Determination of Residual Ethylene Oxide in Methyl Methacrylate Polymer Powders by GLC

LEE A. ZAGAR

Abstract □ Residual ethylene oxide was determined in methyl methacrylate polymer powders utilizing a gas sample procedure for GLC analysis. The procedure involves the use of a styrene–divinylbenzene column under isothermal conditions. Only relatively large microgram quantities are necessary to determine residual ethylene oxide in the parts per million range. Recovery studies showed that residual ethylene oxide determinations for a "hold/release" situation can be accomplished in less than 30 min.

Keyphrases □ Ethylene oxide residues in methyl methacrylate polymer powders—GLC analysis □ Methacrylate polymer powders—GLC analysis, ethylene oxide residues □ Sterilization by ethylene oxide—determination of residues in polymethacrylate powders □ GLC—analysis, ethylene oxide residues in methyl methacrylate polymer powders

Previous methods for determining residual ethylene oxide in products that had been gas sterilized are not generally suitable for methyl methacrylate polymer powders (1–5), which are components of recently introduced "Omniplastic" bone cement kits[1]. These kits usually consist of a plastic bag which contains the methyl methacrylate polymer powder, a glass ampul which contains a solvent, and either a plastic bag or tray for mixing.

The problem of residual ethylene oxide arises from the entrapment of the ethylene oxide in the complex surface structure of the polymer beads[2]. Since the methyl methacrylate polymers cannot be dissolved in, or "wet" with water, an aqueous extraction of the ethylene oxide is not possible. The only way to recover the ethylene oxide for analysis is by dissolving the polymer beads in an organic solvent such as acetone; however, the limited solubility of the polymer does not allow sufficient concentration of the residual ethylene oxide for direct GLC analysis using microliter-size samples[3]. This paper, therefore, deals with a gas sampling procedure for the determination of residual ethylene oxide.

EXPERIMENTAL

Instrumentation—A dual flame-ionization gas chromatograph was used[4].

Column—A coiled stainless steel column, 0.63-cm. (0.25-in.) i.d. × 0.91 m. (3 ft.), packed with a styrene–divinylbenzene copolymer resin, 80–100 mesh[5], was used.

Column Conditioning—The column was conditioned in the chromatograph with helium flow overnight at 200°. The column was then allowed to remain at 120° for several hours, during which several 1-2-μl. injections of acetone were made until a stable baseline was established.

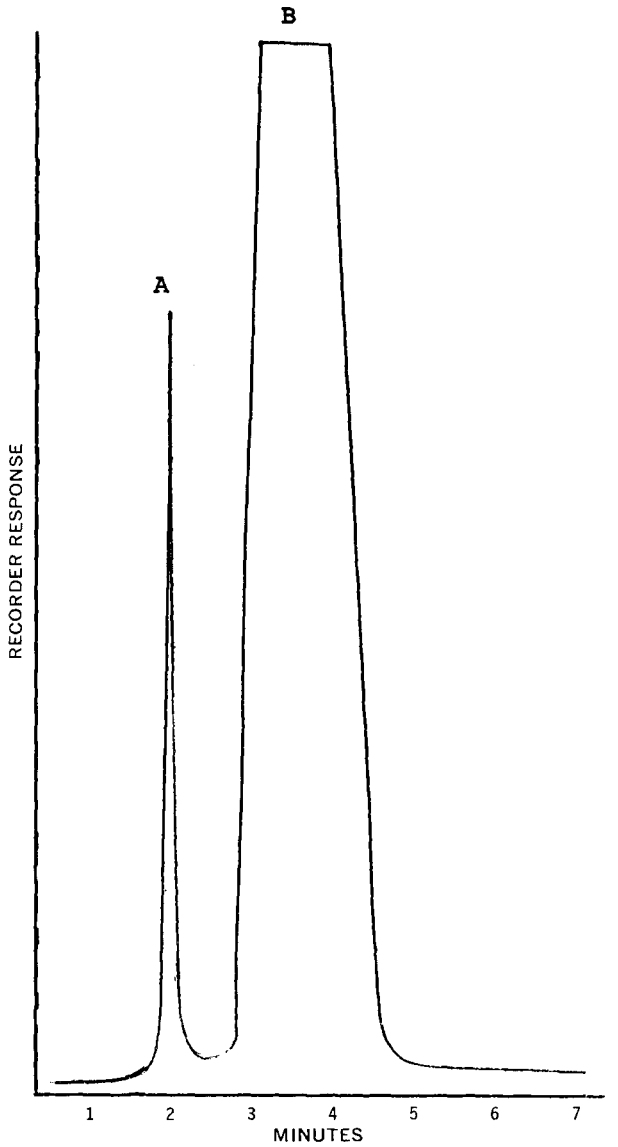

Figure 1—*Typical chromatogram from either a gas or liquid injection. As shown, the retention time for ethylene oxide is about 2 min., allowing for a rapid analysis. Key: A, ethylene oxide; and B, solvent.*

Instrument Parameters—The instrument was operated isothermally at 120° with a helium flow of 30 ml./min. The air flow and the hydrogen flow were regulated by a restrictor built into the instrument. It was only necessary to optimize the flame once and, thereafter, to maintain the hydrogen and air flows by use of the regulators at the respective gas cylinders. The injector temperature was 200° and the manifold temperature was 220°. The attenuation range was on the order of 10 × 16 to 10 × 64. The recorder was operated at a chart speed of 5 min./in.

[1] Supplied by the L. D. Caulk Co., Milford, Del.
[2] Private communication, Dr. L. DeMerre, Bureau of Drugs, Food and Drug Administration, Rockville, MD 20852
[3] Private communication, J. Cresson, L. D. Caulk Co., Milford, Del.
[4] A Perkin-Elmer model 990, equipped with dual flame-ionization detectors, and a Honeywell "Electronic 19" 1-mv. recorder were used.
[5] Chromosorb 101, Johns-Manville Products Corp., New York, N. Y.

Table I—Experimental Results for Residual Ethylene Oxide from Sterilized Samples

Time after Sterilization	Vacuum Aeration[a], hr.	Residual Ethylene Oxide, p.p.m.
1 hr.	0	>200
24 hr.	0	>200
2 days	0	>100
8 days	0	>100
22 days	2	65–70
27 days	8	10–12
35 days	60	2–3

[a] Performed in vacuum chamber at 25.4-cm. (10-in.) vacuum while allowing filtered air to enter chamber at such a rate as to maintain the 25.4-cm. (10-in.) vacuum.

Standard Solutions—Standard solutions were prepared by dissolving a suitable quantity of ethylene oxide[6] in acetone and diluting to give final concentrations of 10.0 mcg./μl. (1.0 g./100 ml.) and 1.0 mcg./μl. When measuring the ethylene oxide and making dilutions, all glassware must be cold (placed in freezer for 10–15 min. prior to use) so that the ethylene oxide will not boil off.

Procedure—*Standard Curve*—Seven 1-g. samples of the methyl methacrylate powder, which had not been sterilized, were accurately weighed and placed in 10-ml. serum vials. To six of these vials was added 0.5, 1.0, 2.0, 4.0, 8.0, and 10.0 mcg., respectively, of ethylene oxide (using standard solutions). One vial served as a blank. To each of the seven vials was added 1.0 ml. of acetone (analytical reagent). The vials were then sealed with butyl rubber stoppers[7], swirled, and allowed to stand for 5–10 min. A 1.0-ml. gas sample was withdrawn from each vial with a gastight syringe and was injected into the chromatograph, and the chromatogram was recorded. Figure 1 shows a typical chromatogram from such a gas injection. It is slightly unusual in that the ethylene oxide elutes before the solvent (acetone). Exactly 1 μl. of each standard solution was injected into the chromatograph, and the chromatograms were recorded. These served as references for 10.0 and 1.0 mcg. of ethylene oxide, respectively.

Sample—One gram of the methyl methacrylate powder, which had been ethylene oxide sterilized, was accurately weighed and placed in a 10-ml. serum vial. One milliliter of acetone was added, and the vial was sealed with a butyl rubber stopper, swirled, and allowed to stand for 5–10 min.

A 1.0-ml. gas sample was withdrawn from the vial and was injected into the chromatograph, and the chromatogram was recorded.

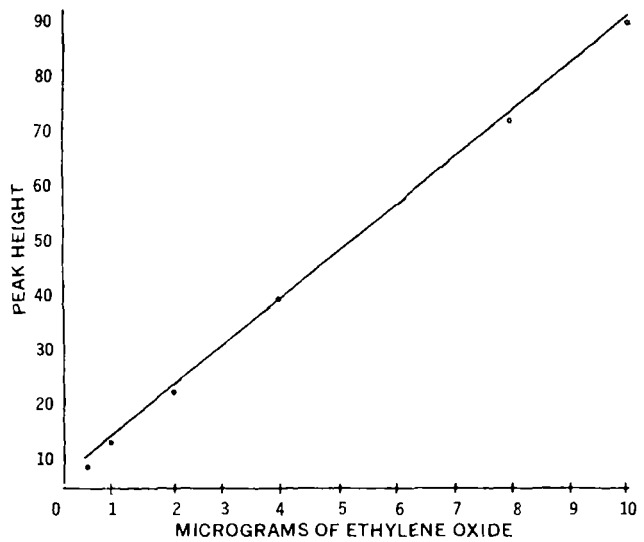

Figure 2—*Standard curve for ethylene oxide recovery.*

[6] Available from K & K Laboratories, Plainfield, N. Y.
[7] Stock No. B0857, Tompkins Rubber Co., Plymouth Meeting, Pa.

Table II—Recovery of Ethylene Oxide from Samples to Which Known Quantities Were Added

Samples	Ethylene Oxide Added, mcg.	Recovery, mcg.	Recovery, %
1	0	0	—
2	0.50	0.46	92.00
3	1.00	0.95	95.00
4	2.00	1.96	98.00
5	4.00	3.97	99.25
6	8.00	7.65	95.63
7	10.00	9.96	99.61

After the chromatograms of the eight samples were recorded, the residual ethylene oxide recovered was calculated as follows:

$$\frac{H_1 - H_2}{H_s - H_2} \times 10.0 \text{ (or } 1.0) = \text{mcg. ethylene oxide} \quad \text{(Eq. 1)}$$

where H_s = ethylene oxide peak height for standard (10.0 or 1.0 mcg.), H_1 = ethylene oxide peak height for sample, and H_2 = ethylene oxide peak height for blank (should be zero).

RESULTS AND DISCUSSION

Figure 2 is a standard curve of ethylene oxide recovery in the 0.5–10.0-mcg. range. The good linearity shown is the basis for using peak height measurements as the method of calculating ethylene oxide recovery.

The data in Table I show analytical results obtained from ethylene oxide-sterilized samples of methyl methacrylate polymer powders, which were allowed to stand at ambient conditions and which were aerated by use of a vacuum chamber held at 25.4-cm. (10-in.) vacuum while allowing filtered air to flow through the chamber at the same time. This was accomplished by adjusting the vacuum valve and the air intake valve until the specified conditions were met.

Table II shows recovery data for samples to which were added various amounts of ethylene oxide. These results indicate good recovery, so that for a quick analysis, only one standard solution made up to a predetermined reference concentration should be necessary. Since the retention time for ethylene oxide is approximately 2 min., the analysis can be carried out very quickly for a "hold/release" situation. In this instance, the upper limit for residual ethylene oxide can be chosen as the concentration for the standard so that no calculations would be necessary.

Since this procedure utilizes relatively large quantities (micrograms) of ethylene oxide, the detection level can be very low, on the order of 1 p.p.m., which certainly is well below most acceptance standards for residual ethylene oxide.

If very accurate values are to be determined, the actual volume of the serum vials should be measured. This volume is divided by 10.0 to develop a factor that should be multiplied by the numerator of the calculation to obtain the correct result. In a hold/release situation, this can generally be neglected if the residue is well below the maximum allowable tolerance.

The only problem encountered was in the type of acetone used. To avoid an extraneous peak that is eluted from the column prior to the ethylene oxide peak, only reagent grade or redistilled acetone should be used. Butyl rubber stoppers were selected for their superior properties for decreased moisture vapor transmission.

Additional work is being done to determine the feasibility of using this procedure to determine ethylene chlorohydrin and ethylene glycol, which are sometimes encountered in ethylene oxide sterilization.

REFERENCES

(1) H. D. Spitz and J. Weinberger, *J. Pharm. Sci.*, **60**, 271(1971).
(2) D. J. Brown, *J. Amer. Offic. Anal. Chem.*, **53**, 263(1970).
(3) R. K. O'Leary, W. D. Watkins, and W. L. Guess, *J. Pharm. Sci.*, **58**, 1007(1969).
(4) D. A. Gunther, *Anal. Chem.*, **37**, 1172(1965).
(5) N. Adler, *J. Pharm. Sci.*, **54**, 735(1965).

ACKNOWLEDGMENTS AND ADDRESSES

Received May 12, 1972, from *Elkins-Sinn, Inc., Cherry Hill, NJ 08034*

Accepted for publication June 27, 1972.

Presented to the Pharmaceutical Analysis and Control Section, APhA Academy of Pharmaceutical Sciences, Houston meeting, April 1972.

The author thanks Dr. John Clenn and Mr. James Cresson for their assistance.

Column Chromatographic Determination of Polymyxin B Sulfate

A. HAEMERS▲ and P. De MOERLOOSE

Abstract □ A column chromatographic method for the quantitative determination of polymyxin B sulfate in bulk samples and pharmaceutical formulations is presented. The method is based on the absorption of polymyxin B on a weak cation-exchange resin and elution with an ionic strength gradient. Polymyxin B is determined in the eluate with ninhydrin by means of an Auto-Analyzer. The results are in good agreement with those obtained by the microbiological method.

Keyphrases □ Polymyxin B sulfate—analysis, column chromatography and ninhydrin, compared to microbiological method □ Column chromatography—analysis, polymyxin B sulfate □ Ninhydrin—analysis, polymyxin B sulfate after column chromatography

Polymyxin B sulfate is a cyclic heteropeptide antibiotic, valuable in the treatment of infections caused by Gram-negative bacteria. It is frequently combined with other antibiotics to extend the antimicrobial spectrum.

The microbiological diffusion method (1) is the common method for assaying polymyxin B sulfate bulk samples and pharmaceutical formulations. Several attempts have been made to develop chemical assay methods. The procedures that can be used include: colorimetric determination with ninhydrin reagent (2), biuret reagent (3), and Folin reagent (4); gravimetric determination with phosphotungstic acid (5); UV spectrophotometric determination (6); amino acid (7) or fatty acid (8) analyses; and methods based on optical rotation (9).

Assay methods used in the pharmaceutical control of drugs must be specific and stability indicating. In the case of antibiotics, a chemical method is valueless if the results differ from those obtained by the microbiological method. The above-mentioned chemical assay methods were tested (10). Results from these methods were found to be in poor agreement with microbiological data, especially with samples containing relatively large amounts of degraded polymyxin B.

Polymyxin B with low potency was prepared by heating active samples. A decrease in activity of up to 30% was obtained by heating polymyxin B sulfate powder in a closed bottle at 100° for 3–21 days. A stronger fall in activity (up to 80%) was obtained by heating a 2% aqueous solution of polymyxin B sulfate in a sealed tube at 100° for from 6 hr. to 7 days.

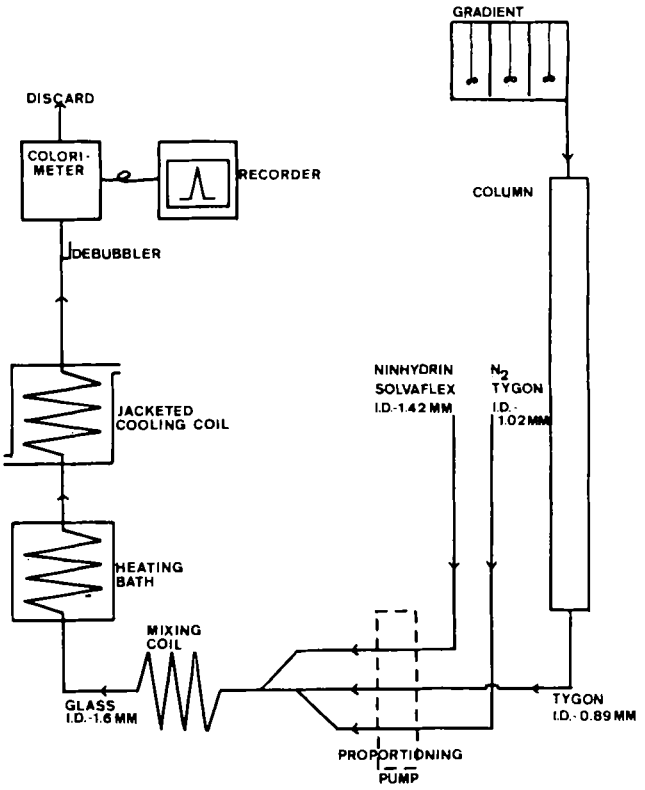

Figure 1—*Flow diagram of apparatus for automatic determination of polymyxin B sulfate.*

Only optical rotation methods had a certain degree of stability-indicating value. The Cotton-curve obtained with polymyxin B as a nickel complex showed a change in shape if degraded polymyxin B was present. Nevertheless, this method could not be used as an assay method. Ivashkiv (11) described a chromatographic method based on the separation of polymyxin B and impurities by means of a cation exchanger. The antibiotic was eluted with a sodium chloride, methanol, and water mixture and determined with ninhydrin. Although in some samples this method appeared