typical infrared spectrum below

Gas chromatography of resin alcohols and glycerol Passes test
See description under TESTS

PURITY

Test for absence of tall oil rosin (sulfur test) Passes test
See description under TESTS

Specific gravity (Vol. 4) d (20, 25): Not less than 0.935 when determined in a 50% solution in d-limonene (97%, boiling point 175.5-176.0º, d (20, 4): 0.84)

Ring and ball softening point Between 82 and 90º
See description under TESTS

Acid value (Vol. 4) Between 3 and 9

Hydroxyl number	Between 15 and 45 See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

TESTS

IDENTIFICATION
TESTS

Gas chromatography of resin alcohols and glycerol

The complex ester groups in the glycerol esters of wood rosin are reduced by reaction with a metal hydride (sodium bis(2-methoxy-ethoxy) aluminium dihydride) in toluene solution to form a mixture of resin alcohols and glycerol. Excess reagent is then hydrolyzed with aqueous acid forming two phases. Gas chromatography of the toluene phase produces a chromatogram of the resin alcohols that is characteristic of wood rosin ester and readily distinguishable from gum and tall oil rosin esters. Wood rosin can be differentiated from tall oil rosin by the proportions of abietyl and dehydroabietyl alcohols: in wood rosin, abietyl predominates, while in tall oil rosin, dehydroabietyl predominates. Wood rosin can be differentiated from gum rosins by the relative proportions of isopimaryl and palustryl alcohols: in wood rosin, isopimaryl predominates, while in gum rosins, palustryl predominates. Chromatography of the neutralized aqueous phase on a different column verifies the presence of glycerol.

Apparatus
- Gas Chromatograph equipped with a flame ionization detector.
- Chromatographic Column I: DB-1 methyl silicone (bonded and cross-linked), 15 m, wide-bore capillary (0.53 mm i.d.), film thickness 1.5 µm, temperature range 60^{o} to 300/320^{o} (e.g., J & W Scientific Inc,. Cat. No. 125-1012). A direct flash vaporization injection port liner is recommended.
- Chromatographic Column II: DB-WAX polyethyleneglycol (bonded and cross-linked), 15 m, wide- bore capillary (0.53 mm i.d.), film thickness 1.0 µm, temperature range 20^{o} to 230^{o}(e.g., J & W Scientific Inc., Cat, No. 125-7012).
- Recorder: 0 to 1 V
- Syringe: 5 µl
- Pipet (transfer): 3.0 ml, 5.0 ml
- Flask: Erlenmeyer, 25 ml
- Vial: 17 ml
- Analytical Balance: capable of weighing to the nearest 0.001 g
- Tube: centrifuge, 15 ml, graduated
- Centrifuge: table top, capable of achieving 3200 rpm
- Stirring Bars: Teflon coated, 1 inch
- Magnetic Stirrer

Reagents
- Toluene, reagent grade.

- Sodium Vitride™ Reagent [Sodium bis(2-methoxyethoxy) aluminium dihydride, pract. ~ 70% in toluene (~ 3.5 mol/l)] (Fluka Chemical Corp., Hauppage, NY, USA). Pipet 10.0 ml into a 100 ml volumetric flask. Dilute to volume with toluene and mix thoroughly.
- Hydrolysis Solution: Slowly add 50 ml of concentrated sulfuric acid, reagent grade, to 200 ml distilled water while stirring in an ice bath. Cool to room temperature.
- Phenolphthalein Solution: 1% in ethanol.
- Sodium Hydroxide Solution: Dissolve 16 g of reagent grade NaOH in 70-80 ml of distilled water and cool to room temperature. Dilute to 100 ml with distilled water and mix thoroughly. Store in a polyethylene bottle.
- 1,4-Butanediol: 99+%
- Glycerol: 99+%
- Internal Standard Solution: weigh 0.1 g 1,4-butanediol into a 100 ml volumetric flask. Dilute to volume with distilled water and mix thoroughly.
- Glycerol Solution: weigh 0.1 g of 1,4-butanediol and 0.1 g glycerol into a 100 ml volumetric flask. Dilute to volume with distilled water and mix thoroughly.

Gas Chromatograph Operating conditions

I (Resin alcohols)

Temperatures
- Column: Isothermal, 190º
- Inlet: 250º
- Detector: 250º

Flow Rates
- Carrier Gas (He): 30 ml/min at 63 psi
- Hydrogen: 30 ml/min
- Air: 240 ml/min

II (Glycerol)

Temperatures
- Column: Programmed, 120 to 200° at 6°/min
- Inlet: 250º
- Detector: 250º

Flow Rates
- Carrier Gas (He): 30 ml/min at 60 psi
- Hydrogen: 30 ml/min
- Air: 240 ml/min

Procedure I (Resin)

Weigh 250-300 mg sample into a 25 ml Erlenmeyer flask containing a stirring bar. Pipet 5.0 ml toluene into the flask and stir magnetically until sample is dissolved. Pipet 5.0 ml of Sodium Vitride™ Reagent into the flask, cap, and stir for 30 min. Uncap and, while stirring, pipet 3.0 ml of Hydrolysis Solution into the flask. Continue stirring for 3 min. Transfer contents of flask to centrifuge tube, stopper, and shake vigorously. Vent and centrifuge at 2800-3200 rpm for 5 min. Inject 0.5 µl of the upper layer into the gas chromatograph operating under the specified conditions and record the chromatogram. Compare with the chromatograms shown below to verify the approximate retention order of the resin alcohols.

Procedure II (Glycerol)

Using a pipet or hypodermic syringe, remove the toluene layer and part of

the aqueous layer leaving approximately 2 ml of the aqueous layer in the centrifuge tube. Add 1 drop of phenolphthalein solution and neutralize with the Sodium Hydroxide Solution. Aluminium salts will precipitate. Pipet 5 ml of the Internal Standard Solution into the tube, dilute to 15 ml with distilled water, stopper, shake, and then centrifuge at 2800-3200 rpm for 5 min. Inject 1 µl of the clear supernatant liquid into the gas chromatograph operating under the specified conditions and record the chromatogram. Inject 1 µl of the Glycerol Solution and record the chromatogram. Measure the retention times of any observed peaks relative to 1,4-butanediol. Compare retention times to that of glycerol.

PURITY TESTS

Test for absence of tall oil rosin (sulfur test)

When sulfur-containing organic compounds are heated in the presence of sodium formate, the sulfur is converted to hydrogen sulfide which can readily be detected by the use of lead acetate paper. A positive test indicates the use of tall oil rosin instead of wood rosin.

Apparatus
- Test Tube: Use a standard, 10 x 75 mm, heat-resistant, glass test tube.
- Burner, Bunsen: A small size burner of the microflame type is preferred.

Reagents
- Sodium Formate Solution: Dissolve 20 g of reagent grade sodium formate, NaOOCH, in 100 ml of distilled water.
- Lead Acetate Test Paper: Commercially available from most chemical supply houses.

Procedure
Weigh 40-50 mg of sample into a test tube and add 1- 2 drops of a 20% (w/v) solution of sodium formate. Place a strip of lead acetate test paper over the mouth of the test tube. Heat the tube with a flame until fumes are formed that contact the test paper. Continue heating for 2-5 min. There must be no formation of a black spot of lead sulfide indicating the presence of sulfur-containing compounds. (Detection Limit: 50 mg/kg sulfur).

Ring and ball softening point

The ring-and-ball softening point is defined as the temperature at which a disk of the sample held within a horizontal ring is forced downward a distance of 1 in. (25.4 mm) under the weight of a steel ball as the sample is heated at a prescribed rate in a water or glycerol bath.

Apparatus
The apparatus illustrated in Figs.1 and 2 consists of the components described in the following paragraphs.

Ring
A brass-shouldered ring conforming to the dimensions shown in Figure 1a should be used. If desired, the ring may be attached by brazing or other convenient manner to a brass wire of about 13 B & S gauge (0.06 to 0.08 in., or 1.52 to 2.03 mm, in diameter) as shown in Figure 2a.

Ball

A steel ball, 3/8 in. (9.53 mm) in diameter, weighing between 3.45 and 3.55 g, should be used.

Ball-Centering Guide
A guide for centering the ball, constructed of brass and having the general shape and dimensions illustrated in Figure 1c, may be used if desired.

Container
Use a heat-resistant glass vessel, such as an 800-ml low-form Griffin beaker, not less than 3.34 in. (8.5 cm) in diameter and not less than 5 in. (12.7 cm) in depth from the bottom of the flare.

Support for Ring and thermometer
Any convenient device for supporting the ring and thermometer may be used, provided that it meets the following requirements: (1) the ring shall be supported in a substantially horizontal position; (2) when using the apparatus shown in Figure 1d, the bottom of the ring shall be 1.0 in. (25.4 mm) above the horizontal plate below it, the bottom surface of the horizontal plate shall be at least 0.5 in. (13 mm) and not more than 0.75 in. (18 mm) above the bottom of the container, and the depth of the liquid in the container shall be not less than 4.0 in. (10.2 cm); (3) when using the apparatus shown in Figure 1e, the bottom of the ring shall be 1.0 in. (25.4 mm) above the bottom of the container, with the bottom end of the rod resting on the bottom of the container, and the depth of the liquid in the container shall be not less than 4.0 in. (10.2 cm), as shown in Figure 1 a, b and c; and (4) in both assemblies, the thermometer shall be suspended so that the bottom of the bulb is level with the bottom of the ring and within 0.5 in. (13 mm) but not touching the ring.

Thermometers (mercury-in-glass)
Depending upon the expected softening point of the sample, use either an ASTM 15C low-softening-point thermometer (-2º to 80º) or an ASTM 16C high-softening-point thermometer (30º to 200º).

Stirrer
Use a suitable mechanical stirrer rotating between 500 and 700 rpm. To ensure uniform heat distribution in the heating medium, the direction of the shaft rotation should move the liquid upward. (See Figure 2d for recommended dimensions.)

Sample Preparation
Select a representative sample of the material under test consisting of freshly broken lumps free of oxidized surfaces. Scrape off the surface layer of samples received as lumps immediately before use, avoiding inclusion of finely divided material or dust. The amount of sample taken should be at least twice that necessary to fill the desired number of rings, but in no case less than 40 g. Immediately melt the sample in a clean container, using an oven, hot plate, or sand or oil bath to prevent local overheating. Avoid incorporating air bubbles in the melting sample, which must not be heated above the temperature necessary to pour the material readily without inclusion of air bubbles. The time from the beginning of heating to the pouring of the sample shall not exceed 15 min. Immediately before filling the rings, preheat them to approximately the same

temperature at which the sample is to be poured. While being filled, the rings should rest on an amalgamated brass plate. Pour the sample into the rings so as to leave an excess on cooling. Cool for at least 30 min, and then cut off the excess material cleanly with a slightly heated knife or spatula. Use a clean container and a fresh sample if the test is repeated.

Procedure

Materials Having Softening Points above 80º: Fill the glass vessel with glycerol to a depth of not less than 4.0 in. (10.2 cm) and not more than 4.25 in. (10.8 cm). The starting temperature of the bath shall be 32º. For resins (including rosin), the glycerol should be cooled to not less than 45º below the anticipated softening point, but in no case lower than 35º. Position the axis of the stirrer shaft near the back wall of the container, with the blades clearing the wall and with the bottom of the blades 0.75 in. (18 mm) above the top of the ring. Unless the ball-centering guide is used, make a slight indentation in the center of the sample by pressing the ball or a rounded rod, slightly heated for hard materials, into the sample at this point. Suspend the ring containing the sample in the glycerol bath so that the lower surface of the filled ring is 1.0 in. (25.4 mm) above the surface of the lower horizontal plate (see Figure 1d), which is at least 0.5 in. (13 mm) and not more than 0.75 in. (18 mm) above the bottom of the glass vessel, or 1.0 in. (25.4 mm) above the bottom of the container (see Figure 2e). Place the ball in the glycerol but not on the test specimen. Suspend an ASTM high-softening-point thermometer (16C) in the glycerol so that the bottom of its bulb is level with the bottom of the ring and within 0.5 in. (13 mm) but not touching the ring. Maintain the initial temperature of the glycerol for 15 min, and then, using suitable forceps, place the ball in the center of the upper surface of the sample in the ring. Begin stirring, and continue stirring at 500 to 700 rpm until completion of the determination. Apply heat at such a rate that the temperature of the glycerol is raised 5º per min, avoiding the effects of drafts by using shields if necessary.

[Note: The rate of rise of the temperature shall be uniform and shall not be averaged over the test period. Reject all tests in which the rate of rise exceeds ±0.5º for any minute period after the first three.]

Record as the softening point the temperature of the thermometer at the instant the sample touches the lower horizontal plate (see Figure 1d) or the bottom of the container (see Figure 2e). Make no correction for the emergent stem of the thermometer.

Materials Having Softening Points of 80º or Below: Follow the above procedure, except use an ASTM low-softening-point thermometer (15C) and use freshly boiled water cooled to 5º as the heating medium. For resins (including rosins), use water cooled to not less than 45º below the anticipated softening point, but in no case lower than 5º.

Hydroxyl Number

Hydroxyl number is defined as the number of mg of potassium hydroxide required to neutralize a sulfonyl carbamate reagent capable of combining with the hydroxyl groups in 1 g of sample. Hydroxyl groups in an organic compound react rapidly with excess p-toluenesulfonyl isocyanate (p-TSI) in an inert solvent to form a sulfonyl carbamate. After reaction of the

excess reagent with water to form p-toluenesulfonamide, the acidic sulfonyl carbamate is titrated potentiometrically or visually with methanolic KOH. Corrections must be made for reagent blank and the presence of any acidic compounds.

Apparatus

- Automatic Recording Potentiometric Titrator: Use pH range 0-14, a speed setting of 4 (about 2 ml/min) and a 20 ml buret.
- Glass Electrode
- Calomel Electrode
- Flasks, Erlenmeyer: 250 ml, with ground glass joints
- Allihn Condenser: 300 mm, with ground glass joint to fit Erlenmeyer flask.

Reagents

- Benzoic Acid, primary standard grade
- Methanol, reagent grade, anhydrous
- Toluene, reagent grade, dried over molecular sieve, 3Å
- Methanolic KOH, 0.1N: Dissolve 7 g KOH in one litre methanol. Standardize by weighing, to the nearest 0.0001 g, about 0.12 g of benzoic acid into a 150 ml beaker. Add a stirring bar and 80 ml of 1:1 toluene:methanol. Stir the solution thoroughly while titrating with the alcoholic KOH solution. The titrant should be introduced beneath the surface of the liquid away from the electrodes. Calculate the normality of the alcoholic KOH solution according to the equation shown under calculations
- Tetrahydrofuran (THF): Dry overnight over molecular sieve, 3Å
- p-Toluenesulfonyl isocyanate (p-TSI)
- p-Toluenesulfonyl isocyanate solution: Approximately 0.22 M or 4.5 meq. per 20 ml aliquot. Pipet 15.0 ml p-TSI into 500 ml of dry tetrahydrofuran (THF) and mix thoroughly

Caution

Because of the toxic nature of isocyanates, reagent preparation and subsequent operations using the reagent solution should be carried out in a hood. Gloves should be worn when handling the reagent and its solution in order to avoid contact with the skin.

- Bromocresol purple indicator solution: Add 0.1 g bromocresol purple to 18.5 ml 0.1N NaOH and dilute to 250 ml with distilled water

Procedure

Weigh a 1.0-1.5 g sample to the nearest 0.0001 g into a dry 250 ml Erlenmeyer flask fitted with a ground glass joint. Pipet 10.0 ml of dry THF into the flask and dissolve the sample. Pipet 20.0 ml of the p-TSI solution into the flask and swirl to mix. Add a boiling chip, attach a condenser, and place the flask on a hot plate. Heat to boiling and reflux for 10 min. While still refluxing, add through the condenser, in a single portion, the appropriate volume of water:

	Blank	*Sample*
Visual Titration	2	2
Potentiometric Titration	1	1

Remove the flask and condenser from the hot plate and allow to cool to room temperature. Rinse down the condenser with 5 ml THF. Disconnect the condenser and quantitatively transfer the contents of the flask to a 150 ml beaker with the aid of 50 ml of THF. Add a stirring bar and insert the electrodes or add 20 drops of Indicator Solution. Titrate potentiometrically or visually with the alcoholic KOH while stirring magnetically. The titrant should be introduced beneath the surface of the liquid away from the electrodes. Determine the volume of titrant required by the sample. Determine the volume of titrant required by a reagent blank carried through the entire procedure.

Calculations

Calculate the normality of the alcoholic KOH as follows:

$$Normality,\ N = \frac{W \times 1000}{122.1 \times KOH}$$

where
W = g benzoic acid
122.1 = equivalent weight of benzoic acid
KOH = ml alcoholic KOH

Calculate the hydroxyl number of the sample as follows:

$$Hydroxyl\ Number = \frac{(A - B) \times N \times 56.1}{g\ sample} - AV$$

where
A = sample titration, ml
B = reagent blank titration, ml
N = normality of methanolic KOH
56.1 = mg of KOH per milliequivalent
AV = Acid value

Arsenic (Vol. 4)

Prepare the sample solution as follows: Transfer a 1 g sample, accurately weighed, into a Kjeldahl flask, rest the open end of the flask in a Kjeldahl fume bulb attached to a water aspirator, add 5 ml of sulfuric acid and 4 ml of 30% hydrogen peroxide, and digest over a small flame. Continue adding the peroxide in 2 ml portions, allowing the reaction to subside between additions, until all organic matter is destroyed, fumes of sulfuric acid are copiously evolved, and the solution becomes colourless. Maintain oxidizing conditions at all times during the digestion by adding peroxide whenever the mixture turns brown or darkens. (The amount of peroxide required to completely digest the samples will vary, but as much as 200 ml may be required in some cases, depending upon the nature of the material). Cool, cautiously add 10 ml of water, again evaporate to strong fuming, and cool, Transfer the solution into an arsine generator flask, wash the Kjeldahl flask and bubble with water, adding the washings to the generator flask, and dilute to 35 ml with water. This solution meets the requirements of the Limit Test.

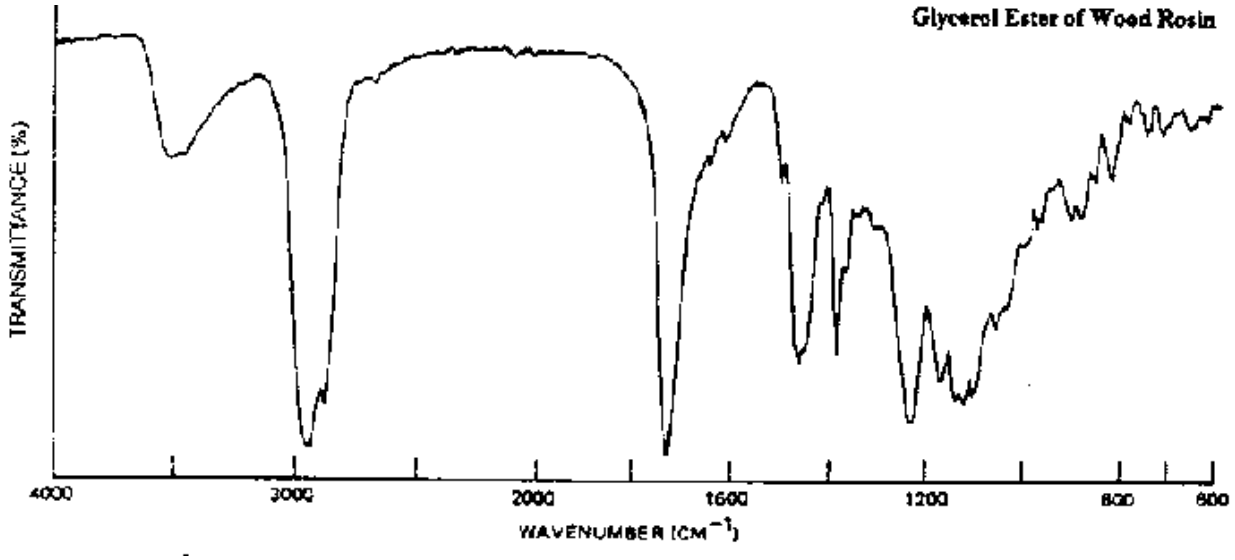
Lead (Vol. 4)

Transfer a 5 g sample, accurately weighed, into a porcelain dish or casserole, heat on a hot plate until completely charred, then heat in a

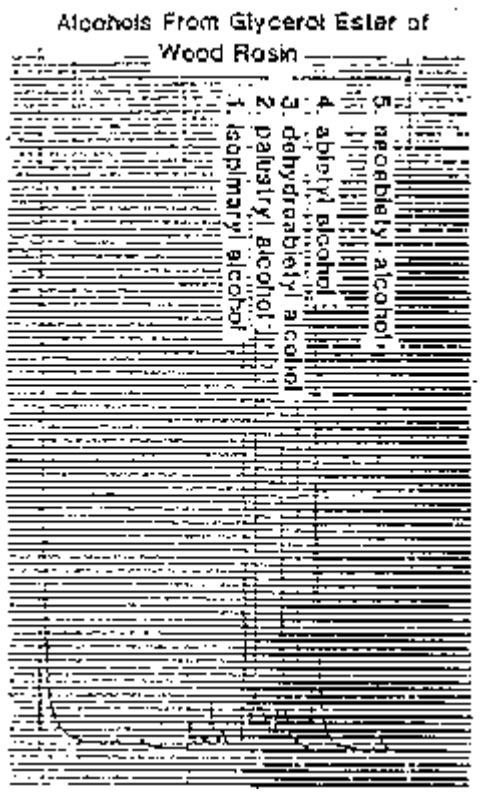
muffle furnace at 480º for 8 h or overnight, and cool. Cautiously add 5 ml of nitric acid, evaporate to dryness on a hot plate, then heat again in the muffle furnace at 480º for exactly 15 min, and cool. Extract the ash with two 10-ml portions of water, filtering each extract into a separator. Leach any insoluble material on the filter with 6 ml of ammonium citrate TS, 2 ml of hydroxylamine hydrochloride TS, and 5 ml of water, adding the filtered washings to the separator. Continue as directed under Procedure in the Limit Test, beginning with "To the separator add 2 drops of phenol red TS...", using 10 µg lead ion (Pb) in the control.

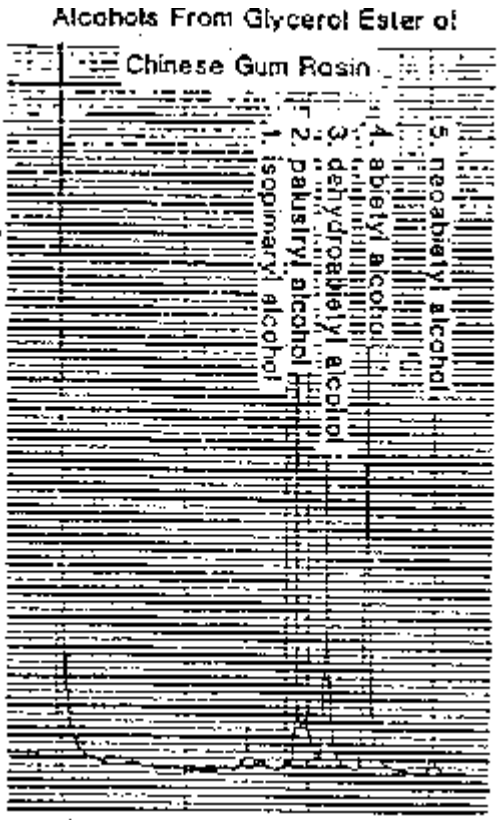
Infrared spectrum

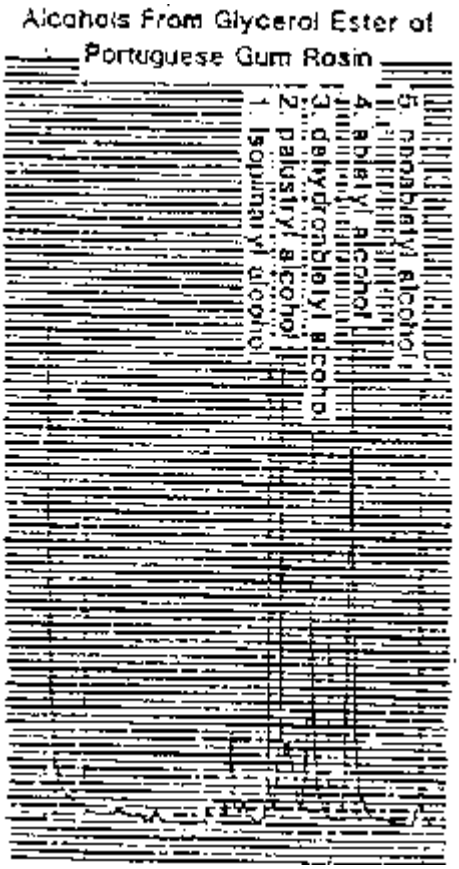
Glycerol ester of wood rosin

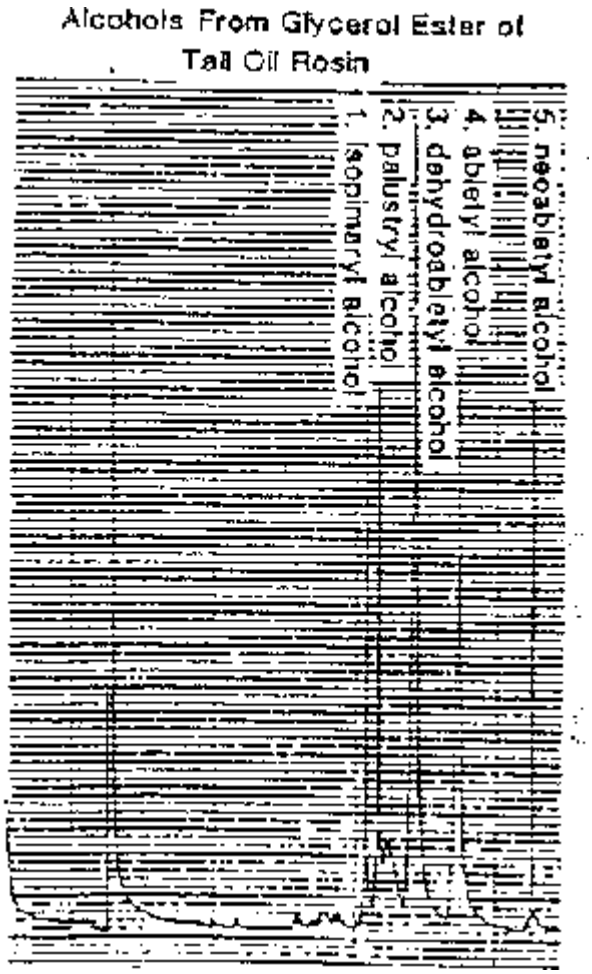


Gas chromatography of resin alcohols



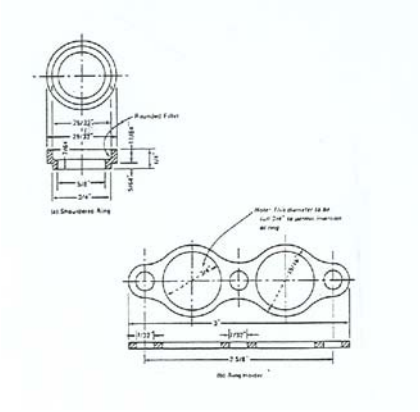






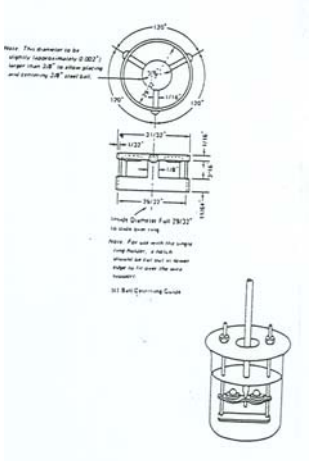
Apparatus - Ring and Ball Softening Point

(a)Shouldered Ring



(b)Ring Holder

(c)Bell Centering Guide

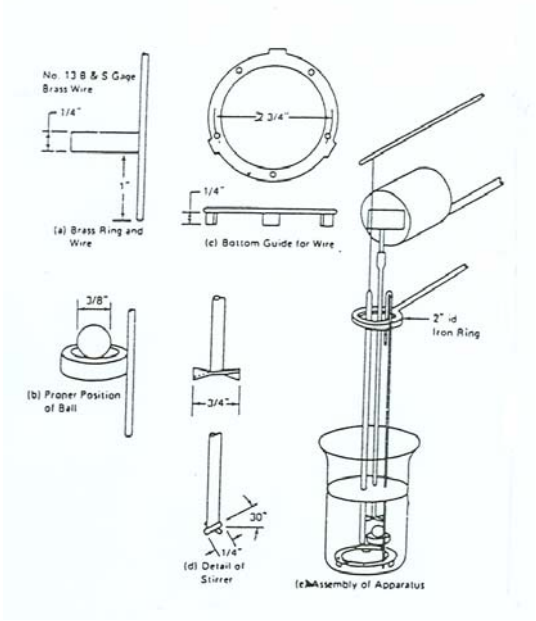


(d)Assembly Apparatus with Two Rings

Figure 1. Shouldered Ring, Ring Holder Ball-Centering Guide, and Assembly of Apparatus Showing Two Rings

(a)Brass Ring and Wire

(c)Bottom Guide for Wire



(b)Proper Position of Ball

(e)Assembly of Apparatus

(d)Detail of Stirrer

Figure 2. Assembly of Apparatus Showing Stirrer and Single Shouldered Ring
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