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Simple GLC Determination of Ethylene Oxide and Its Reaction Products in Drugs and Formulations

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Abstract □ Convenient rapid GLC methods for the estimation of residual ethylene oxide, ethylene chlorohydrin, and ethylene glycol in ethylene oxide-sterilized bulk drugs and formulations prepared therefrom are described. Ethylene oxide was chromatographed using a porous polymer column; ethylene chlorohydrin and ethylene glycol were chromatographed using either polyethylene glycol 400 or a porous polymer column. All three residuals were determined from the same sample preparation for each type of drug or formulation examined. Recoveries of each of the three residuals were greater than 95% for all samples examined. Detection limits, at moderate electrometer sensitivities, were 2 μg/g or ml for ethylene oxide and ethylene chlorohydrin and 5 μg/g or ml for ethylene glycol. The most likely interferences are discussed.

Keyphrases □ Ethylene oxide—and reaction products, GLC analysis, sterilized bulk drugs and formulations □ GLC—analysis, ethylene oxide and reaction products in sterilized bulk drugs and formulations □ Sterilizers—ethylene oxide and reaction products, GLC analysis in bulk drugs and formulations

The determination of residual levels of ethylene oxide and its reaction products, ethylene chlorohydrin and ethylene glycol, has received a great deal of attention. However, most work has dealt with the estimation of the residual levels in plastic medical devices and foods (1, 2).

Essentially all recently published procedures depended on GLC for separation and quantitation. Differences in the methods were primarily in the sample preparations (3). Natural and synthetic polymers have the capacity to absorb large quantities of ethylene oxide, and various methods have been proposed to measure accurately ethylene oxide, ethylene chlorohydrin, and ethylene glycol in these materials (4). However, drugs and pharmaceutical formulations have received scant attention. A GLC procedure was described for the determination of residual ethylene oxide in steroids that involved distillation and thermal conductivity detection (5).

Recent work centered on the use of porous polymer packings (Chromosorb 101 or Porapak) for ethylene oxide and of polyethylene glycols for ethylene chlorohydrin and ethylene glycol.

To test large numbers of samples, a method was desired that would require minimal sample preparation and analyst time without compromising the requirements for quantitative results. Since the measurement of residual levels is more of a limits test than a precise quantitation for a potency measurement, simple sample preparations and minimum chromatographic time were desired. In this paper, procedures for water-insoluble bulk drugs, aqueous suspensions, aqueous solutions, and ointments are described. For each material or formulation discussed, a single sample preparation was suitable for the quantitation of ethylene oxide, ethylene chlorohydrin, and ethylene glycol.

For quantitation of ethylene oxide, a Porapak R column was employed; a polyethylene glycol 400 column was used for the other two compounds. The use of the two columns was advantageous, since both were operated at the same temperature. With a dual-column dual-electrometer instrument, all three residuals could be determined simultaneously.

EXPERIMENTAL

Instrument—A gas chromatograph[1] equipped with a flame-ionization detector and U-shaped glass columns, 180 cm × 3 mm i.d., was used. The column packings used were Porapak R[2] (80–100 mesh) for ethylene oxide determinations and 15% Carbowax 400 on 80–100-mesh Gas Chrom Q[3] for ethylene chlorohydrin and ethylene glycol measurements. The carrier gas was helium at a flow rate of approximately 60 ml/min, with hydrogen and air flows adjusted for maximum flame response.

The oven temperature was approximately 110° for both columns. A Porapak R column at 170° was also used for ethylene chlorohydrin and ethylene glycol. The flash heater and the detector were operated about 10 and 30° above the column temperature, respectively. The instrument sensitivity was range 10 and attenuation 1, 2, or 4, depending upon sample concentration.

Reagents and Chemicals—Distilled water, hexane[4], ethylene oxide[5] (liquid), ethylene chlorohydrin[5], and ethylene glycol[6] were used. The hexane was washed with distilled water prior to use.

Reference Preparations—The ethylene oxide reference solution was prepared by diluting an ethylene oxide stock solution with distilled water to a concentration of 30 μg/ml. The stock solution of ethylene oxide was prepared by adding about 0.5 ml of liquid ethylene oxide to a 50-ml volumetric flask containing about 25 ml of distilled water. The flask was weighed before and after addition of the ethylene oxide; the increase in weight was the amount of ethylene oxide dissolved. The ethylene oxide solution was diluted to volume and mixed prior to dilution.

A combined ethylene chlorohydrin and ethylene glycol reference solution was prepared by dilution of a stock solution containing both compounds to a concentration of 30 μg/ml each. The stock solution was

[1] Model 402, Hewlett-Packard Corp., Avondale, Pa.
[2] Waters Associates, Milford, Mass.
[3] Applied Science Labs, State College, Pa.
[4] Burdick and Jackson, Muskegon, Mich.
[5] Eastman Organic Chemicals, Rochester, N.Y.
[6] Matheson, Coleman and Bell, Norwood, Ohio.

Table I—Representative Recovery Data

Ethylene Oxide			Ethylene Chlorohydrin			Ethylene Glycol		
Added, μg/g	Found, μg/g	Recovery, %	Added, μg/g	Found, μg/g	Recovery, %	Added, μg/g	Found, μg/g	Recovery, %
				Bulk Drug				
0.0	0.0	—	0.0	0.0	—	0.0	0.0	—
8.7	8.9	102.3	20.5	19.5	95.1	18.8	20.6	109.6
17.4	17.2	98.9	41.2	38.9	94.5	37.6	30.5	81.1
34.9	30.1	86.2	82.4	79.1	96.0	75.6	79.3	104.9
Mean recovery		95.8			95.2			98.5
				Aqueous Suspension				
0.0	0.0	—	0.0	0.0	—	0.0	0.0	—
10.3	10.1	98.1	15.8	15.0	94.9	15.4	15.2	98.7
20.6	21.3	103.3	31.6	33.7	106.7	30.8	29.5	95.8
41.3	40.4	97.8	63.2	63.6	100.6	61.7	64.2	104.1
Mean recovery		99.7			100.7			99.5
				Ointment				
0.0	0.0	—	0.0	0.0	—	0.0	0.0	—
9.9	10.3	104.0	15.5	15.0	96.8	15.3	15.0	98.7
19.8	18.6	93.9	31.0	31.5	101.8	30.6	29.0	91.3
39.5	38.2	96.6	62.0	60.6	97.7	61.2	63.0	102.4
Mean recovery		98.2			98.8			97.5

prepared by combining about 0.3 g each, accurately weighed, of ethylene chlorohydrin and ethylene glycol in a 100-ml volumetric flask and diluting to volume with distilled water.

Sample Preparations—Aqueous solutions were chromatographed directly. Aqueous suspensions were centrifuged, and portions of the clear supernates were removed for chromatography.

One-gram samples of water-insoluble bulk drugs were placed in disposable vials, and 4.0 ml of distilled water was added. The samples were mixed thoroughly with a vortex mixer and shaken vigorously for at least 5 min. The samples were centrifuged, and portions of the clear supernates were withdrawn for chromatography.

Three-gram samples of ointments were placed into 35-ml centrifuge tubes and dissolved in 6.0 ml of water-washed hexane. Exactly 3.0 ml of distilled water was added, and the samples were mixed for 5 min with a vortex mixer and centrifuged. The upper organic layer was removed by aspiration, and the lower aqueous layer was retained for chromatography. A 3.0-ml portion of the ethylene oxide reference solution was washed with 6.0 ml of water-washed hexane before chromatography in this case.

Equal aliquots, usually 6.0 μl, of the sample preparations and the appropriate reference preparations were chromatographed using the specified conditions. It was necessary to equilibrate the columns by injecting two or three aliquots of the reference solutions prior to each day's analyses. Peak heights were determined directly from the chromatograms.

Calculations—The level of each residual, ethylene oxide, ethylene chlorohydrin, and ethylene glycol, was calculated by a peak height comparison with the corresponding reference.

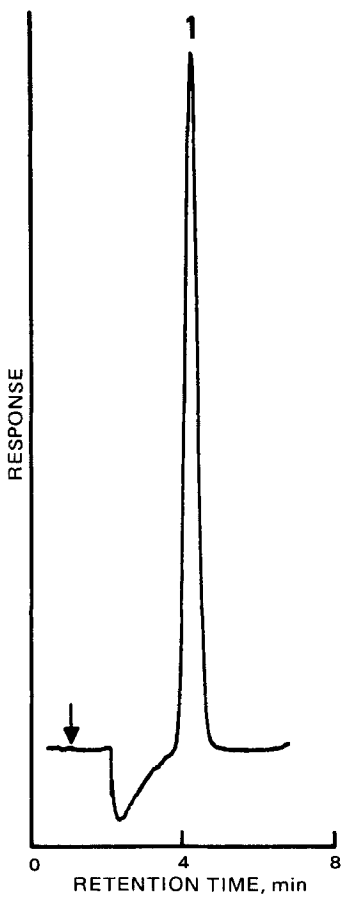

Figure 1—*Chromatogram of 40 μg/ml of ethylene oxide (1) added to an aqueous suspension of a steroid (Porapak R column). The arrow indicates the point of injection.*

RESULTS AND DISCUSSION

GLC—A representative chromatogram for ethylene oxide is shown in Fig. 1. Although water should not give a response in the flame-ionization detector, an apparent solvent front was observed, probably due to pertubation of flows by injection and vaporization. The shape of this solvent front varied from one sample type to another. Chromatographic response was linear over the 2–80-μg/ml range. The limit of detection was about 2 μg/ml for ethylene oxide.

Under the standard chromatographic conditions, ethylene oxide was not resolved from methanol. Methanol is a common recrystallization solvent for steroids and could be present in residual amounts. If a significant ethylene oxide response was observed, chromatography at 70° resolved the methanol. At a column temperature of 70°, ethylene oxide and methanol had retention times of 7 and 8 min, respectively. Acetone, another common recrystallization solvent, also was detected in some cases. This peak, which had a retention time of about 13 min at 110°, did not interfere with the ethylene oxide determination but did require extended chromatography time. No other interferences were observed.

Either of two sets of chromatographic conditions was used for the simultaneous determination of ethylene chlorohydrin and ethylene glycol. Typical chromatograms for the Carbowax 400 and Porapak R columns are shown in Figs. 2 and 3, respectively. Chromatographic response was linear for both columns over the 2–110-μg/ml range for ethylene chlorohydrin and the 5–200-μg/ml range for ethylene glycol. Limits of detection were estimated to be 2 μg/ml for ethylene chlorohydrin and 5 μg/ml for ethylene glycol.

Chromatography on the Carbowax 400 column was preferred over the Porapak R column because fewer extraneous peaks were observed and

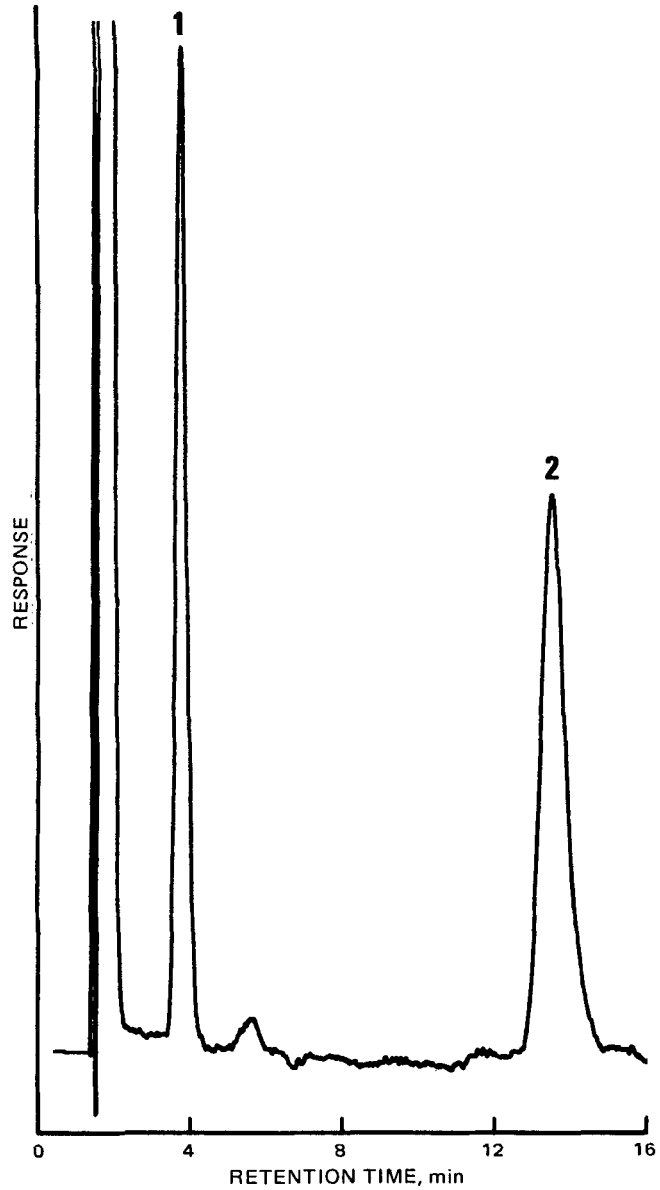

Figure 2—*Chromatogram of ethylene chlorohydrin and ethylene glycol added to an aqueous suspension of a steroid (polyethylene glycol 400 column). Key: 1, ethylene chlorohydrin (63 μg/ml); and 2, ethylene glycol (63 μg/ml).*

the resolution and shape of the peaks were superior. The Carbowax column was tried first in all cases. However, some aqueous suspensions and solutions were formulated with benzyl alcohol as a preservative, and the large benzyl alcohol peak masked the ethylene glycol peak when the Carbowax column was used. The retention time of benzyl alcohol on the Porapak R column was about 90 min at 170°, thus permitting several samples to be run before the benzyl alcohol eluted. It was necessary to chromatograph two or three aliquots of the reference solutions to establish a reproducible response, probably due to on-column adsorption of these compounds.

A buildup of components from the various sample formulations on the column packings was observed after extended use and caused a deterioration of the chromatographic performance. Original column efficiency was restored by replacing the front 4–5 cm of column packing and allowing the column to reequilibrate for at least 16 hr.

No attempt was made to identify extraneous peaks (other than those previously discussed) because they were always resolved from the peaks of interest. As a precautionary measure, the hexane was washed with distilled water prior to use to eliminate impurities that interfered.

Recovery—Recovery data were obtained for each of the 12 bulk drugs, 16 aqueous suspensions and solutions, and 11 ointments studied. Various spiking techniques were used to add three different levels each of ethylene oxide, ethylene chlorohydrin, and ethylene glycol to separate samples of every sample type studied.

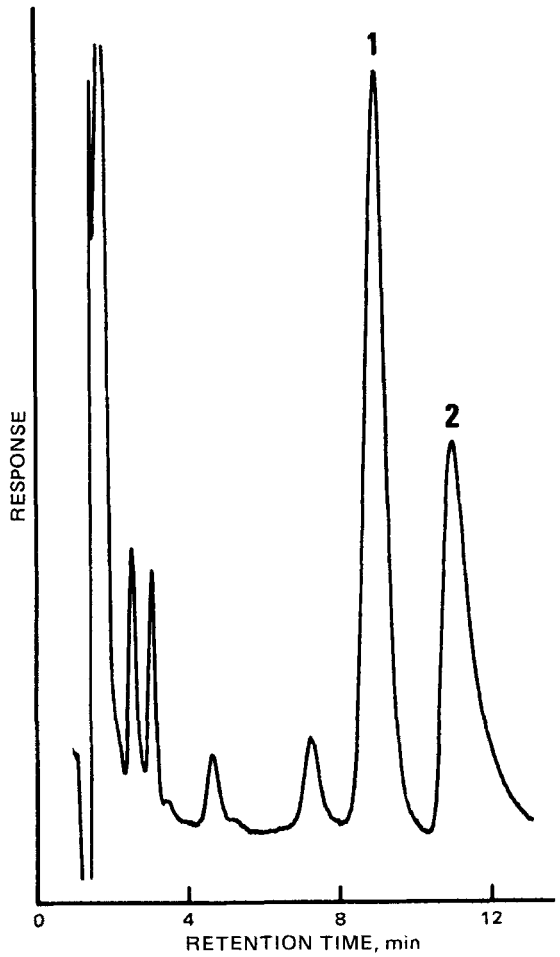

Figure 3—*Chromatogram of ethylene chlorohydrin and ethylene glycol added to a sterile infusion solution of a steroid (Porapak R column). Key: 1, ethylene chlorohydrin (61 μg/ml); and 2, ethylene glycol (62 μg/ml). Excipient peaks are not identified, and the benzyl alcohol peak is not shown.*

For bulk drugs, recovery samples were simulated by equilibrating aqueous solutions of the residuals with samples of non-ethylene-oxide-treated bulk drugs. The vortex mixer produced a homogeneous mixture of the bulk drug powder and the aqueous phase. This mixture was separated by centrifugation, and the clear supernates were chromatographed. Adler (5) found that the retention of ethylene oxide on steroids is due to sorption on the surface of the individual drug particles. Since ethylene oxide is highly water soluble and the drugs tested were generally micronized, the removal of this residual from the drug should have been highly efficient.

Aqueous suspensions and solutions for the recovery studies were prepared using vehicles containing non-ethylene-oxide-treated materials. Microliter aliquots of concentrated solutions of the residuals were added to produce the desired concentrations. The samples were mixed, centrifuged if necessary, and chromatographed under the appropriate conditions.

Recovery for the ointments was demonstrated by extracting hexane solutions of a nonsterilized formulation with aqueous solutions containing the residuals.

Typical recovery data are presented in Table I. In most cases, the recovery for individual samples was greater than 90%. The average recovery for each formulation or bulk material was greater than 95%. Due to the significant solubility of ethylene oxide in hexane, it was necessary to wash the ethylene oxide reference solution with hexane prior to analysis of ointments. Thus, the recoveries reported for ethylene oxide are actually apparent recovery.

The reference solutions were quite stable. The combined ethylene chlorohydrin and ethylene glycol reference preparation was retained for several weeks with no noticeable change in concentration. The ethylene

oxide reference solution was stable for about 1 week when prepared in distilled water. However, a change in pH or contamination with inorganic halide would induce rapid conversion of the ethylene oxide to ethylene glycol or ethylene halohydrin. The same concern existed for the sample preparations. Therefore, samples were always chromatographed on the same day that they were prepared.

Precision—Reproducibility of the chromatographic response was about 2% in each case, determined by replicate injections of reference solutions. The variation in results for actual samples was about 5%.

Simplicity—The measurement of the levels of residual ethylene oxide, ethylene chlorohydrin, and ethylene glycol present in pharmaceutical preparations is more of a limits test than a precise quantitation for potency determination. Thus, it is desirable to use rapid, simple, sensitive, and fairly accurate methods for these measurements. The procedures as described satisfy these requirements.

The sample preparation was as simple as possible. All three residuals were extracted simultaneously by a single aqueous extraction.

The chromatographic systems were ideal. Two columns in one dual column instrument provided the necessary instrumentation for estimating all three residuals without changing oven temperature.

Chromatographic time was held to a minimum. Since the highest precision was not required, internal standards were not employed which minimized chromatographic time and permitted a single sample preparation to be employed for all three residuals. Water proved to be the ideal solvent. It provided the highest extraction efficiency, yet did not respond in the flame-ionization detector. If an organic solvent had been required, it would probably have eluted after ethylene oxide and time would have been lost waiting for a large solvent peak to clear the column. With water, this delay was eliminated.

These procedures are versatile and applicable to a large number of aqueous suspensions, aqueous solutions, ointments, and water-insoluble bulk drugs.

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Enzymatic Activity of Pig Heart Mitochondrial Malate Dehydrogenase Monomolecular Films by Surface Exchange Technique

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Abstract □ A technique for studying the catalytic activity of enzymes spread as a film at an air–water interface, by exchanging the subphase under the film to remove unspread enzyme molecules, was developed, and its effectiveness was studied using surface-spread mitochondrial malate dehydrogenase. Mitochondrial malate dehydrogenase formed stable films which gave reproducible π–A curves. The enzyme activity was measured by the oxidation rate of reduced nicotinamide adenine dinucleotide (NADH) in the presence of the substrate oxalacetic acid. Oxalacetic acid and NADH were injected into the subphase. The catalytic activity of the enzyme was dependent on the surface pressure of the film. The maximum catalytic activity was observed at a surface pressure of 4.4 dynes/cm. The activity was higher at intermediate surface pressures than at very low or very high surface pressures. A high bulk catalytic activity was observed in the unstable region, *i.e.*, at a high degree of compression, of the film. The catalytic activity of the surface-spread enzyme was only a fraction of an equivalent amount of enzyme in solution.

Keyphrases □ Catalytic activity—enzymes spread as a monomolecular film at an air–water interface, surface exchange technique developed □ Enzymes—mitochondrial malate dehydrogenase, spread as a monomolecular film at an air–water interface, surface exchange technique developed □ Malate dehydrogenase, mitochondrial—spread as a monomolecular film at an air–water interface, surface exchange technique developed □ Films, monomolecular—of enzymes at an air–water interface, surface exchange technique developed □ Surface exchange technique—enzymes spread as a monomolecular film at an air–water interface

Monomolecular films offer a useful model system for studying the behavior of membrane components at an air–water interface (1). The application of monomolecular films to the study of interactions that may occur at the membranes of cells and cell organelles is a flexible and versatile system whereby numerous effects can be observed. The relative simplicity of this system permits analysis and understanding of the effects that often cannot be observed in the extremely complex environment of living systems.

BACKGROUND

Modification of the film can be employed to gain further insight into cellular function. Since membrane-bound enzymes are situated at interfaces, it must be assumed that they are in a partially unfolded state and that the side chains are oriented in relation to the interface. Monolayer studies also afford a method to study the conformational change effects of proteins by monitoring the surface pressure, since the orientation of the side chains of surface-spread protein would be expected to vary with the surface pressure of the film. A dependence of the catalytic activity on surface pressure was reported for catalase and acetylcholinesterase films (2). The activity of surface-spread trypsin was dependent on the surface concentration of the enzyme (3).

Estimation of catalytic activity of surface-spread enzymes presents unique difficulties, because the fraction of the enzyme that escapes into the bulk phase must first be removed. Previously (4), an apparatus and technique for studying monomolecular films were reported; the subphase can be exchanged without disturbing the film, thereby removing any dissolved contaminants in the bulk phase. Data were presented to demonstrate the utility of this system for studying protein monolayers and its applicability to the study of ion-exchange properties of phospholipid monolayers.

In this study, the subphase exchange technique was used to investigate the enzymatic behavior of surface-spread pig heart mitochondrial malate