MICROCRYSTALLINE WAX

Prepared at the 55th JECFA (2000) and published in FNP 52 Add 8 (2000), superseding specifications prepared at the 49th JECFA (1997) and published in FNP 52 Add 5 (1997). A group ADI of 0-20 mg/kg bw was established at the 44th JECFA (1995).

SYNONYMS Petroleum wax; INS No. 905c(i)

DEFINITION Microcrystalline Wax is a refined mixture of solid, saturated hydrocarbons, mainly branched paraffin, obtained from petroleum

DESCRIPTION Colourless or white, somewhat translucent, tasteless and odourless wax

FUNCTIONAL USES Chewing gum base, protective coating, defoaming agent, surface finishing agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) — Insoluble in water, very slightly soluble in ethanol, sparingly soluble in diethyl ether and hexane

Refractive index (Vol. 4) — n (100, D): 1.434 - 1.448

Infrared absorption — The infrared absorbance spectrum of the sample melted and prepared on a caesium or potassium bromide plate corresponds to the spectrum in the Appendix

PURITY

Viscosity, 100° — Not less than 11 mm^2/sec
See description under TESTS

Carbon number at 5% distillation point — Not more than 5% of molecules with carbon number less than 25
See description under TESTS

Average molecular weight — Not less than 500
See description under TESTS

Residue on ignition — Not more than 0.1%
See description under TESTS

Colour — Passes test
See description under TESTS

Sulfur — Not more than 0.4%
See description under TESTS

Lead (Vol. 4)

Not more than 3 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 on Volume 4, "Instrumental methods".

Polycyclic aromatic hydrocarbons

The sample shall meet the following ultraviolet absorbance limits when subjected to the analytical procedure described under the TESTS.

nm	max. absorbance per cm path length
280 - 289	0.15
290 - 299	0.12
300 - 359	0.08
360 - 400	0.02

TESTS

PURITY TESTS

Viscosity, 100°

(ASTM D 445 Adopted with permission from the Annual Book of ASTM Standards copyright American Society for Testing and Materials, 100 Harbor Drive, West Conshohocken, PA 19428. Copies of the complete ASTM standard may be purchased direct from ASTM, phone 610-832-9585, fax: 610-832-9555, e-mail: service@astm.org , website: http://www.astm.org)

Use a viscometer of the glass capillary type, calibrated and capable of measuring kinematic viscosity with a repeatability exceeding 0.35% only in one case in twenty. Immerse the viscometer in a liquid bath at the temperature required for the test ± 0.1° ensuring that at no time of the measurement will any portion of the sample in the viscometer be less than 20 mm below the surface of the bath. Charge the viscometer with sample in a manner directed by the design of the instrument. Allow the sample to remain in the bath for about 30 min. Where the design of the viscometer requires it, adjust the volume of sample to the mark. Use pressure to adjust the head level of the sample to a position in the capillary arm of the instrument about 5 mm ahead of the first mark. With the sample flowing freely, measure, in seconds (±0.2 sec), the time required for the meniscus to pass from the first to the second timing mark. If the time is less than 200 s, select a viscometer with a capillary of smaller diameter and repeat the operation. Make a second measurement of the flow time. If two measurements agree within 0.2%, use the average for calculating the kinematic viscosity. If the measurements do not agree, repeat the determination after thorough cleaning and drying the viscometer.

Viscosity, 100° (mm^2/sec) = C x t

where
C = calibration constant of the viscometer (mm^2/s)
t = flow time (s)

Carbon number at 5% distillation point

(ASTM D 5442 See TEST for Viscosity, 100° for Copyright permission) "Carbon number" is number of carbon atoms in a molecule. Determine the carbon number distribution of the sample by gas chromatography. Below is shown some typical working conditions for determination of up to carbon number 45.

Column length (m)	25	30	15
inside diameter (mm)	0.32	0.53	0.25
stationary phase	DB-1	RTX-1	DB-5
	methyl silicone	methyl silicone	5% phenyl methyl silicone
film thickness (µm)	0.25	0.25	0.25
Carrier gas	helium	helium	helium
flow (ml/min)	1.56	5.0	2.3
Linear velocity (cm/sec)	33	35	60
Temperature program			
initial temperature	80°	80°	80°
rate (°/min)	10	8	5
final temperature	380°	340°	350°
Injection technique	cool on-column	cool on-column	cool on-column
Detector	FID	FID	FID
temperature	380°	340°	375°
Sample size (µl)	1.0	1.0	1.0

NOTE: By optimizing the length of separation column and/or column temperature, waxes with carbon number higher than 45 can also be included.

Standards for calibration and identification

Standard samples of normal paraffins covering the carbon number range of the sample of purity greater than 95%.

Linearity standard

Prepare a weighed mixture of n-paraffins covering the range between C_{16} to C_{65} and dissolve the mixture in cyclohexane. Use approximately equal amounts of each of the paraffins weighed with 1% accuracy. Solutions of 0.01 % (w/w) may be used. It is not necessary to include every n-paraffin in this mixture so long as it contains C_{16}, C_{44}, (C_{60} if determination of higher carbon numbers is relevant) and at least one of every fourth n-paraffin. This standard must be capped tightly to prevent solvent loss.

Internal standard solution

Prepare a stock solution containing 0.5% (w/w) n-C_{16} in n-hexane. (minimum purity of 98%) by accurately weighing approximately 0.4 g n-C_{16} into a 100 ml volumetric flask. Add 100 ml of cyclohexane and reweigh. Record the mass of n-C_{16} (W_{ISTD}) to within 0.001 g and the mass of the

stock solution (Ws) to within 0.1 g. Prepare a dilute solution of internal standard by diluting one part of stock solution with 99 parts of cyclohexane. Calculate the concentration of internal standard using the following equation:

$$C_{INST} = \frac{W_{ISTD}}{Ws} \times \frac{100}{100} \% \text{ (w/w)}$$

Where
C_{INST} = concentration of n-C_{16} in the internal standard dilute solution in % (w/w)
W_{ISTD} = weight of n-C_{16} used for the stock solution in g.
Ws = Weight of the stock solution in g

Check of solvent blank
Inject 1 µl of the solvent. No peaks must be detected within the retention time range over which the wax elutes.

Column resolution
Inject 1 µl of a solution of 0.05 % each of n-C_{20} and n-C_{24} in cyclohexane. The column resolution R not be less than 30 as calculated by the following equation:

$$R = \frac{2d}{1.699\ (W1 + W2)}$$

Where
d = the distance in mm between the peak maxima of n-C_{20} and n-C_{24}
W1 = the peak with in mm of half height of n-C_{20}
W2 = the peak with in mm of half height of n-C_{24}

Linearity
Analyze the linearity standard. The calculated mass response factors relative to hexadecane must be between 0.90 and 1.10.

Retention time repeatability
Analyze the linearity standard in duplicate. The retention times for duplicate analysis must not differ more than 0.10 min between duplicate runs.

Calibration for n-paraffin identification
Determine the retention time of each n-paraffin in the range from C_{16} to C_{44} (or C_{60} if determination of higher carbon numbers is relevant) by injecting small amounts of each paraffin either separately or in known mixtures.

Sampling
Heat the sample to 10° above the temperature at which the wax is completely molten. Mix well by stirring.. Using a clean eyedropper, transfer a few drops to the surface of a clean sheet of aluminium foil, allow to solidify and break into pieces. Aluminium foil usually contains a thin film of oil from processing. This oil must be removed by rinsing the foil with solvents such as hexane or mineral spirits, prior to use.

Procedure

Accurately weigh about 0.0100 g of the sample (W_{sample}) obtained as described under sampling into a glass vial of approximately 15 ml capacity. Add approximately 12 ml of dilute internal standard solution, cap the vial and determine the exact weight of the added dilute internal standard solution (W_{INSTD}). Agitate the vial until the wax is completely dissolved using gentle heating if necessary.

Inject 0.5 to 1.0 µl of the sample solution. Record the chromatogram and store the detector signal. The peak from the internal standard must be completely resolved from the wax sample area. Based on the retention time as obtained under Calibration for n-paraffin identification, identify the normal paraffin peaks. Using a vertical drop to a horizontal baseline construction (see Figure 1), integrate the detector signal. Sum the area of all the peaks of each carbon number. By convention, the peaks assigned the carbon number n are those that elute between the valley immediately following the normal paraffin peak (C_{n-1}) and the corresponding valley following the next normal paraffin peak (C_n).

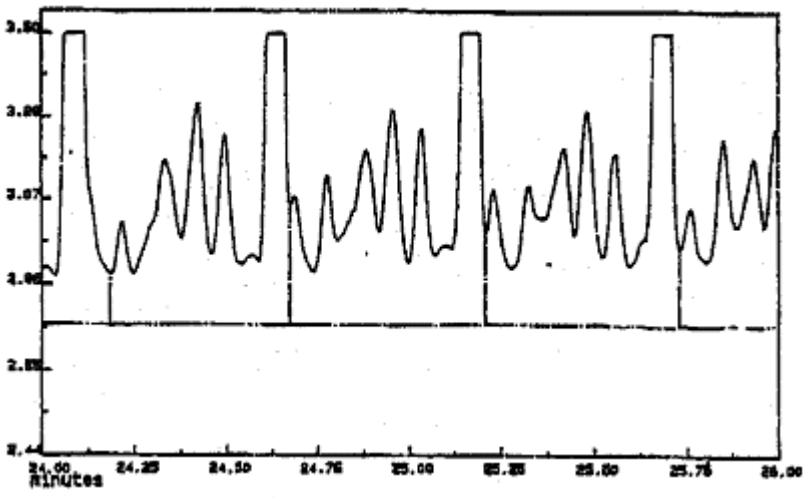

Figure 1. Carbon number summation (vertical drop to horizontal baseline)

Calculation

For each carbon number, calculate the content in % (w/w) by using the following equation:

$$C_i = \frac{A_i}{A_{ISTD}} \times RRF_i \times \frac{W_{ISTD}}{W_{sample}} \times C_{ISTD}$$

Where

C_i = content in % (w/w) of hydrocarbons with carbon number i
A_i = area sun of hydrocarbons with carbon number I
A_{ISTD} = the area of n-C_{16} internal standard peak
RRFi = the response factor relative to n-C_{16}
W_{INSTD} = the weight of dilute internal standard solution
Wsample = the weight of wax sample
C_{ISTD} = the concentration of n-C_{16} in the dilute internal standard solution

The combined contents of components with carbon number less than 25 is not more than 5%.

Average molecular weight

Using the carbon number distribution obtained in the test for "Carbon number at 5% distillation point" calculate the average molecular weight by the following formula:
Average molecular weight =

$$\frac{\sum_{i=17}^{i=i} C_i(14i+2)}{100}$$

where
i = the carbon number
C_i = the content in % of components having a carbon number of i

Residue on ignition

Accurately weigh about 2 g of the sample in a tared porcelain or platinum dish and heat over a flame. The sample volatilizes without emitting an acrid odour. Ignite to not exceeding a very dull redness until free from carbon. Cool in a desiccator and weigh.

Colour

Melt about 10 g of the sample on a steam bath, and pour 5 ml of the liquid into a clear-glass, 16 x 150-mm bacteriological test tube: the warm, melted liquid is not darker than a solution made by mixing 3.8 ml of ferric chloride TS(FNP 5) and 1.2 ml of cobaltous chloride TS (FNP 5) in a similar tube, the comparison of the two being made in reflected light against a white background, the tubes being held directly against the background at such an angle that there is no fluorescence.

Sulfur

(ASTM D 2622 See TEST for Viscosity, 100° for Copyright permission)
Determine by X-ray spectrometry using the following conditions:

Apparatus
- X-ray spectrograph, equipped for soft ray detection in the 537 Å range
- Optical path of helium
- Pulse height analyzer or other means of energy discrimination
- Detector designed for detection of long wavelength X-rays

Analyzing crystal suitable for the dispersion of sulfur Kα X-rays within the angular range of the spectrometer employed. Pentaerythritol and germanium are the most popular although less reflective materials such as EDDT, ADP and quartz may be used.
X-ray tube of exiting sulfur Kα radiation.
X-ray tube with tungsten, platinum or chromium target

Sensitivity standards
Liquid petroleum materials containing sulfur in concentrations approximately in the middle of the calibration graph used for the test.

Calibration standards
Prepare calibration standards by careful weight dilution of di-n-butyl sulfide (high purity standard with a certified analysis, manufactured especially as a

calibration material for this method, available from Philips Petroleum Co., Bartlesville, OK, USA) with white oil (containing less than 5 mg/kg). Exact standards of approximately 0.100, 0.250, 0.500 and 1.0 % (w/w) should be prepared.

Calibration curve

Measure the net emitted sulfur radiation from each of the calibration standards. Plot the intensity, in terms of net counts per sec, against sulfur concentration.

To maintain the validity of the calibration curve with slight changes in the instruments sensitivity, measure the sensitivity standard at frequent intervals during the course of the days run. Establish the counting rate of this standard by measuring its intensity at frequent intervals during the preparation of the calibration curve. Calculate the correction factor for changes in daily instrument sensitivity by using the following equation:

$$F = \frac{A}{B}$$

Where

A = the counting rate of the sensitivity standard determined at the time of calibration

B = the counting rate of the sensitivity standard determined at the time of analysis

Procedure

Place the sample in an appropriate cell. Although sulfur radiation will penetrate through only a small distance in the sample, scatter from the sample cup and the sample may vary to such an extent that a specific amount or a minimum amount of the sample must be used.

Place the sample in the X-ray beam and allow the X-ray optical atmosphere to come to equilibrium. Determine the intensity of the sulfur Kα radiation at 5.373 Å by making counting rate measurements at the precise angular settings for this wavelength. Measure background count-rate at 5.190 Å.

Calculation

Calculate the content of sulfur in the sample using the following equation:

$$R = \left[\frac{C_K}{S_1} - \frac{C_B \times F'}{S_2}\right] \times F$$

R = the corrected net counting rate

C_K = total counts collected at 5.373 Å for the sample

S_1 = the time in sec required to collect C_K counts

C_B = total counts collected at 5.190 Å for background

S_2 = the time in sec required to collect C_B counts

F' = count-rate at 5.373 Å / count-rate at 5.190 Å for a sample not containing sulfur

F = the correction factor for changes in daily instrument sensitivity

Using the corrected net counting rate, read the sulfur concentration from the calibration curve.

Polycyclic aromatic hydrocarbons

General Instructions

Because of the sensitivity of the test, the possibility of errors arising from contamination is great. It is of the greatest importance that all glassware be scrupulously cleaned to remove all organic matter such as oil, grease, detergent residues, etc. Examine all glassware, including stoppers and stopcocks, under ultraviolet light to detect any residual fluorescent contamination. As a precautionary measure it is a recommended practice to rinse all glassware with purified isooctane immediately before use. No grease is to be used on stopcocks or joints. Great care to avoid contamination of wax samples in handling and to assure absence of any extraneous material arising from inadequate packaging is essential. Because some of the polynuclear hydrocarbons sought in this test are very susceptible to photo-oxidation, the entire procedure is to be carried out under subdued light.

Apparatus

- Separatory funnels: 250-ml, 500-ml, 1,000-ml, and preferably 2000-ml capacity, equipped with tetrafluoroethylene polymer stopcocks.
- Reservoir: 500 ml capacity, equipped with a 24/40 standard taper male fitting at the bottom and a suitable balljoint at the top for connecting to the nitrogen supply. The male fitting should be equipped with glass hooks.
- Chromatographic tube: 180 mm in length, inside diameter to be 15.7 mm ± 0.1 mm, equipped with a coarse, fritted-glass disc, a tetrafluoroethylene polymer stopcock, and a female 24/40 standard tapered fitting at the opposite end. (Overall length of the column with the female joint is 235 mm). The female 24/40 standard tapered fitting at the opposite end.
- Disc: Tetrafluoroethylene polymer 2-inch diameter disc approximately 3/16-inch thick with a hole bored in the center to closely fit the stem of the chromatographic tube.
- Heating jacket: Conical, for 500-ml separatory funnel. (Used with variable transformer heat control).
- Suction flask: 250-ml or 500-ml filter flask.
- Condenser: 24/40 joints, fitted with a drying tube, length optional.
- Evaporation flask (optional): 250-ml or 500-ml capacity all-glass flask equipped with standard taper stopper having inlet and outlet tubes permitting passage of nitrogen across the surface of the liquid to be evaporated.
- Vacuum distillation assembly: All glass (for purification of dimethyl sulfoxide); 2 litre distillation flask with heating mantle; Vigreaux vacuum-jacketed condenser (or equivalent) about 45 cm in length and distilling head with separable cold finger condenser. Use of tetrafluoroethylene polymer sleeves on the glass joints will prevent freezing. Do not use grease on stopcocks or joints.
- Spectrophotometric cells: Fused quartz cells, optical path length in the range of 5.000 ± 0.005 cm; also for checking spectrophotometer performance only, optical path length in the range 1.000 ± 0.005 cm. With distilled water in the cells, determine any absorbance differences.
- Spectrophotometer: Spectral range 250-400 nm with spectral slit width of 2 nm or less, under instrument operating conditions for these absorbance measurements, the spectrophotometer shall also meet the following

performance requirements:
Absorbance repeatability: ±0.01 at 0.4 absorbance
Absorbance accuracy: ±0.05 at 0.4 absorbance
Wavelength repeatability: ±0.2 nm
Wavelength accuracy: ±1.0 nm
- Nitrogen cylinder: Water-pumped or equivalent purity nitrogen in cylinder equipped with regulator and valve to control flow at 5 p.s.i.g.

Reagents and materials
- Organic solvents: All solvents used throughout the procedure shall meet the specifications and tests described in this specification. The isooctane, benzene, acetone, and methyl alcohol designated in the list following this paragraph shall pass the following test:

To the specified quantity of solvent in a 250-ml Erlenmeyer flask, add 1 ml of purified n-hexadecane and evaporate on the steam bath under a stream of nitrogen (a loose aluminium foil jacket around the flask will speed evaporation). Discontinue evaporation when not over 1 ml of residue remains. (To the residue from benzene add a 10 ml portion of purified isooctane, reevaporate, and repeat once to insure complete removal of benzene).

Alternatively, the evaporation time can be reduced by using the optional evaporation flask. In this case the solvent and n-hexadecane are placed in the flask on the steam bath, the tube assembly is inserted, and a stream of nitrogen is fed through the inlet tube while the outlet tube is connected to a solvent trap and vacuum line in such a way as to prevent any flow-back of condensate into the flask.

Dissolve the 1 ml of hexadecane residue in isooctane and make to 25 ml volume. Determine the absorbance in the 5 cm path length cells compared to isooactane as reference. The absorbance of the solution of the solvent residue (except for methyl alcohol) shall not exceed 0.01 per cm path length between 280 and 400 nm. For methyl alcohol this absorbance value shall be 0.00.

- Isooctane (2,2,4-trimethylpentane): Use 180 ml for the test described in the preceding paragraph. Purify, if necessary, by passage through a column of activated silica gel (Grade 12, Davison Chemical Company, Baltimore, Maryland, or equivalent) about 90 cm in length and 5 cm to 8 cm in diameter.
- Benzene, reagent grade: Use 150 ml for the test. Purify, if necessary, by distillation or otherwise.
- Acetone, reagent grade: Use 200 ml for the test. Purify, if necessary, by distillation.
- Eluting mixtures:
1. 10% benzene in isooctane: Pipet 50 ml of benzene into a 500-ml glass-stoppered volumetric flask and adjust to volume with isooctane, with mixing.
2. 20% benzene in isooctane: Pipet 50 ml of benzene into a 250-ml glass-stoppered volumetric flask, and adjust to volume with isooctane, with mixing.
3. Acetone-benzene-water mixture: Add 20 ml of water to 380 ml of

acetone and 200 ml of benzene, and mix.
- n-Hexadecane, 99% olefin-free: Dilute 1.0 ml of n-hexadecane to 25 ml with isooctane and determine the absorbance in a 5-cm cell compared to isooctane as reference point between 280-400 nm. The absorbance per cm path length shall not exceed 0.00 in this range. Purify, if necessary, by percolation through activated silica gel or by distillation.
- Methyl alcohol, reagent grade: Use 10.0 ml of methyl alcohol. Purify, if necessary, by distillation.
- Dimethyl sulfoxide: Pure grade, clear, water-white, m.p. 18° minimum. Dilute 120 ml of dimethyl sulfoxide with 240 ml of distilled water in a 500-ml separatory funnel, mix and allow to cool for 5-10 min. Add 40 ml of isooctane to the solution and extract by shaking the funnel vigorously for 2 min. Draw off the lower aqueous layer into a second 500 ml separatory funnel and repeat the extraction with 40 ml of isooctane. Draw off and discard the aqueous layer. Wash each of the 40 ml extractives three times with 50 ml portions of distilled water. Shaking time for each wash is 1 min. Discard the aqueous layers. Filter the first extractive through anhydrous sodium sulfate prewashed with isooctane (see Sodium sulfate under "Reagents and Materials" for preparation of filter), into a 250-ml Erlenmeyer flask, or optionally into the evaporating flask. Wash the first separatory funnel with the second 40 ml isooctane extractive, and pass through the sodium sulfate into the flask. Then wash the second and first separatory funnels successively with a 10 ml portion of isooctane, and pass the solvent through the sodium sulfate into the flask. Add 1 ml of n-hexadecane and evaporate the isooctane on the steam bath under nitrogen. Discontinue evaporation when not over 1 ml of residue remains. To the residue, add a 10 ml portion of isooctane and reevaporate to 1 ml of hexadecane. Again, add 10 ml of isooctane to the residue and evaporate to 1 ml of hexadecane to insure complete removal of all volatile materials. Dissolve the 1 ml of hexadecane in isooctane and make to 25 ml volume. Determine the absorbance in 5 cm path length cells compared to isooctane as reference. The absorbance of the solution should not exceed 0.02 per cm path length in the 280-400 nm range. (Note - Difficulty in meeting this absorbance specification may be due to organic impurities in the distilled water. Repetition of the test omitting the dimethyl sulfoxide will disclose their presence. If necessary to meet the specification, purify the water by redistillation, passage through an ion-exchange resin, or otherwise). Purify, if necessary, by the following procedure: To 1.5 L of dimethyl sulfoxide in a 2 l glass-stoppered flask, add 6.0 ml of phosphoric acid and 50 g of Norit A (decolorizing carbon, alkaline) or equivalent. Stopper the flask, and with the use of a magnetic stirrer (tetrafluoroethylene polymer coated bar) stir the solvent for 15 min. Filter the dimethyl sulfoxide through four thicknesses of fluted paper (18.5 cm) (Schleicher & Schuell No. 597, or equivalent). If the initial filtrate contains carbon fines, refilter through the same filter until a clear filtrate is obtained. Protect the sulfoxide from air and moisture during this operation by covering the solvent in the funnel and collection flask with a layer of isooctane. Transfer the filtrate to a 2-l separatory funnel and draw off the dimethyl sulfoxide into the 2-l distillation flask of the vacuum distillation assembly and distil at approximately 3 mm Hg pressure or less. Discard the first 200 ml fraction of the distillate and replace the distillate collection flask with a clean one. Continue the distillation until approximately 1 litre of the sulfoxide has been collected. At completion of the distillation, the reagent should be stored in glass-

stoppered bottles since it is very hygroscopic and will react with some metal containers in the presence of air.
- Phosphoric acid, 85% reagent grade
- Sodium borohydride, 98%
- Magnesium oxide (Sea Sorb 43, Food Machinery Company, Westvaco Division, distributed by chemical supplier firms, or equivalent): Place 100 g of the magnesium oxide in a large beaker, add 700 ml of distilled water to make a thin slurry, and heat on a steam bath for 30 min with intermittent stirring. Stir well initially to insure that all the absorbent is completely wetted. Using a Buchner funnel and a filter paper* of suitable diameter, filter with suction. Continue suction until water no longer drips from the funnel. Transfer the absorbent to a glass trough lined with aluminium foil (free from rolling oil). Break up the magnesia with a clean spatula and spread out the absorbent on the aluminium foil in a layer about 1-2 cm thick. Dry at 160±1° for 24 h. Pulverize the magnesia with mortar and pestle. Sieve the pulverized absorbent between 60-180 mesh. Use the magnesia retained on the 180-mesh sieve.
- Celite 545 (Johns-Manville Company, diatomaceous earth, or equivalent)
- Magnesium oxide-Celite 545 mixture (2+1) by weight: Place the magnesium oxide (60-180 mesh) and the Celite 545 in 2 to 1 proportions, respectively, by weight in a glass-stoppered flask large enough for adequate mixing. Shake vigorously for 10 min. Transfer the mixture to a glass trough lined with aluminium foil (free from rolling oil) and spread it out on a layer about 1 to 2 cm thick. Reheat the mixture at 160±1° for 2 h, and store in a tightly closed flask.
- Sodium sulfate, anhydrous, reagent grade, preferably in granular form: For each bottle of sodium sulfate reagent used, establish as follows the necessary sodium sulfate prewash to provide such filters required in the method: Place approximately 35 g of anhydrous sodium sulfate in a 30 ml coarse, fritted-glass funnel or in a 65 ml filter funnel with glass wool plug; wash with successive 15 ml portions of the indicated solvent until a 15 ml portion of the wash shows 0.00 absorbance per cm path length between 280 nm and 400 nm when tested as prescribed under "Organic solvents." Usually three portions of wash solvent are sufficient.

Procedure

Before proceeding with the analysis of a sample, determine the absorbance in a 5 cm path cell between 250 nm and 400 nm for the reagent blank by carrying out the procedure, without a wax sample, at room temperature, recording the spectra after the extraction stage and after the complete procedure as prescribed. The absorbance per centimeter path length following the extraction stage should not exceed 0.040 in the wavelength range from 250 to 400 nm; the absorbance per cm path length following the complete procedure should not exceed 0.070 in the wavelength range from 250 to 299 nm, inclusive, nor 0.045 in the wavelength range from 300 nm to 400 nm. If in either spectrum the characteristic benzene peaks in the 250-260 nm region are present, remove the benzene by the procedure under "Organic solvents" and record absorbance again.

Place 300 ml of dimethyl sulfoxide in a 1liter separatory funnel and add 75 ml of phosphoric acid. Mix the contents of the funnel and allow to stand for 10 min. (The reaction between the sulfoxide and the acid is exothermic.

Release pressure after mixing, then keep funnel stoppered). Add 150 ml of isooctane and shake to preequilibrate the solvents. Draw off the individual layers and store in glass-stoppered flasks.

Place a representative 1 kg sample of wax, or if this amount is not available, the entire sample, in a beaker of a capacity about three times the volume of the sample and heat with occasional stirring on a steam bath until the wax is completely melted and homogenous. Weigh four 25 ± 0.2 g portions of the melted wax in separate 100 ml beakers. Reserve three of the portions for later replicate analyses as necessary. Pour one weighed portion immediately after remelting (on the steam bath) into a 500 ml separatory funnel containing 100 ml of the preequilibrated sulfoxide-phosphoric acid mixture that has been heated in the heating jacket at a temperature just high enough to keep the wax melted. (Note: In preheating the sulfoxide-acid mixture, remove the stopper of the separatory funnel at intervals to release the pressure).

Promptly complete the transfer of the sample to the funnel in the jacket with portions of the preequilibrated isooctane, warming the beaker, if necessary, and using a total volume of just 50 ml of the solvent. If the wax comes out of solution during these operations, let the stoppered funnel remain in the jacket until the wax redissolves. (Remove stopper from the funnel at intervals to release pressure).

When the wax is in solution, remove the funnel from the jacket and shake it vigorously for 2 min. Set up three 250 ml separatory funnels with each containing 30 ml of preequilibrated isooctane. After separation of the liquid phases, allow to cool until the main portion of the wax-isooctane solution begins to show a precipitate. Gently swirl the funnel when precipitation first occurs on the inside surface of the funnel to accelerate this process. Carefully draw off the lower layer, filter it slowly through a thin layer of glass wool fitted loosely in a filter funnel into the first 250 ml separatory funnel, and wash in tandem with the 30 ml portions of isooctane contained in the 250 ml separatory funnels. Shaking time for each wash is 1 min. Repeat the extraction operation with two additional portions of the sulfoxide-acid mixture, replacing the funnel in the jacket after each extraction to keep the wax in solution and washing each extractive in tandem through the same three portions of isooctane.

Collect the successive extractives (300 ml total) in a separatory funnel (preferably 2 liter), containing 480 ml of distilled water, mix, and allow to cool for a few minutes after the last extractive has been added. Add 80 ml of isooctane to the solution and extract by shaking the funnel vigorously for 2 min. Draw off the lower aqueous layer into a second separatory funnel (preferably 2 litre) and repeat the extraction with 80 ml of isooctane. Draw off and discard the aqueous layer. Wash each of the 80 ml extractives three times with 100 ml portions of distilled water. Shaking time for each wash is 1 min. Discard the aqueous layers. Filter the first extractive through anhydrous sodium sulfate prewashed with isooctane (see Sodium Sulfate under "Reagents and Materials" for preparation of filter) into a 250-ml Erlenmeyer flask (or optionally into the evaporation flask). Wash the first separatory funnel with the second 80 ml isooctane extractive and pass through the sodium sulfate. Then wash the second and first separatory

funnels successively with a 20 ml portion of isooctane and pass the solvent through the sodium sulfate into the flask. Add 1 ml of n-hexadecane and evaporate the isooctane on the steam bath under nitrogen. Discontinue evaporation when not over 1 ml of residue remains. To the residue, add a 10 ml portions of isooctane, reevaporate to 1 ml of hexadecane, and repeat this operation once more.

Quantitatively transfer the residue with isooctane to a 25 ml volumetric flask, make to volume, and mix. Determine the absorbance of the solution in the 5 cm path length cells compared to isooctane as reference between 280-400 nm (take care to lose none of the solution in filling the sample cell). Correct the absorbance values for any absorbance derived from reagents as determined by carrying out the procedure without a wax sample. If the corrected absorbance does not exceed the limits prescribed in the Characteristics, the wax meets the ultraviolet absorbance specifications. If the corrected absorbance per centimeter path length exceeds the limits prescribed in the Characteristics, proceed as follows: Quantitatively transfer the isooctane solution to a 125 ml flask equipped with 24/40 joint and evaporate the isooctane on the steam bath under a stream of nitrogen to a volume of 1 ml of hexadecane. Add 10 ml of methyl alcohol and approximately 0.3 g of sodium borohydride (Minimize exposure of the borohydride to the atmosphere. A measuring dipper may be used). Immediately fit a water-cooled condenser equipped with a 24/40 joint and with a drying tube into the flask, mix until the borohydride is dissolved, and allow to stand for 30 min at room temperature, with intermittent swirling. At the end of this period, disconnect the flask and evaporate the methyl alcohol on the steam bath under nitrogen until the sodium borohydride begins to come out of the solution. Then add 10 ml of isooctane and evaporate to a volume of about 2-3 ml. Again, add 10 ml of isooctane and concentrate to a volume of approximately 5 ml. Swirl the flask repeatedly to assure adequate washing of the sodium borohydride residues.

Fit the tetrafluoroethylene polymer disc on the upper part of the stem of the chromatographic tube, then place the tube with the disc on the suction flask and apply the vacuum (approximately 135 mm Hg pressure). Weigh out 14 g of the 2+1 magnesium oxide-Celite 545 mixture and pour the adsorbent mixture into the chromatographic tube in approximately 3 cm layers. After the addition of each layer, level off the top of the adsorbent with a flat glass rod or metal plunger by pressing down firmly until the adsorbent is well packed. Loosen the topmost few ml of each adsorbent layer with the end of a metal rod before the addition of the next layer. Continue packing in this manner until all the 14 g of the adsorbent is added to the tube. Level off the top of the adsorbent by pressing down firmly with a flat glass rod or metal plunger to make the depth of the adsorbent bed approximately 12.5 cm in depth. Turn off the vacuum and remove the suction flask. Fit the 500 ml reservoir onto the top of the chromatographic column and prewet the column by passing 100 ml of isooctane through the column. Adjust the nitrogen pressure so that the rate of descent of the isooctane coming off of the column is between 2-3 ml per min. Discontinue pressure just before the last of the isooctane reaches the level of the adsorbent. (Caution: Do not allow the liquid level to recede below the adsorbent level at any time). Remove the reservoir and decant the 5 ml isooctane concentrate solution onto the column and with slight pressure again allow the liquid level to

recede to barely above the adsorbent level. Rapidly complete the transfer similarly with two 5 ml portions of isooctane, swirling the flask repeatedly each time to assure adequate washing of the residue. Just before the final 5 ml wash reaches the top of the adsorbent, add 100 ml of isooctane to the reservoir and continue the percolation at the 2-3 ml per minute rate. Just before the last of the isooctane reaches the adsorbent level, add 100 ml of 10% benzene in isooctane to the reservoir and continue the percolation at the aforementioned rate. Just before the solvent mixture reaches adsorbent level, add 25 ml of 20% benzene in isooctane to the reservoir and continue the percolation at 2-3 ml per minute until all this solvent mixture has been removed from the column. Discard all the elution solvents collected up to this point. Add 300 ml of the acetone-benzene-water mixture to the reservoir and percolate through the column to elute the polynuclear compounds. Collect the eluate in a clean 1-l separatory funnel. Allow the column to drain until most of the solvent mixture is removed. Wash the eluate three times with 300 ml portions of distilled water, shaking well for each wash. (The addition of small amounts of sodium chloride facilitates separation). Discard the aqueous layer after each wash. After the final separation, filter the residual benzene through anhydrous sodium sulfate prewashed with benzene (see *Sodium sulfate* under "Reagents and Materials" for preparation of filter) into a 250-ml Erlenmeyer flask (or optionally into the evaporation flask). Wash the separatory funnel with two additional 20 ml portions of benzene which are also filtered through the sodium sulfate. Add 1 ml of n-hexadecane and completely remove the benzene by evaporation under nitrogen, using the special procedure to eliminate benzene as previously described under "Organic Solvents". Quantitatively transfer the residue with isooctane to a 25 ml volumetric flask and adjust the volume. Determine the absorbance of the solution in the 5 cm path length cells compared to isooctane as reference between 250 - 400 nm. Correct for any absorbance derived from the reagents as determined by carrying out the procedure without a wax sample. If either spectrum shows the characteristic benzene peaks in the 250 - 260 nm region, evaporate the solution to remove benzene by the procedure under "Organic Solvents". Dissolve the residue, transfer quantitatively, and adjust to volume in isooctane in a 25 ml volumetric flask. Record the absorbance again. If the corrected absorbance does not exceed the limits prescribed in the Characteristics the wax meets the ultraviolet absorbance specifications.

Appendix