Novel Applications for Headspace Gas Chromatography*

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Key Words

Headspace analysis
Gas chromatography
Residual analysis of polyethylene terephthalate
Acetylene reduction assay
Ethylene evolution in germinating seed
Acetaldehyde generation in polyester resin

Summary

New application areas for headspace gas chromatography in agricultural and polymer degradation research are described. Specific examples are drawn from the various forms of headspace analysis with emphasis on the automated static equilibrium method.

Introduction

Headspace gas chromatography (HSGC) is a powerful technique for the analysis of volatiles in solids or viscous liquids. The theory and application of this technique have been reviewed in the literature [1–3]. Commercial headspace analyzers and sampling accessories have been developed for both the static equilibrium [4] and the purge-and-trap modes [5]. With the advent of automated instrumentation, HSGC is rapidly becoming the method of choice for the analysis of residual solvents and monomers in polymers [2, 6, 7] and volatile water pollutants [8]. As described by Kolb [2], quantitative HSGC requires more extensive calibration efforts than conventional GC, and therefore for repetitive analysis, the use of automated equipment is essential.

HSGC has been used in this laboratory extensively for both routine analyses and problem solving. Presented here are new application areas for HSGC in agricultural and polymer degradation research. Examples are drawn which illustrate different headspace techniques with emphasis on the use of automated equilibrium methods.

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Experimental

Reagents

Acetaldehyde (>99%) was obtained from Aldrich Chemical Co. (Milwaukee, Wisconsin). Pure ethylene and acetylene were obtained from Matheson Gas Co. (East Rutherford, NJ). Certified gas mixtures of ethylene in acetylene (2.0 and 0.20 mole%) and ethylene in nitrogen (9.9, 0.88, 0.09 ppm) were also obtained from Matheson.

Apparatus

For equilibrium headspace analysis, either a Perkin Elmer (Norwalk, CT) Model F-42 or F-45 headspace analyzer was used. Detailed descriptions of these instruments are given elsewhere [4]. Each instrument consists of a thermostatted sample turntable, an electropneumatic dosing head for automatic injection and an F-22 Gas Chromatograph with flame ionization detector (FID). The detector block on the F-22 was modified to permit installation of both thermal conductivity detector (TCD) and FID in series. These headspace analyzers were coupled to a Hewlett Packard (Avondale, PA) Model 3354 laboratory data system for automatic data acquisition and calculations. A discontinuous gas extraction apparatus (Perkin Elmer accessory) was used to cross-check static HSGC results. For the purge-and-trap method, a Varian (Walnut Creek, CA) Model 2700 gas chromatograph with FID was used. For the acetaldehyde generation rate study, a custom-designed, precision-controlled injection port was employed (New Jersey Technical Consultants, Middlesex, NJ). For grinding polymers at liquid nitrogen temperature, a Spex freezer/mill (Spex Industries, Metuchen, NJ) was used.

Applications

Automated Acetylene Reduction Assay

The acetylene reduction assay has gained wide-spread acceptance for the measurement of nitrogen fixation [9]. This assay, proposed by Hardy in 1967, involves the nitrogenase catalyzed reduction of acetylene to ethylene and subsequent measurement by GC [10]. The assay has been applied to entire plants, bacterial cultures, excised root systems, detached root nodules and enzyme preparations

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[11]. Soya-bean roots were used in this study to demonstrate the feasibility of HSGC for performing this assay. A direct application was the rapid screening of numerous mutant Rhizobia to identify strains having superior nitrogen fixation abilities [12].

The screening procedure is summarized below:

Soya-bean, inoculated with a Rhizobium strain, was grown into plants under standardized conditions [12]. After two weeks, nodulated root systems of each plant were excised and sealed inside a glass vial. One milliliter of acetylene was introduced immediately into the vial with a gas-tight syringe. After an incubation period of 45 min at 25 °C, the acetylene reduction assay was performed. Typical HSGC traces of such an assay are shown in Fig. 1. Analysis time is one minute, with a precision of better than ±2%. Calibration was accomplished by analyzing an empty vial containing 1.0 ml of 2.0 mole% ethylene in acetylene. Results of each analysis were expressed as mole % of ethylene in acetylene. Since all Rhizobia strains were assayed under identical conditions, the mole % can be directly correlated to the nitrogen fixation rates. Because of large plant/plant variation, batches of 25 plants were evaluated in each experiment to reduce the standard error of the entire procedure. A standard Rhizobia strain was always tested along with each sample strain. Typical results are shown in Table I. In comparison to manual sampling with a gas-tight syringe, HSGC was found to be faster and more precise while permitting automatic data reduction.

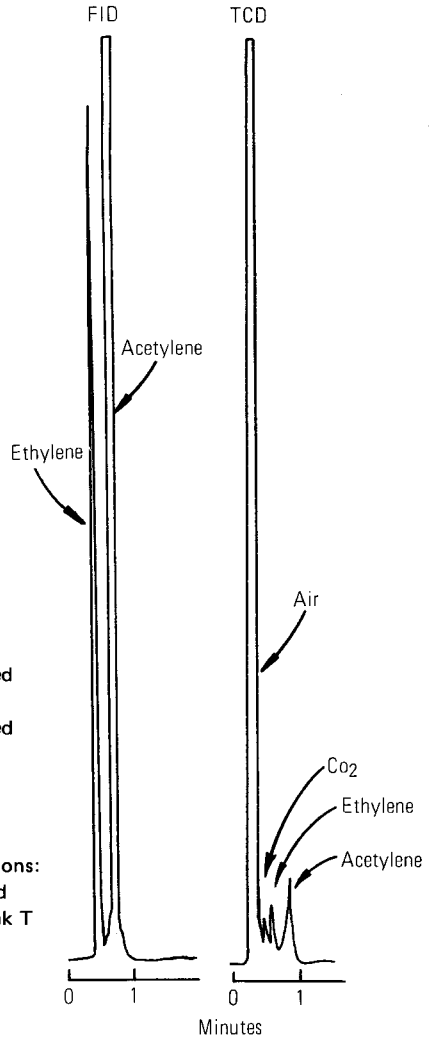

Fig. 1

Automated acetylene reduction assay.

Roots from 2-week old soya been plant inoculated with Rhizobia.

HSGC analysis was carried out 45 min after the injection of 1.0 ml of acetylene into the vial containing the sample.

Chromatographic conditions: 1.3 m x 1.8 mm i.d. packed column containing Porapak T 60/80 mesh. Column temperature: 100 °C.

Ethylene Generation During Seed Germination

Ethylene, a product of fatty acid metabolism, is produced by many seeds during the germination process [13–16]. These trace levels of ethylene subsequently act as a stimulant and contribute to the breaking of dormancy. Esashi and Leopold [16] showed that as little as 0.01 ppm of ethylene can drastically increase the percentage of germination in clover seeds.

In this laboratory, an HSGC procedure was devised to study the ethylene generation phenomenon in alfalfa and cotton seeds [17]. Conventional headspace sampling using a gas-tight syringe suffers from poor sensitivity and memory effects. With the improved sampling device of the automated headspace analyzer, a sensitivity of 10 ppb ethylene was achieved. Typical HSGC traces of 100 mg alfalfa seed 4 h after inbibition of 0.2 ml of water are shown in Fig. 2. The effect of different seed treatments on the ethylene generation rate was also studied (Table II).

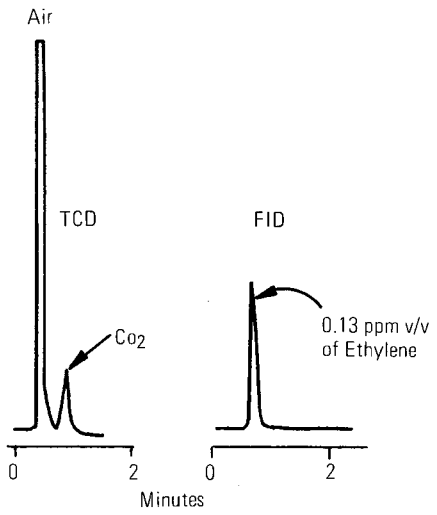

Fig. 2

Ethylene generation during seed germination.

The sample vial contained 100 mg of alfalfa Dekalb 123. HSGC analysis was carried out 4 h after the addition of 0.2 ml of water into the vial.

Chromatographic conditions: 1.3 m x 1.8 mm i.d. packed column containing Porapak Q 80/100 mesh. Column temperature: 100 °C.

Acetaldehyde Analysis in Polyethylene Terephthalate (PET) Bottles and Resins

Acetaldehyde is a major degradation product formed during the polycondensation and melt processing of PET [18]. Because of its organoleptic properties, residual acetaldehyde in PET containers can impart off-taste to cola beverages. The residual acetaldehyde produced at molding temperatures must therefore be controlled and followed throughout processing [19]. Several HSGC procedures were developed for these measurements which were subsequently

Table I. Comparison of acetylene reaction rate of Rhizobia strain under our testing conditions

Strain of Rhizobia	Mole of Ethylene*	Standard deviation
Standard Strain 1 (day 1)	4.2	1.9
Standard Strain 1 (day 2)	4.0	2.3
Standard Strain 1 (day 3)	5.9	1.7
Strain A	0.8	0.34
Strain B	0.3	0.25
Strain C	5.9	2.3
Strain D	5.8	2.0
Strain E	1.4	1.8
Strain F	1.4	0.9
Strain G	0.2	0.9
Strain H	2.0	0.65
Strain I	2.2	1.0

* Each value is average of 25 samples.

Table II. Comparison of different seed treatments on ethylene generation rate

	Ethylene (ppm)*			
	4 h	28 h	52 h	76 h
DeKalb alfalfa –				
untreated	0.09	0.5	2.4	2.8
1% NaOCl	0.04	0.7	2.6	2.9
coated	0.02	1.0	2.4	2.5

* 100 mg of DeKalb alfalfa seed inbided with 0.20 ml of H_2O. Result of duplicate analysis.

adopted as specification testing methods for the production of bottle grade PET resins. The methods are summarized below.

Bottle headspace test. This test was originally used by the soft-drink industry as an evaluation tool because it was simple and appeared to measure the acetaldehyde diffusing out of the bottle wall. The test has been adopted as part of a quality control specification by most suppliers (bottle molders). The test itself is quite straight-forward though the results are often severely influenced by outside factors other than the actual acetaldehyde level in the bottle walls [19].

In this laboratory, a molded preform is blown into a bottle which is then purged with nitrogen, sealed with a septum and placed in an environmental chamber (70 °F, 65% relative humidity). After 24 h, the bottle headspace is analyzed. A 1-ml gas sample is taken with a gas-tight syringe and injected into a GC. Calibration is accomplished with a standard solution of acetaldehyde in water [20]. A typical HSGC trace is shown in Fig. 3. Analytical precision is about ±10%. However, results are often affected by outside factors such as equilibration temperature and age of preforms. Acceptable bottle headspace values usually range from 2.5 to 3.0 μg/l. Though the results of the bottle headspace test are fairly accurate under rigidly standardized conditions and can be correlated with taste panel results, the author believes that direct residual acetaldehyde testing on the preforms and bottles is more convenient and accurate.

Residual acetaldehyde (RAC). As a resin supplier, a more direct and rapid measurement on the residual acetaldehyde on the resin or bottle is required. A method based on purge and trap GC was developed and used for a number of years [21]. Briefly, the procedure involved solid-injecting 25 mg of powdered PET in a glass sleeve into a GC injector port at 145 °C for 20 min, trapping the off gas on a cold column and then eluting the acetaldehyde by heating the column. Recently, an improved method using an automated headspace GC was developed. Details of the procedure have been published elsewhere [22] Briefly, the analysis sequence entails sample grinding in a liquid nitrogen mill, storage in a septum capped vial, thermal treatment to liberate acetaldehyde and subsequent headspace analysis for acetaldehyde content. This method offers significant advantages in sensitivity (50 ppb), precision (±7%) and sample throughput (40 sample/shift) compared to solid-injection GC. A comparison of the two RAC methods is summarized in Table III. Discontinuous gas extraction [22], a new technique based on stepwise gas extraction, was used to cross-check RAC results (Fig. 4).

Generation Rate of Acetaldehyde (GRA Test)

As mentioned above, acetaldehyde can be generated during the melt processing step. Low values for both GRA and RAC in PET resin are necessary therefore for an acceptable commercial product. In this laboratory, a rapid and accurate

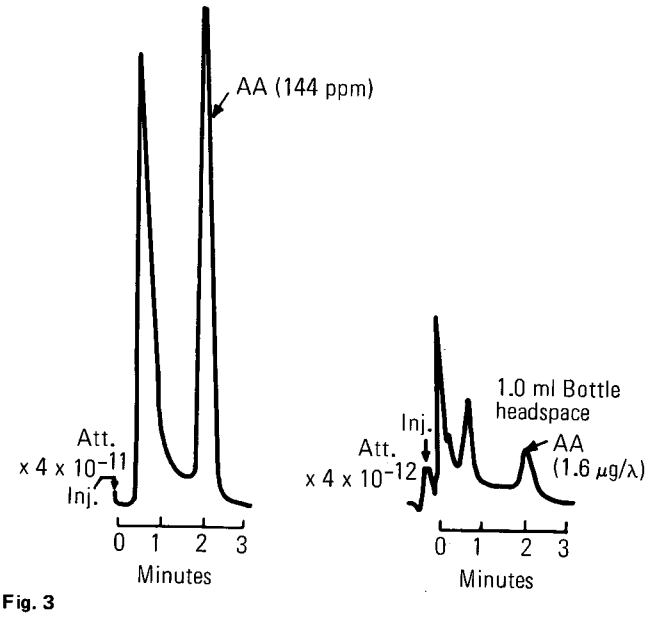

Fig. 3

Typical HSGC chromatogram of PET bottle headspace.

Left: 1 μl of aqueous standard solution. Right: 1.0-ml aliquot of the bottle headspace. AA acetaldehyde.

Chromatographic conditions: 2 m x 1.8 mm i.d. packed column containing Porapak Q 80/100 mesh. Column temperature: 135 °C.

Fig. 4
Discontinuous gas extraction analysis of PET resin.
Chromatographic conditions:
1.3 m x 1.8 mm i.d. packed column containing Porapak Q 80/100 mesh.
Column temperature: 135 °C.
AA acetaldehyde.

Table III. Comparison of residual acetaldehyde analyses

Step No.	Solid injection GC method	Automated headspace method
1	Grind resin under liquid nitrogen	Grind resin under liquid nitrogen
2	Weigh 20–30 mg of sample into glass injection sleeve	Weigh 2g of sample into vial and cap
3	Insert tube into 145 °C injection port	a) Place vial in 130 °C oven for one hour b) Place vial into 92 °C turntable of the HSGC for indexing and analysis
4	Degas onto cold GC column for 20 min	Pressurize vial
5	Heat GC column to 150 °C	Depressurize vial into GC column
6	Detect peak with FID	Detect peak with FID
Calibration	Inject aqueous acetaldehyde standard solutions into gas chromatograph	Inject acetaldehyde standard solutions into 2.0-g of "dried" resin and repeat steps 3–6, above
No of samples per shift	Approximately six samples	Up to 40 single analysis or 20 samples
Advantages	Simple equipment	Good sensitivity and precision; automated analysis

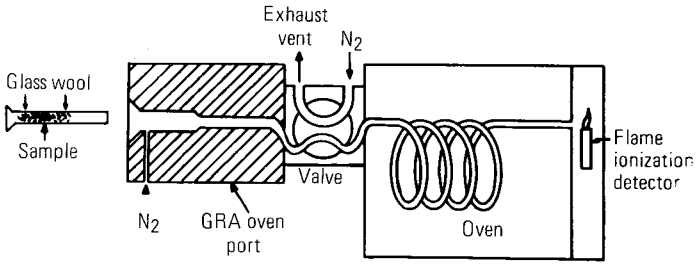

Fig. 5
GRA test apparatus.

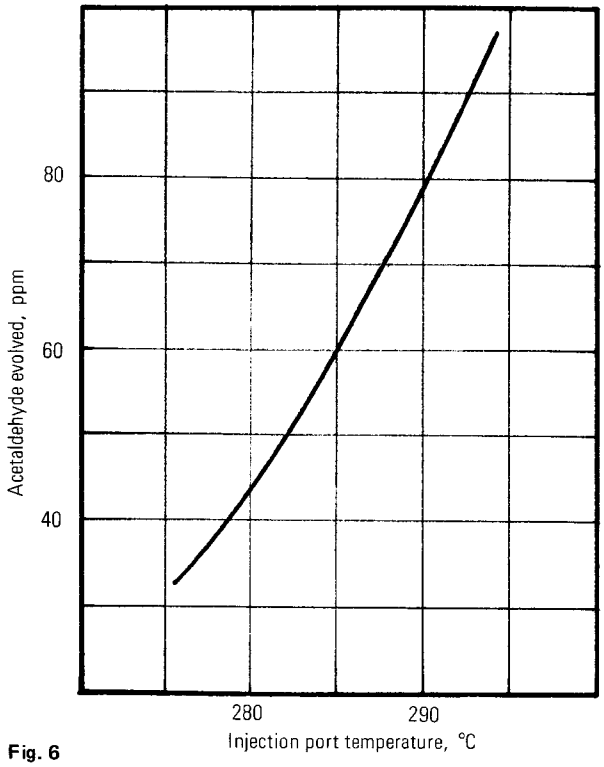

Fig. 6
Effect of temperature on the generation rate of acetaldehyde.

method to measure GRA was developed [23]. A schematic of the GRA apparatus is shown in Fig. 5. The GRA oven port, a custom-built precison controlled port constructed from an aluminium block (±0.2 °C set point) replaces the normal GC injection port. This is critical because data obtained showed that GRA values increase exponentially with temperature (Fig. 6). The procedure of using the GRA apparatus is as summarized below:

(a) Degas powdered sample at 145 °C for 20 min;
(b) place sample in port at 280 °C and vent into cold column for 10 min;
(c) valve sample to exhaust and nitrogen to column;
(d) heat column to 145 °C and measure acetaldehyde as evolved.

Results are expressed as ppm of acetaldehyde generated per minute. The precision of the test is ±10% for duplicate analyses. Sensitivity is about 0.2 ppm/min. Typical GRA traces are shown in Fig. 7. The range of GRA and RAC in commercial PET bottle resins is shown in Table IV.

Table IV. RAC and GRA values for commercial PET bottle resins

		Resin			
		A	B	C	D
Acetaldehyde	ppm	1.7	0.8	2.4	2.1
Generation rate	ppm/min	2.4	4.0	3.2	3.4

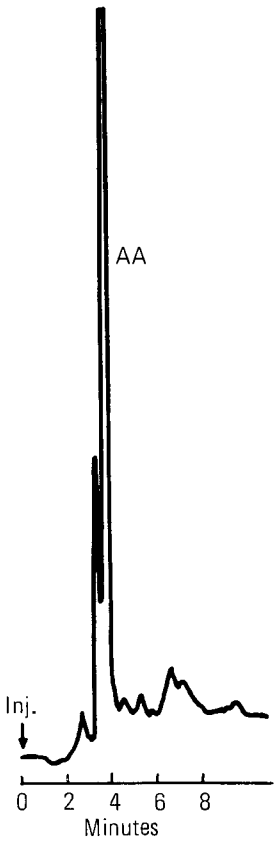

Fig. 7
Typical chromatogram obtained in the GRA test.
Chromatographic conditions:
2m x 1.8mm i.d. packed column containing Porapak Q 80/100mesh.
Column temperature: 145 °C.
AA acetaldehyde.

Conclusion

Headspace GC methods were developed for the acetylene reduction assay, ethylene generation during seed germination and acetaldehyde analysis of PET bottles and resins. The use of the automated headspace analyzer allows rapid sample turnaround and generally yields better precision and sensitivity than manual headspace sampling methods.

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