Determination of Ethylene Oxide in Surgical Materials by Vacuum Extraction and Gas Chromatography

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Abstract □ A method for the determination of ethylene oxide sterilized plastic is described. The ethylene oxide is extracted from the plastic by vacuum and condensed in a cold trap. The gas is subsequently analyzed by GLC. Other methods are compared.

Keyphrases □ Ethylene oxide in plastics—determination □ Vacuum extraction—ethylene oxide □ GLC—analysis

Ethylene oxide (EO) gaseous sterilization has found increased use since the first patent was applied for by Schrader and Bossert in 1933 (1). Its advantages such as low temperature, good penetration, effectiveness against all organisms, and effectiveness at low humidities are well known. At present, many items made of temperature-sensitive materials, which should not be exposed to heat, are sterilized by means of EO. One disadvantage of sterilization with EO is the residual EO that remains in the plastic. Attempts to determine these residual amounts have resulted in a variety of analytical methods.

One early method was used to determine residual EO in fumigated grain (2). Later, a colorimetric method (3) for determining EO in spices was used. After this, in succession, appeared a distillation and titration method (4), a water extraction method (5), and an acetone extraction method (6, 7).

The authors have developed a method by which residual EO is removed from the EO sterilized plastic or silastic material by vacuum. The eluted gas is liquified and collected in a cold trap under vacuum, using liquid nitrogen as the coolant. The condensate is subsequently revaporized by mild heat and injected into the helium stream of a gas chromatograph.

EXPERIMENTAL

Apparatus—The gas chromatograph was a Perkin-Elmer model 154 equipped with a gas sampling valve. A 0.95-cm. (0.375-in.) o.d. × 20.32-cm. (8-in.) long condenser U-tube was connected to the gas sampling valve, which was further modified by removing the metal hose connectors, in place of which two stainless steel valves were fitted. To each valve was connected a 7.62-cm. (3-in.) piece of 0.635-cm. (0.25-in.) o.d. stainless steel tubing. A union (Swagelok) to adapt glass to steel ended in a 24/40 ground-glass fitting, connected to a 250-ml. round-bottom flask. The upper stainless steel tubing was connected to a Welch duo-seal pump. A McLeod vacuum gauge was located in front of the pump by means of a T-connection. The apparatus is shown in Fig. 1.

A 1.8288-m. (6-ft.) × 0.635-cm. (0.25-in.) stainless steel standard B-column (Perkin-Elmer) consisted of a 25% di(2-ethylhexyl) sebacate on a GC-22 super support (60/80 mesh) operated at 70° at a power setting of 70. The operating parameters were: helium flow rate, 165 ml./min. at 25 psig.; and thermal conductivity detector, 8 v. The indium tubing was 0.1524 cm. (0.06 in.) o.d. × 0.0762 cm. (0.03 in.) i.d.[1]

[1] Catalog No. 37-000094-00; purchased from Wilkins Instrument and Research, Inc., a division of Varian Aerograph.

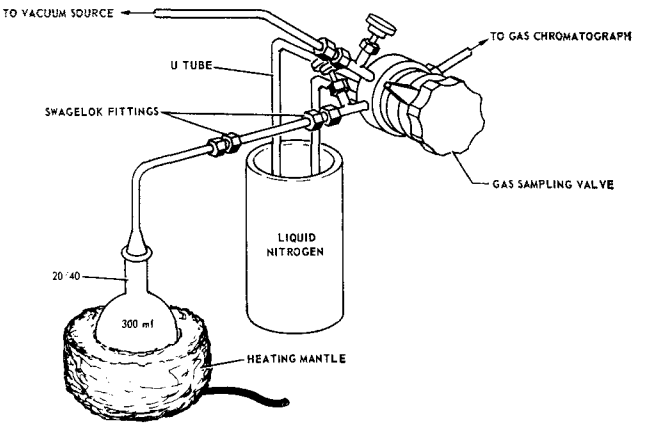

Figure 1—*Test apparatus.*

For comparing acetone, water, and chloroform extraction methods, determinations of EO were accomplished using the model 800 flame-ionization gas chromatograph (Perkin-Elmer). The columns, 1.8288 m. (6 ft.) × 0.3175 cm. (0.125 in.) o.d., were 10% polyethylene glycol[2] on Teflon (35 mesh) solid support. The operating parameters were: helium flow rate, 29 ml./min.; 40 psig. air, zero gas; 17 psig., hydrogen; temperature block, 200°; and detector temperature, 160°.

Procedure—The plastic and rubber tested were exposed in a commercial sterilizer to 750–800 mg./l. EO for 4 hr.

After sterilization, samples were cut, weighed, and placed in a round-bottom flask. Plastic tubing and other thicker materials were first chilled in liquid N_2 and crushed to increase surface area. The flask was attached to a U-collection tube *via* the glass tube and fitting. The Dewar flask, with liquid nitrogen in it, was raised into position so that the condenser tube was partially immersed in the cooling liquid. When the condenser tube was cooled, the valves connecting the system to the vacuum were opened, and the sample flask was subjected to mild heat (80–90°). Condensation proceeded for 1 hr. The valves were closed at the end of the sampling period, and the cold trap was removed and replaced by a water bath of 50–60° for 3 min. Then the gas sampling valve was turned to the sampling position, allowing the carrier gas to sweep the sample into the gas chromatograph. The average retention time was 1.4 min. for EO.

A calibration curve was made by determining the instrument response to various amounts of EO by the following procedure. A short piece of indium tubing was cut and crimped at one end and then weighed. The tube was cooled along with a syringe and needle. Cold EO was drawn into the syringe, and the needle of the syringe was inserted into the open end; the cold EO was gently forced into the tubing as the needle was gradually withdrawn. The remaining opening of tubing was quickly crimped. The tubing was allowed to warm to ambient temperature and was again weighed. The net weight was EO.

Subsequently, the indium tubing with the EO in it was placed in the round-bottom flask, which was positioned in the vacuum system, and vacuum was applied. The bottom of the flask was heated with a plastic welding torch until the small piece of indium tubing softened and the EO escaped. This was noted by a slight movement of the tube when the gas escaped. The sampling then proceeded as described. The round-bottom flask was always covered with a safety shield. A second vacuum of 0.5 hr. was applied to the same

[2] Carbowax 1540, Union Carbide Corp., New York, N. Y.

Table I—Comparison of Three EO Determination Methods[a]

Sample	Distillation and Titration	Vacuum Extraction	H_2O Extraction
1	20,651 p.p.m. EO	27,005 p.p.m. EO	22,107 EO; 3043 p.p.m. EG
2	21,213 p.p.m. EO	26,960 p.p.m. EO	23,662 EO; 3082 p.p.m. EG

[a] Nearly identical samples were used, all exposed 2 hr. to 1200 mg./l. EO at 54° with no poststerilization vacuum. The plastic was 0.051-cm. (0.020-in.) PVC squares.

Table II—Recovery of EO after Aeration

Sample	Aeration, hr.	EO, p.p.m. Vacuum Extraction	EO, p.p.m. Acetone Extraction	Ratio, Vacuum/Acetone
1	0	14,598	12,029	1.21
2	18.5	7,481	3,117	2.40
3	72	1,653	1,559	1.06

sample to assure that no EO remained in the flask. The procedure was repeated with different weights of EO. A plot of peak areas in millivolts *versus* concentration at an attenuation of ×128 produced a straight-line graph. The areas were determined on a Hewlett Packard 3370A integrator.

In the comparative acetone extraction method, the volume of the extracting liquid varied. The extraction at the zero aeration time used 100 ml. acetone and 25 ml. at other times. The duration of extraction was 18 hr. for both acetone and chloroform.

Samples of 0.2 μl. were injected into the gas chromatograph, and the amount of EO in the extracting liquid was determined by referring to a standard curve. This standard curve, comparing areas to milligrams of EO, was prepared by injecting into the gas chromatograph various known levels of EO in acetone.

RESULTS

A summary of the data is given in Tables I–IV. Table I shows a comparison of the Gunther (4), Bartak and Kulkarni (5), and vacuum methods. The same plastic and the same sterilization cycles were used in all cases. The plastic was 20-mil polyvinyl chloride (PVC) sheeting. The extraction solvent used in the Bartak and Kulkarni method (5) was also analyzed for ethylene glycol (EG), using a modification of the Critchfield–Johnson (3) method. If the EO had not hydrolyzed to EG during the 3-day H_2O extraction, this method would conceivably recover amounts equivalent to the vacuum method. However, the extraction time is severely long.

Table II shows the EO residuals as determined by vacuum and acetone extraction methods over an aeration period. Aeration is defined as the desorption in time of the sterilant gas from polymeric materials. All aeration for this work was performed at ambient temperature. The plastic was a 60-mil plastic vinyl sheet. No wrap was used when sterilizing and aerating these samples. The first two samples in Table II were compounded from Formula A. The third sample was compounded from Formula B. The plastic formulations were as follows:

Sample	Epoxy Content	Dioctyl Phthalate (DOP)	Total
A	10	50	60
B	10	30	40

Table III—Determination of EO by Weight Difference

Sample	Sample Wt., g., Presterilization	Sample Wt., g., Poststerilization	EO Determination Method	EO, p.p.m.
A	0.1044	0.1062	Weight	17,241
B	0.0875	—	Bartak and Kulkarni (5)	27,143
C	0.1143	0.1143	Weight	—

Table IV—Cycle Parameters *versus* Recovery and Recovery Ratio

Plastic Material	Cycle[a]	EO Recovered, p.p.m. Vacuum	Acetone	Ratio, Vacuum/Acetone
A	1	26,570	21,670	1.25
A*[b]	1	14,712	10,835	1.36
A	2	18,519	13,124	1.4
B	3 (100% RH)	75,599	69,696	1.10
B	2	24,732	22,526	1.10
B	3 (excess of 100%)	116,762	111,053	1.05
B	3 (50% RH)	42,636	28,991	1.47
C	4	13,232	8,740	1.50
C	5	18,352	10,364	1.76
C**[b]	4	384	273	1.41

[a] Cycle 1: 60°, 1200 mg./l. EO, 50% RH, 3 hr. of exposure. Cycle 2: similar to 1; 2 hr. of exposure. Cycle 3: similar to 1; 25.5° (78°F.), 2 hr. of exposure. Cycle 4: 63.5 cm. (25 in.) prevacuum, 50% RH, 30 min. humidification under vacuum, 8 psig. 12/88 EO F_{-12} mix., 4 hr. of exposure, 63.5 cm. (25 in.) postvacuum. Cycle 5: similar to 4; 50 psig., 12/88 EO F_{-12} mix., 1 hr. of exposure. [b] (*) 20 hr. or (**) 30 hr., respectively, elapsed prior to sampling. Plastic material without asterisks was sampled at zero aeration time.

Sample A was a phthalate epoxy combination at the 60 per hundred resin (phr.) level and was typical of commercially used compounds. Sample B was similar to A, but the total plasticizer level was lowered to only 40 phr. to determine the effect of using harder compounds.

Further results are portrayed graphically in Figs. 2 and 3. Figure 2 shows the elution rates obtained when various extraction methods were used. Samples [0.95-cm. (0.375-in.) o.d. × 0.711-cm. (0.281-in.) i.d.; 0.1188-cm. (0.047-in.) wall] of the same PVC plastic tubing were sterilized under identical conditions in the commercial sterilizer, using 750–800 mg./l. EO concentration for 4 hr. of exposure.

Extraction with water was for a period of 1 day. The data indicate that the vacuum method gives superior results in the determination

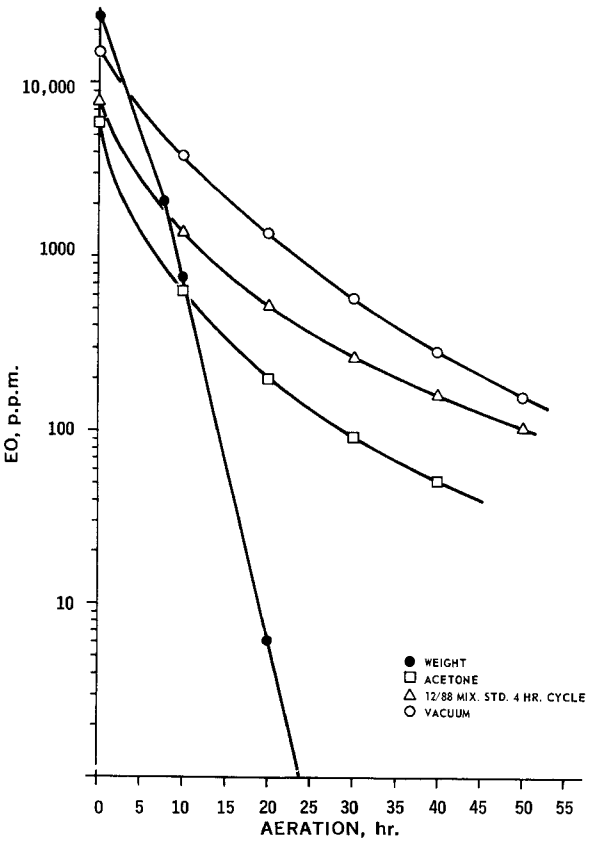

Figure 2—*Comparison of EO extraction methods for similar PVC formulations over extended periods, including EO retention values for pure EO* versus *12/88 EO/freon 12 mixture determined by acetone extraction.*

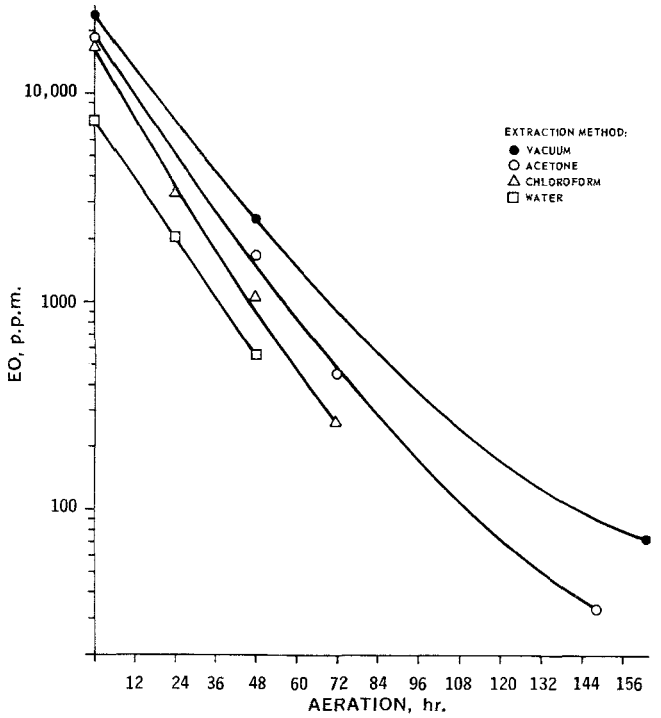

Figure 3—*Comparison of EO extraction methods for similar PVC formulations over extended aeration periods.*

of EO residuals in this material. Data in Table III on 5-mil polyurethan (Thermoplastic MP 1485) show the difficulty of determining by weight analysis the total amount of EO absorbed. This is also graphically portrayed in Fig. 3. Samples A and B were weighed and placed in a sterilizer chamber at 55°. Undiluted EO (1200 mg./l.) was added and the cycle was run for 2 hr. A control sample, C, was run later in the same chamber with no EO at 55° for 2 hr. In this test, the sample was weighed immediately after removal from the sterilizer. The other sample was then treated by the Bartak and Kulkarni (5) water extraction method of analysis. A PVC tubing of 0.237-cm. (0.094-in.) wall gave similar values for determinations by weight and by chloroform extraction. This was not true of all PVC samples. A PVC plastic obtained from one company lost weight following a long aeration period.

Table IV shows additional results of comparing the vacuum and acetone extraction methods using PVC. Plastic A is compared at 0-hr. aeration and at 20-hr. aeration by both methods. Plastic A is also compared at 0-hr. aeration where the duration of the EO exposure in the sterilizer was reduced from 3 to 2 hr. The humidity in the sterilizing cycle was varied for Sample B. Zero-aeration residual values are shown. Plastic C is compared at 0-hr. aeration and at 30-hr. aeration. The plastics used had the following dimensions:

Plastic	o.d.	i.d.	Wall
A	1.106 cm. (0.438 in.)	0.790 cm. (0.313 in.)	0.158 cm. (0.063 in.)
B	0.95 cm. (0.375 in.)	0.711 cm. (0.281 in.)	0.118 cm. (0.047 in.)
C	1.004 cm. (0.4 in.)	0.632 cm. (0.25 in.)	0.079 cm. (0.031 in.)

The vacuum-to-acetone ratio for Plastic A is approximately 1.3–1.4; for Plastic B, it is 1.0; and for Plastic C, it is 1.5.

SUMMARY

Many methods have appeared in the literature for the determination of EO and other toxic residues (reaction products of EO such as EG and ethylene chlorohydrin) in polymeric materials, rubber, and food. In most of these methods, the residue is extracted and then analyzed using GLC.

The data indicate that the vacuum method extracts as large or larger amounts of EO in almost all cases, as compared with other extraction techniques. The vacuum method has additional advantages: (*a*) large samples may be used to determine low p.p.m. EO residual; (*b*) fluorocarbons used as diluents for EO are absorbed by plastic and may also be determined, eluting from the column in 0.58 min.; and (*c*) extraction time and, consequently, the entire determination are faster and more reliable. Determinations based on weight determinations alone would be very unreliable because of water absorption; the absorption or elution of other volatile components such as freon, CO_2, air, and plasticizers. These effects become more pronounced, as indicated in Fig. 2, at lower retention levels.

REFERENCES

(1) H. Schrader and E. Bossert, U. S. pat. 2,037,439 (1936).

(2) S. E. El Shishen, *Sci. Food Agr.*, **1**, 71(1950).

(3) F. E. Critchfield and J. B. Johnson, *Anal. Chem.*, **29**, 797 (1957).

(4) D. A. Gunther, *ibid.*, **37**, 1172(1965).

(5) D. E. Bartak and R. K. Kulkarni, "Determination of Ethylene Oxide in Sterilized Catheters by Gas–Liquid Chromatography," Technical Report 6704, U. S. Army Medical Biochemical Research Laboratory, Walter Reed Medical Center, Washington, D. C., 1967.

(6) D. Cleary, unpublished report, Medical Div., 3-M Co., St. Paul, Minn., 1968.

(7) S. Ben-Yehoshua and P. Krinsky, *J. Gas Chromatogr.*, **6**, 350 (1968).

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