POLYETHYLENE GLYCOLS

Prepared at the 31st JECFA (1987), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). An ADI of 0-10 mg/kg bw was established at the 23rd JECFA (1979)

SYNONYMS PEG, Macrogol; INS No. 1521

DEFINITION Addition polymers of ethylene oxide and water usually designated by a number roughly corresponding to the molecular weight.

Chemical names alpha-Hydro-omega-hydroxypoly (oxy-1,2-ethanediol)

C.A.S. number 25322-68-3

Chemical formula $(C_2H_4O)_{n+1}H_2O$

Structural formula $HOCH_2 - (CH_2 - O - CH_2)_n - CH_2OH$

Formula weight 200-9500

DESCRIPTION PEG's below 700 molecular weight occur as clear to slightly hazy, colourless, slightly hygroscopic liquids with a slight characteristic odour. PEG's between 700-900 are semi-solid. PEG's over 1000 molecular weight are creamy white waxy solids, flakes, or free-flowing powders.

FUNCTIONAL USES Carrier solvent, excipient

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Polyethylene glycols having a molecular weight of 1000 or above are freely soluble in water; polyethylene glycols are soluble in many organic solvents, including aliphatic ketones and alcohols, chloroform, glycol ethers, esters, and aromatic hydrocarbons; they are insoluble in ether and in most aliphatic hydrocarbons; with increased molecular weight, water solubility and solubility in organic solvents decrease

Molecular weight PEG's having molecular weight below 1000: not less than 95.0% and not more than 105.0% of the declared value
PEG's having molecular weight between 1000 and 7000: not less than 90.0% and not more than 110.0% of the declared value
PEG's having molecular weight above 7000: not less than 87.5% and not more than 112.5% of the declared value
See description under TESTS

Viscosity The viscosity ranges at 100±0.3°, in cSt for PEG's of various molecular weight should be:

Average MW	Viscosity range, cSt

200	4.1-4.8
300	5.4-6.4
400	6.8-8.0
500	8.3-9.6
600	9.9-11.3
700	11.5-13.0
800	12.5-14.5
900	15.0-17.0
1000	16.0-19.0
1100	18.0-22.0
1200	20.0-24.5
1300	22.0-27.0
1400	24.0-30.0
1450	25.0-32.0
1500	26.0-33.0
1600	28.0-36.0
1700	31.0-39.0
1800	33.0-42.0
1900	35.0-45.0
2000	38.0-49.0
2100	40.0-53.0
2200	43.0-56.0
2300	46.0-60.0
2400	49-65
2500	51-70
2600	54-74
2700	57-78
2800	60-83
2900	64-88
3000	67-93
3250	73-105
3350	76-110
3500	87-123
3750	99-140
4000	110-158
4250	123-177
4500	140-200
4750	150-228
5000	170-250
5500	206-315
6000	250-390
6500	295-480
7000	350-590
7500	405-735
8000	470-900

For PEG's not listed in the table, calculate the limits by interpolation. See description under TESTS

PURITY

pH (Vol. 4) 4.5 - 7.5 (1 in 20 soln)

Sulfated ash (Vol. 4)	Not more than 0.1% w/w Test 5 g of the sample
Acidity	Not more than 0.05% w/w (as acetic acid) Transfer 6 g of the sample into a 250-ml Erlenmeyer flask, add phenolphthalein TS and 50 ml neutral ethanol and titrate with 0.1 N ethanolic potassium hydroxide to a pink end-point that persists for at least 15 sec. Not more than 0.5 ml is required.
1,4-Dioxane	Not more than 10 mg/kg. Proceed as directed in the Limit Test using *Gas chromatography*. See also Headspace gas chromatography method described below under the test method for Ethylene oxide.
Ethylene oxide	Not more than 0.02% See description under TESTS
Ethylene glycol and diethylene glycol	Total not more than 0.25% w/w individually or in combination See description under TESTS
Lead (Vol. 4)	Not more than 1 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

Molecular weight

Phthalic anhydride solution

Place 49 g of phthalic anhydride in an amber bottle and dissolve it in 300 ml of pyridine that has been freshly distilled over phthalic anhydride. Shake the bottle vigorously until solution is effected, and allow to stand overnight before using.

Sample preparation for liquid polyethylene glycols

Carefully introduce 25 ml of the Phthalic anhydride solution into a clean, dry, heat-resistant pressure bottle. To the bottle add an accurately weighed amount of the sample equivalent to its expected molecular weight divided by 160. (Thus, a sample of about 1.3 g would be taken for PEG 200, or about 3.8 g for PEG 600). Stopper the bottle, and wrap it securely in a fabric bag.

Sample preparation for solid polyethylene glycols

Carefully introduce 25 ml of the Phthalic anhydride solution into a clean, dry, heat-resistant pressure bottle. To the bottle add an accurately weighed amount of the sample, previously melted, equivalent to its expected molecular weight divided by 160; because of limited solubility, however, do not use more than 25 g of any sample. Add 25 ml of pyridine, freshly distilled over phthalic anhydride, swirl to effect solution, stopper the bottle, and wrap it securely in a fabric bag.

Procedure

Immerse the sample bottle in a water bath, maintained between 96° and 100°, to the same depth as that of the mixture in the bottle. Heat it in the water bath for 30 to 60 min., then remove the bottle from the bath and allow it to cool to room temperature. Uncap the bottle carefully to release any pressure, remove the bottle from the fabric bag, add 5 drops of a 1 in 100 solution of phenolphthalein in pyridine, and titrate with 0.5 N sodium hydroxide to the first pink colour that persists for 15 sec, recording the volume, in ml of 0.5 N sodium hydroxide required as S. Perform a blank determination on 25 ml of the Phthalic anhydride solution plus any additional pyridine added to the sample bottle, and record the volume, in ml of 0.5 N sodium hydroxide required as B. Calculate the molecular weight of the sample by the formula:

$$Molecular\ weight = \frac{2000\,W}{(B - S)\,N}$$

where
W = weight of the sample in g
B = volume of 0.5 N NaOH consumed by the blank, in ml
S = volume of 0.5 N NaOH consumed by the sample, in ml
N = exact normality of NaOH

Alternative Tentative method using size exclusion chromatography (gel permeation chromatography)

1. Polyethylene glycols having nominal molecular weight below 1000

Apparatus

Use a suitable HPLC chromatograph equipped with a differential refractometer fitted with a 0.60 m x 7.7 mm (inside diameter) column packed with PL gel 10 µm 50 Å with tetrahydrofuran as the mobile phase.

Operating Conditions

The operating parameters may vary depending upon the particular instrument used but a suitable chromatogram may be obtained using the following conditions:
-Mobile phase flow rate: 1 ml/min
- Pressure: 35 bars
- Injected volume: 20 µl of a 1% (w/v) solution
- Temperature of detection: 25° ± 0.1°
The procedure allows the identification of PEG by comparison with a standard and to examine mixtures of PEG.

2. Polyethylene glycols having a nominal molecular weight of 1000 and higher

The determination is carried out with the same procedure but with a mobile phase: Tetrahydrofuran/n-heptane (80/20).
The elution volumes of PEG are approximately as follows depending on the particular instrument and operating conditions.

Molecular Mass	Elution Volume ml
35 000	21.2

10 000	22.8
6 000	24.2
4 000	25.1
2 000	26.8
1 000	28.4

Viscosity

Apparatus

The Ubbelohde suspended level viscometer, shown in the Figure is efficient for the determination of viscosity in the case of polyethylene glycols. This apparatus is preferred for the determination of viscosity. For use in the range of 300 to 600 centistrokes, a number 3 size viscometer, having a capillary diameter of 2.00±0.04 mm, is required. The viscometer should be fitted with holders which satisfy the dimensional positions of the separate tubes as shown in the diagram, and which hold the viscometer vertical. Filling lines in bulb A indicate the minimum and maximum volumes of liquid to be used for convenient operation. The volume of bulb B is approximately 5 ml.

Calibration of the Viscometer

Determine the viscosity constant (C) for each viscometer by using an oil of known viscosity. Charge the viscometer by tilting the instrument about 30 degrees from the vertical, with bulb A below the capillary, and then introduce enough of the sample into tube 1 to bring the level up to the lower filling line. The level should not be above the upper filling line when the viscometer is returned to the vertical position and the sample has drained from tube 1. Charge the viscometer in such a manner that the U-tube at the bottom fills completely without trapping air. After the viscometer has been in a constant temperature bath long enough for the sample to reach temperature equilibrium, place a finger over tube 3 and apply suction to tube 2 until the liquid reaches the center of bulb C. Remove suction from tube 2, then remove the finger from tube 3 and place it over tube 2 until the sample drops away from the lower end of the capillary. Remove the finger from tube 2, and measure the time, to the nearest 0.1 sec., required for the meniscus to pass from the first timing mark (T_1) to the second (T_2). In order to obtain accurate results within a reasonable time, the apparatus should be adjusted to give an elapsed time of from 80 to 100 sec.

Calculate the viscometer constant C by the equation $C = cSt/t_1$ in which cSt is the viscosity, in centistokes, and t_1 is the efflux time, in sec, for the standard liquid.

Determine the viscosity of the sample, maintaining the constant temperature bath at 100±0.3^o and using a capillary viscometer having a flow time of at least 200 sec for the PEG being tested. The viscosity must be within the limits specified in the table, or interpolated from the table.

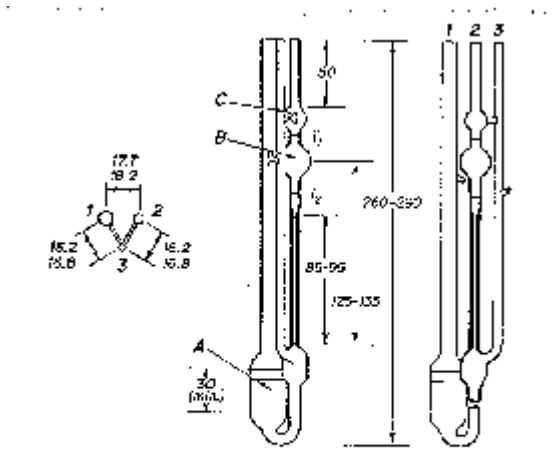

Figure. Ubbelohde Viscometer (all dimensions are in mm)

PURITY TESTS

Ethylene oxide

Morpholine solution
Mix one part of redistilled morpholine with nine parts of anhydrous methanol.

Mixed indicator
Weigh 0.050 g of 4,4'bis-(amino-1-naphthylazo-2,2'-stilbenedisulfonic acid) and 0.010 g of brilliant yellow into a 60-ml vial. Pipet 1.5 ml of 0.1 N sodium hydroxide into the vial, and mix. Add 3.5 ml of water, and mix. Transfer the mixture to a storage bottle with the aid of 45 ml of methanol as a rinse, and mix.

Standard methanolic hydrochloric acid
Mix 8.5 ml of hydrochloric acid and 1000 ml of anhydrous methanol, and standardize by titrating 9.00 ml with 0.1 N sodium hydroxide TS to a phenolphthalein end-point. Restandardize if this solution is used more than 48 h after standardization.

Procedure
Place 50 ml of anhydrous methanol into a 250-ml conical flask. Add 4 to 6 drops of Mixed indicator, and titrate with Standard methanolic hydrochloric acid to a clear blue colour.

Transfer to the flask about 25 g of the sample, accurately weighed, to provide the specimen blank, swirl to effect complete solution. Titrate with Standard methanolic hydrochloric acid to a clear blue colour, approaching the end-point carefully using small increments of titrant. Place 50 ml of Morpholine solution into a heat-resistant pressure bottle, and place an equal amount into a similar bottle to provide the reagent blank. To the first bottle add about 25 g of the sample, accurately weighed, and swirl to effect complete solution. Wrap the bottles securely in a cloth bag, and place them

close together in a water bath maintained at 98±2° for 30 min, keeping the water level in the bath just above the liquid level in the bottles. Remove the bottles from the bath, and allow them to cool in air to room temperature. When the bottles have cooled, loosen the wrappers, uncap to release any pressure, and remove the wrappers. Slowly add 20 ml of acetic anhydride to each bottle, and swirl to effect complete solution. Allow to stand at room temperature for 15 min. If the bottles are still warm, cool them to room temperature. To each bottle add 4 to 6 drops of Mixed indicator and titrate with Standard methanolic hydrochloric acid to a clear blue colour, adding very small increments when approaching the end-point.

Calculate the percentage of ethylene oxide by the formula:

$$4.41 \times N \times \frac{(A - B)}{W_1 - \frac{C}{W_2}}$$

where
N = the normality of the Standard methanolic hydrochloric acid
A, B, and C = the volumes (ml) required in the titration of the specimen, the reagent blank, and the specimen blank, respectively
W_1 and W_2 = the weights (g) of the sample taken for the reaction and the specimen blank, respectively

Headspace gas chromatography:
Alternative tentative method for 1,4-Dioxane and Ethylene oxide

Principle:
After addition of water to the sample, ethylene oxide and 1,4-dioxane are analysed by headspace gas chromatography.

Standard Solutions
- 1,4-Dioxane Standard Stock Solution
Standard solutions of 1,4-dioxane in water are prepared by weighing out about 1.00 g 1,4-dioxane/100 ml distilled water (stock solution) with successive dilutions. Two standard solutions of about 20 and 100 µg 1,4-dioxane/ml water are used.
- Ethylene Oxide (EO) Standard Stock Solution
In a 25 ml multidose injection vial, introduce 25 ml of distilled water. Close the vial with septum and cap with a gas tight syringe. Introduce slowly into the liquid 20 ml of ethylene oxide gas (about 40 mg). Determine the exact amount of ethylene oxide added by weighing the vial before and after the introduction of the gas (stock solution).

Prepare two working standard solutions by dilution with about 2 and 10 µg ethylene oxide per ml of water by successive dilutions.

Apparatus
Use a suitable gas chromatograph equipped with a flame-ionization detector (FID) containing a 30 m fused silica capillary column coated with dimethylpolysiloxane, internal diameter 0.25 mm, film thickness 1.0 µm.
Operating Conditions

The operating conditions may vary depending upon the particular instrument used, but the suitable chromatogram can be obtained using the following conditions:

Headspace Sampler Setting
- Temperature equilibration time: 45 min
- Temperature: 70°
- Transfer line temperature: 150°
- Pressurization time: 30 sec
- Injection time: 6 sec
- Analysis time: 45 min

Gas chromatography conditions
- Temperature: Column, 50° (5 min isothermal), then 5°/min to 180°
Detector (FID), 250°
Carrier gas: Helium, 1 ml/min
Carrier pressure: 0.7 bar
Split ratio: 40 : 1
Hydrogen and air: for FID

Sample Preparations
Transfer about 1 g of the sample accurately weighed (± 0.1 mg) in a headspace vial and add 1 ml of distilled water. Seal the vial and insert it into the headspace analyser for equilibration 45 min at 70°.
Prepare in the same conditions 2 vials with each 1 g sample accurately weighed (±0.1 mg) and 1 ml of standard stock solutions of 1,4-dioxane and EO.

Standard Solutions for Work
Two standard solutions A and B (for spiking) are prepared as follows:

A. 1 ml EO stock solution with ca. 200 mg EO/100 ml + 2 ml 1,4-dioxane stock solution with ca. 1000 mg dioxane/100 ml + water make up to 100 ml. This solution will be diluted 1 : 10 with water to yield a concentration of about 2 µg EO/ml and 20 µg 1,4-dioxane/ml.

B. 1 ml EO stock solution with ca. 500 mg EO/100 ml + 5 ml 1.4 dioxane stock solution with ca. 1000 mg dioxane/100 ml + water make up to 100 ml. This solution will be diluted 2 : 10 with water to yield a concentration of about 10 µg EO/ml and 100 µg 1.4-dioxane/ml.

Calculation
The concentration of compound i can be calculated by the following formula:

$$\mu g\,compound\,i\,/\,g = \frac{W_i \times A_i}{A_{is}}$$

where
µg compound i/g = Mass portion of 1,4-dioxane or EO in the sample [µg/g]
W_i = Mass of spiked compound i normalized to 1 g sample [µg/g]
A_i = Peak area of compound i in the sample, normalized to 1 g sample
A_{is} = Peak area of compound i in the spiked sample, normalized to 1 g

sample

Ethylene glycol and diethylene glycol

Polyethylene glycols having molecular weights below 450

Apparatus

Use a suitable gas chromatograph equipped with a hydrogen flame ionization detector, containing a 1.5 m x 3 mm (inside diameter) stainless steel column packed with sorbitol 12%, by weight, on 60/80 mesh non-acid-washed diatomaceous earth (Chromosorb W, or equivalent).

Operating conditions

The operating parameters may vary, depending upon the particular instrument used, but a suitable chromatogram may be obtained using the following conditions: Column temperature: 165º; Inlet temperature: 260º; Carrier gas: nitrogen (or suitable inert gas); flowing at a rate of 70 ml per min; Recorder: -0.5 to 1.05 mv, full span, 1 sec. full response time; Hydrogen and air flow to burner, optimize to give maximum sensitivity.

Standard solutions

Prepare chromatographic standards by dissolving accurately weighed amounts of commercial ethylene glycol and diethylene glycol, previously purified by distillation if necessary, in water. Suitable concentrations range from 1 to 6 mg of each glycol per ml.

Sample preparation

Transfer about 4 g of the sample, accurately weighed, into a 10-ml volumetric flask, dilute to volume with water and mix.

Procedure

Inject a 2 µl portion of each of the Standard solutions into the chromatograph, and obtain the chromatogram for each solution. Under the stated conditions, the elution time is approximately 2 min for ethylene glycol and 6.5 min for diethylene glycol. Measure the peak heights, and record the values as follows:

A = height, in mm, of the ethylene glycol peak;
B = weight, in mg, of ethylene glycol per ml of the Standard solution;
C = height, in mm, of the diethylene glycol peak; and
D = weight, in mg, of diethylene glycol per ml of the Standard solution

Similarly, inject a 2 µl portion of the Sample preparation into the chromatograph, and obtain the chromatogram, recording the height of the ethylene glycol peak as E and that of the diethylene glycol peak as F.

Calculation

Calculate the percent of ethylene glycol in the sample by the formula: (E x B) / A x sample weight in g.

Calculate the percent of diethylene glycol in the sample by the formula: (F x D) / C x sample weight in g.

Polyethylene glycols having molecular weights of 450 or higher

Sample preparation
Dissolve 50 g of the sample in 75 ml of diphenyl ether in a 250-ml distillation flask. Slowly distil at a pressure of 1-2 mm of mercury into a receiver graduated to 100 ml in 1-ml subdivisions, until 25 ml of distillate has been collected. Add 25 ml of water to the distillate, shake vigorously, and allow the layers to separate. Cool the container in an ice bath to solidify the diphenyl ether and to facilitate its removal. Filter the water layer through filter paper into a 50-ml glass-stoppered graduated cylinder, and to the filtrate add an equal volume of freshly distilled acetonitrile.

Standard preparation
Transfer 50 mg of diethylene glycol to a 25-ml volumetric flask, dilute to volume with a 1:1 mixture of freshly distilled acetonitrile and water, and mix.

Procedure
Transfer 10 ml of each of the Sample preparation and of the Standard preparation into separate 50-ml flasks each containing 15 ml of ceric ammonium nitrate TS, and mix. Within 2 to 5 min., determine the absorbance of each solution in a 1-cm cell at 450 nm, with a suitable spectrophotometer, using a blank, consisting of 15 ml of ceric ammonium nitrate TS and 10 ml of a 1:1 mixture of acetonitrile and water. The absorbance of the solution from the Sample preparation does not exceed that from the Standard preparation.

Alternative tentative method for the determination of mono and diethylene glycol

Test solution
In a 100 ml volumetric flask weigh 5.0 g of the substance to be examined, dissolve in acetone and dilute to 100.0 ml with acetone.

Reference solution
In a 100 ml volumetric flask weigh 100 mg of monoethylene glycol and 500 mg of diethylene glycol . Dilute to 100.0 ml with acetone. Dilute 1.0 ml of this solution to 10.0 ml with acetone.

Procedure
Gas chromatographic procedure may be carried out using:
- Glass column 1.8 m and 2 mm internal diameter, packed with diatomaceous earth for gas chromatography, washed and silanised (Chromosorb G.AW.DMCS 100-125 mesh is suitable), impregnated with 4% (m/m) of polyethylene glycol 20.000 (Carbowax 20 M is suitable).
- Nitrogen as carrier gas with a flow rate of 30 ml/min,
- Flame ionization detector

If necessary, preconditioning of the column may be carried out by heating at 200^{o} for about 15 h.

Adjust the initial temperature to obtain a retention time of 14 min to 16 min for diethylene glycol. Lower the temperature of the column to 140^{o}. Inject the solutions and raise the temperature of the column to 170^{o}, at a rate of 2^{o} per min. Maintain the temperature of the injection port at 250^{o} and that

of the detector at 250°. Inject 2.0 µl of the test solution and of the reference solution. Verify after five injections the repeatability of the response.

Calculation

Measure the peak areas of the mono and diethyleneglycol in the test and reference solutions. Calculate the concentration of the mono and diethylene glycol in the test solution from the peak areas.