Separation of Prostaglandin A_2 and Prostaglandin B_2 by Ion-Exchange Liquid Chromatography

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Abstract □ Separation of prostaglandin A_2 (PGA_2) and prostaglandin B_2 (PGB_2) was achieved by liquid chromatography on triethylaminoethyl cellulose as well as on a strong anion-exchange pellicular support. The stability of prostaglandin E_2 (PGE_2) can be monitored easily by following the appearance of the UV active compounds PGA_2 and PGB_2 using the peak height method.

Keyphrases □ Prostaglandins—separation of PGA_2 and PGB_2 by ion-exchange liquid chromatography, comparison of two columns □ Liquid chromatography—separation of prostaglandin A_2 and B_2, comparison of two columns □ Ion-exchange liquid chromatography—separation of prostaglandins A_2 and B_2, comparison of two columns

High-resolution chromatographic systems are required for separation of many closely related prostaglandins. The isomeric pair prostaglandin A_2 (PGA_2) and prostaglandin B_2 (PGB_2) show little resolution using TLC, although separation is complete with GLC (1, 2). Prostaglandin E_2 (PGE_2) undergoes rapid dehydration to yield PGA_2 and PGB_2 in consecutive fashion (3, 4) (Scheme I). GLC is not generally used to monitor the stability of PGE_2 since protection of the C_9-carbonyl group is required and the commonly used methoxime derivative gives rise to two peaks for the oximino *syn-anti*-isomers (5). PGA_2 and PGB_2 also undergo methoximation with formation of *syn-anti*-isomers and, as a result, degraded samples of PGE_2 show a complicated GLC pattern.

This work shows that single peaks are obtained for PGA_2 and PGB_2 using ion-exchange liquid chromatography without protective derivatization.

EXPERIMENTAL

Equipment—Liquid chromatography was conducted on a liquid chromatograph[1] (254-nm detector) using 2.1-mm (i.d.) × 1-m stainless steel columns.

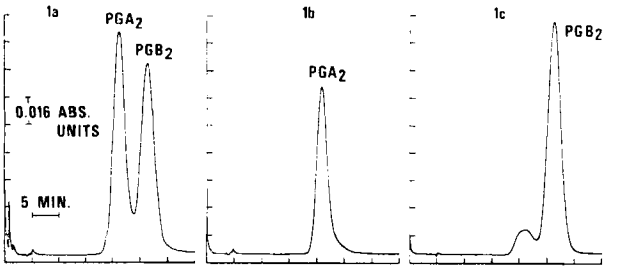


Figure 1—*Liquid chromatograms on triethylaminoethyl cellulose using aqueous 0.05* M *tromethamine acetate (pH 7.20) at 2100 psi and 0.45 ml/min. Key: 1a, mixture of* PGA_2 *(40 μg) and* PGB_2 *(7 μg); 1b,* PGA_2 *(35 μg); and 1c,* PGB_2 *(9 μg).*

[1] DuPont model 820.

Triethylaminoethyl Cellulose Columns—Triethylaminoethyl cellulose[2] (0.58 mEq/g) was freed of fines by slurrying 1–4% suspensions in water and allowing the material to settle for 1–1.5 hr. The supernate was discarded, and the slurry-settling process was repeated six times. The purified support was isolated by filtration, washed with acetone, and air dried for 24 hr.

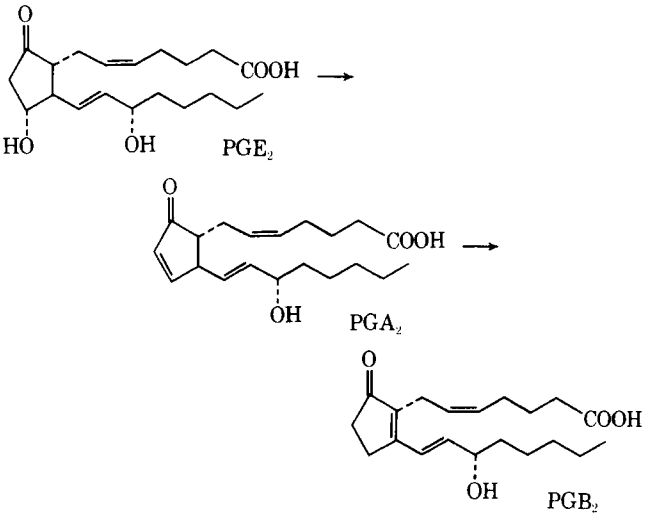
A funnel was attached to the column, and 5–10 mg of triethylaminoethyl cellulose was added from an inverted container supported on a 50-mesh screen by brief mechanical vibration of the screen. The side of the column was vibrated for a few seconds, and the material within was compacted with a loosely fitting stainless steel rod (1.8 mm) with an applied hand force of 0.454–0.681 kg (1–1.5 lb). The tamping rod was turned 120°, and three such compressions were made after each addition of support. Excessive compaction force results in columns with poor flow rates, so practice is required to produce columns with adequate flow (0.4–1.5 ml/min using 2000 psi at room temperature).

The fully packed columns were washed in succession with water, 0.5 *M* sodium acetate, and, finally, 0.5 *M* tromethamine adjusted to pH 7.20 with acetic acid. The mobile phase employed for chromatography of the prostaglandins was aqueous 0.05 *M* tromethamine adjusted to pH 7.20 with acetic acid. The operating condition was 0.45 ml/min at 2100–2300 psi (room temperature).

Pellicular Strong Ion-Exchange Columns—Columns of the strong anion-exchange support[3] were packed by the usual procedure (6). The mobile phase employed was 0.025 *M* tromethamine in acetonitrile–water (1:4) adjusted to pH 7.30 ± 0.02 with acetic acid. The operating condition was 0.50 ml/min at 350–400 psi (room temperature).

Peak Identification—Identity of the peaks was established by fraction collection followed by determination of the λ_{max} (PGA_2, 220 nm; PGB_2, 282 nm).

Compounds—PGB_2 was generated by treatment of either PGE_2[4] or PGA_2[4] with aqueous 0.05 *N* potassium hydroxide at 80° for 30 min (7). After cooling to room temperature, the solution was acidified to pH 3 with citric acid and extracted with ethyl acetate. The organic phase was dried with sodium sulfate, and



Scheme I—Conversion of PGE_2 *to* PGB_2 via PGA_2

[2] Cellex-T, BioRad Laboratories, Richmond, Calif.
[3] AS-Pellionex-SAX, Reeve-Angel, Clifton, N.J.
[4] Research Laboratories, The Upjohn Co.

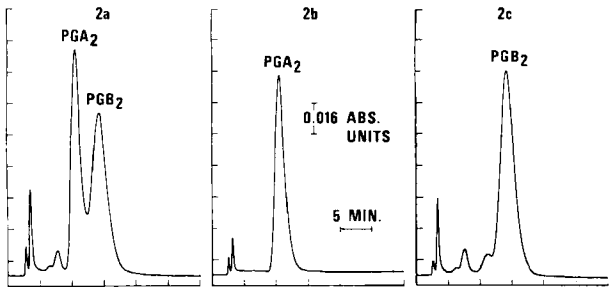


Figure 2—*Liquid chromatograms on the pellicular strong anion-exchange column using 0.025* M *tromethamine acetate (pH 7.30) in acetonitrile–water (1:4) at 350 psi and 0.5 ml/min. Key: 2a, mixture of* PGA_2 *(20 μg) and* PGB_2 *(7 μg); 2b,* PGA_2 *(17 μg); and 2c,* PGB_2 *(9 μg).*

the solvent was removed under vacuum at 45°. The oily residue was dissolved in methanol to provide a stock solution of PGB_2.

Sample Injection—Samples were injected under normal flow conditions using 1–10-μl charges of the prostaglandins dissolved in either methanol or aqueous potassium hydroxide.

RESULTS AND DISCUSSION

PGE_2 could not be detected by liquid chromatography using the 254-nm monitor due to the low molar absorptivity at this wavelength. PGA_2 and PGB_2 are both strongly UV absorbing and were detected easily at 254 nm. Figure 1*a* shows the separation of PGA_2 and PGB_2 on the triethylaminoethyl cellulose column using 0.05 *M* tromethamine acetate, pH 7.20. The separation is estimated to be 98% complete according to the method of Snyder (8). PGA_2 and PGB_2, when chromatographed separately (Figs. 1*b* and 1*c*), showed rather symmetrical peaks with little evidence of tailing.

Figure 2*a* shows the separation of PGA_2 and PGB_2 on the pellicular strong anion-exchange column using a mobile phase of 0.025 *M* tromethamine acetate, pH 7.30, in acetonitrile–water (1:4). Evidence of slight tailing was seen for the compounds when chromatographed separately (Figs. 2*b* and 2*c*). In purely aqueous systems, marked tailing occurred with this column; however, with aqueous–organic solvent systems, the tailing was minimized.

In Figs. 1*c* and 2*c*, the sample of PGB_2 was generated from PGA_2 with base. The extraneous peaks are probably due to the impurities in the initial sample of PGA_2 since generation of PGB_2 from PGE_2 in base shows very little extraneous peak formation.

Excellent linear relationships between peak heights and amount chromatographed were observed for PGA_2 and PGB_2 on both the triethylaminoethyl cellulose and the strong anion-exchange columns. Eight samples of PGA_2 were chromatographed on each column, with a peak height response range of 10–95% of full-scale (0.16 absorbance unit). A plot of micrograms injected *versus* peak height showed the following for the triethylaminoethyl cellulose and the pellicular strong anion-exchange columns, respectively: correlation coefficient 0.998, 0.999; slope 2.019, 2.307; and intercept 0.92, 1.03.

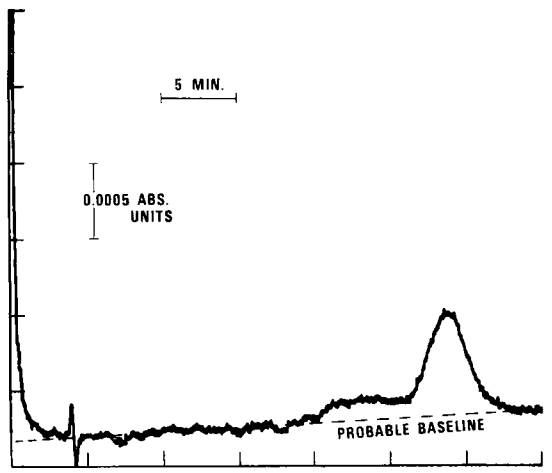


Figure 3—*Liquid chromatogram of 87 ng* PGB_2 *on the triethylaminoethyl cellulose column. Conditions were the same as in Fig. 1.*

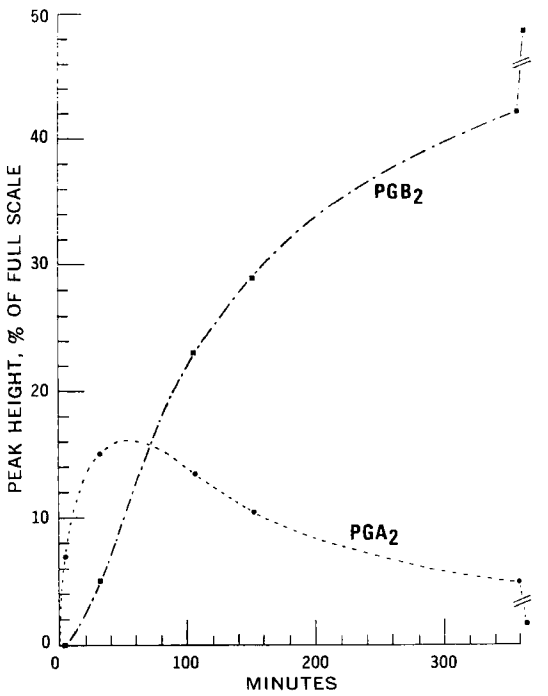


Figure 4—*Peak heights of* PGA_2 *and* PGB_2 versus *time as produced by treatment of* PGE_2 *(12.40 mg) with 1 ml aqueous 0.05* N *potassium hydroxide. Aliquots were injected directly onto the triethylaminoethyl cellulose column. The last data point on both curves was obtained at 1020 min.*

The lower limit of detection of PGB_2 was somewhat less than 87 ng on the triethylaminoethyl cellulose column (Fig. 3). A higher electrolyte concentration would permit detection of even lower levels of PGB_2.

The ease of data acquisition was demonstrated by monitoring the base-catalyzed conversion of PGE_2 to PGA_2 and PGB_2. A solution of PGE_2 (12.40 mg/ml) in 0.05 *N* potassium hydroxide at room temperature was periodically subjected to liquid chromatography by direct injection of a sample of the alkaline mixture onto the triethylaminoethyl cellulose column. The composite peak height *versus* time plot is shown in Fig. 4.

The amount of PGB_2 continually increased, whereas the amount of PGA_2 increased and then decreased as expected from the consecutive first-order reactions involved (Scheme I) (4). A small amount of 8-iso-PGA_2 is formed upon treatment of PGA_2 with base (9); since these two are inseparable by liquid chromatography, the peak heights in Fig. 4 represent a summation of these two compounds.

In conclusion, both columns are quite stable and can be used to monitor the stability of PGE_2 by following the appearance of PGA_2 and PGB_2 using the peak height method. Triethylaminoethyl cellulose gave somewhat better resolution of PGA_2 and PGB_2 as compared with the pellicular strong anion-exchange column, although this advantage is offset by the tedium required to pack columns of the former.

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Effects of γ-Irradiation on 2-Chloroethanol Formation in Ethylene Oxide-Sterilized Polyvinyl Chloride

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Abstract □ γ-Irradiation prior to sterilization with ethylene oxide is shown to enhance 2-chloroethanol formation in a surgical, hospital grade, polyvinyl chloride tubing. The site of formation and the insignificantly low 2-chloroethanol levels produced in this manner are discussed.

Keyphrases □ Ethylene oxide, use as sterilant of polyvinyl chloride—effects of γ-irradiation on 2-chloroethanol formation □ 2-Chloroethanol—effects of γ-irradiation on formation in ethylene oxide-sterilized polyvinyl chloride □ Irradiation, gamma—effects on 2-chloroethanol formation in ethylene oxide-sterilized polyvinyl chloride □ Polyvinyl chloride, ethylene oxide sterilized—effects of γ-irradiation on formation of 2-chloroethanol

When the formation of 2-chloroethanol from the ethylene oxide resterilization of previously γ-irradiated polyvinyl chloride was first reported (1), no data were presented and none have been reported supporting this observation, although references to these findings have been made (2, 3). This study was undertaken because of the lack of definitive data on the effects of γ-irradiation on 2-chloroethanol formation in polyvinyl chloride and the growing concern over the biological impact of this residue in sterilized polyvinyl chloride medical devices.

EXPERIMENTAL

Materials—Surgical, hospital grade, polyvinyl chloride tubing was used[1].

Procedure—The tubing was cut into samples approximately 1 cm long, weighing 120 ± 3 mg. The samples were divided into four groups and placed in glassine envelopes. One group received 2.5 Mrads of γ-irradiation from a ^{60}Co source and one received 5.0 Mrads. The two irradiated groups and one nonirradiated group were then exposed to a 1099-mg/liter concentration of ethylene oxide (12/88, ethylene oxide/freon 12 mixture) for 4.5 hr. A temperature of 55° and relative humidity of 50 ± 5% were maintained under a sterilization pressure of 15 psig. The fourth group remained as the untreated control. Immediately following sterilization with ethylene oxide, samples for 2-chloroethanol analysis from each group were placed in glass vials with rubber septa and crimped metal caps and extracted in 5 ml distilled water for 48 hr at 72°. The same number of treated samples were also extracted after 2 and 4 days of ambient aeration.

Additional samples from the group that was only sterilized with ethylene oxide were placed in vials for ethylene oxide analysis, extracted, and analyzed by the head-space technique (4). These samples were extracted at the same time as the 2-chloroethanol samples to compare the concentrations of both residues.

A separate group of samples was soaked in 2-chloroethanol[2] for 2.5 hr and heated for 3.5 hr at 72°. This material was sampled every 2–3 days over a 28-day ambient aeration period to assess 2-chloroethanol desorption.

Apparatus—Analysis of 2-chloroethanol was accomplished using a published GLC method (5) with slight modifications.

A gas chromatograph[3], equipped with a hydrogen flame-ionization detector connected to a 1-mv strip-chart recorder[4], was employed.

A U-shaped glass column, 1.83 m (6 ft) × 0.20 cm (0.078 in.) i.d., was packed with 3% polyethylene glycol[5] coated on a styrene–divinylbenzene copolymer resin[6] (80–100 mesh, less than 50 m^2/g surface area). The column was prepared according to the method used by Spitz and Weinberger (5) for their Column B.

The column was initially conditioned for 24 hr at 200° with a nitrogen flow rate of 30 ml/min. The column was then connected to the detector, and 2.5-μl injections of distilled water were made approximately every 15 min for several hours at a column temperature of 180°.

The instrument was operated isothermally at a column temperature of 170°, an injector temperature of 195°, and a detector tem-

Table I—Concentration of 2-Chloroethanol and Ethylene Oxide as a Function of Aeration Time (Mean ± *SD*)

Aeration, days	2-Chloroethanol, ppm: Ethylene Oxide Sterilized Only[a]	2-Chloroethanol, ppm: 2.5 Mrads + Ethylene Oxide Sterilized	2-Chloroethanol, ppm: 5.0 Mrads + Ethylene Oxide Sterilized	Ethylene[a] Oxide, ppm: Ethylene Oxide Sterilized Only
0	35 ± 2	329 ± 5	354 ± 7	8954 ± 438
2	n.d.[b]	19 ± 1	21 ± 1	33 ± 2
4	n.d.	n.d.	n.d.	n.d.

[a] Samples from the same ethylene oxide sterilization cycle were analyzed for both residues. [b] Not detected: detection limit 10 ppm.

[1] Tygon, S-50-HL, 4.762 mm (0.1875 in.) o.d. × 3.175 mm (0.125 in.) i.d., Norton Plastics and Synthetics Division, Akron, Ohio.

[2] Baker grade (anhydrous), J. T. Baker Chemical Co.
[3] Varian model 2100.
[4] Varian model 20.
[5] Carbowax 20M, Union Carbide Corp.
[6] Chromosorb 101, Johns-Manville Products Corp.