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Evaluation of the pulsed discharge helium ionization detector for the analysis of hydrogen and methane in breath

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Abstract

Under the appropriate separation conditions the pulsed discharge helium ionization detector (PDHID) was used to detect hydrogen and methane separated from the matrix components of human breath samples. The sensitivity of this method is over an order of magnitude better than published methods using a flame ionization detector (FID) and a thermal conductivity detector (TCD), and has the further advantage of detecting both analytes with only one detector. Limits of detection were 0.3 ppmv for both hydrogen and methane and the method had a linear dynamic range (LDR) of three orders of magnitude (0.3–400 ppm, v/v). The PDHID was also compared to the FID and the TCD in regard to selectivity, sensitivity and reproducibility for high-speed gas chromatography (HSGC). It was shown that the PDHID is as sensitive as the FID for fast separations but is limited by the difficulty of resolving analyte peaks from O_2 and N_2. The PDHID was at least three orders of magnitude more sensitive than the TCD for all of the analytes examined.

Keywords: Detection, GC; Pulsed discharge helium ionization detection; Air analysis; Hydrogen; Methane; Gases

1. Introduction

The pulsed discharge helium ionization detector (PDHID) is a sensitive and universal detector [1]. It has been compared to the argon ionization detector (AID) and the helium ionization detector (HID), which are not suited for routine analysis because of their instability, but have better sensitivity and similar linear dynamic ranges (LDR) [2–5]. The PDHID is 500 times more sensitive than the thermal conductivity detector (TCD) and 50 times more sensitive than the flame ionization detector (FID) [6,7]. Reviews of the detection mechanism and response characteristics of the PDHID have been presented elsewhere [3,8].

The properties described above make the PDHID well suited for certain gas chromatographic measurements. Because it is a universal detector, the PDHID can be used for the analysis of certain gases that cannot be detected with the FID (e.g. O_2, N_2, O_2, Ar, SO_2, CO, CO_2, and H_2CO), and its sensitivity makes it potentially a better choice than a TCD. For samples containing low levels of both volatile organic species and oxidized analytes, which are typically analyzed in two or more separate measurements, the PDHID could be an ideal detector because it would allow all of these analytes to be measured in a single analysis.

Methane and hydrogen levels in human breath have been used to monitor microbial metabolism in the colon [9,10]. Traditionally, after separation on a packed column, a TCD was used for the quantification of hydrogen and an FID for methane [11]. This system is cumbersome and relatively insensitive since the reported limit of detection for each analyte is 6 ppm when a 1 ml or 2 ml injection is made on an 8 m packed column for methane or hydrogen, respectively [12]. It should be possible to make small volume injections on capillary columns in order to reduce analysis time while achieving better limits of detection with the PDHID. In this paper, the PDHID is compared to the TCD in regard to permanent gases and the FID for organic compounds followed by its application to separated breath components, hydrogen and methane. We have given special attention to detector performance for fast separations.

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2. Experimental

2.1. Standards

Gas-phase organic standards were prepared from benzene, toluene (>99.5% purity, Chem Services, West Chester, PA, USA) and hexane (HPLC/spectroscopic grade, EM Science, Gibbstown, NJ, USA). These standards were injected as liquids, using a gas-tight syringe into filled 2 l Tedlar bags (Supelco, Bellefonte, PA, USA) and allowed 60 min to vaporize and equilibrate. The solvent gas in these standards was medical-grade air (Airgas, Radnor, PA, USA). Standards for methane (National Specialty Gases, Durham, NC, USA), hydrogen (Airgas) and carbon monoxide (Union Carbide, New York, NY, USA) were prepared similarly by injecting the appropriate quantities of these gases into filled Tedlar bags using a gas-tight syringe. Successive dilutions were made to prepare samples with concentrations differing by an order of magnitude.

2.2. Instrumentation

2.2.1. Sampling and injection

Gaseous samples were injected using a loop injector. The loop injector consisted of a Valco six-port, two-position valve (Valco, Houston, TX). The sample loop (9 μl), as well as all tubing and connections, were 1/16 in. stainless steel. Samples were pulled through the sampling loop using a Gast model DOA-P104-AA rotary pump (Daigger, Vernon Hills, IL, USA).

2.2.2. Gas chromatographs

GC–PDHID experiments were conducted either with a Hewlett-Packard 5890 oven or with an oven constructed in our laboratory. In each case, a Valco model D-1 PDHID and electrometer were installed (Valco, Houston, TX, USA). Data obtained from both instruments were nearly identical. All data collection occurred on a Hewlett-Packard HP 3396 Series II integrator (Hewlett-Packard, Wilmington, DE, USA). UHP helium (Airgas) was used as both the carrier and discharge gas. An Indicating Oxytrap (Alltech, Deerfield, IL, USA) followed by a Valco model HP helium purifier (Valco) were placed between the cylinder and the flow splitter.

The GC–FID experiments were either performed on the HP 5890 with the factory-installed FID or with the laboratory-built instrument with an FID retrofitted from a Varian Model 3700 GC (Varian Instruments, Palo Alto, CA, USA). Data from both instruments were nearly identical. All data collection occurred on a Hewlett-Packard HP 3396 Series II integrator (Hewlett-Packard). Balloon-grade helium was used as the carrier gas, normal-grade hydrogen was used as the fuel and medical grade compressed air was used as the oxidant (Airgas).

A Hewlett-Packard 5890A GC with a TCD using UHP helium carrier gas was used for comparison and was configured using the six-port loop injector described for the PDHID system.

2.3. Separation conditions

Volatile organic compounds (VOCs) were separated on a 2.5 m × 0.25 mm i.d. DB-5 column with a 0.25 μm stationary phase at 45 °C (J&W Scientific, Folsom, CA, USA). Flow restrictors were used to set the carrier gas flow rate to 7.5 ml/min for comparisons of the PDHID to the FID. Makeup flow on the FID was adjusted to give a total flow of 30 ml/min. Discharge gas on the PDHID was set at 25 ml/min.

H_2, O_2, N_2, CH_4 and CO were separated on a molecular sieve 5A porous-layer open tubular (PLOT) column, 30 m × 0.32 mm, held at 35 °C (Supelco, Bellefonte, PA, USA). Flow restrictors were used to set the carrier gas flow rate at 5.2 ml/min for comparisons of the PDHID to the TCD. The GC–TCD used a carrier gas flow rate of 2 ml/min. Makeup gas was added to reach the recommended 5 ml/min and the reference gas was set at 20 ml/min. The injector, oven and detector temperatures were set at 100, 80 and 200 °C, respectively.

Breath samples were separated on a 30 m × 0.32 mm i.d. RT-Msieve 5A column (Restek, Bellefonte, PA, USA). Flow was set at 2 ml/min using flow restrictors. The oven temperature was 30°C while the detector was 190 °C.

2.4. Human breath samples

The effects of diet on colonic fermentation was assessed by measuring hydrogen and methane production. Samples were collected from non-smoking males ages 36 and 41 years with body mass indexes (BMI) of 28.5 and 34.5, respectively. Both subjects were screened for general health before they were allowed into the study. Subjects gave their informed consent to participate in the study and all aspects of the study were reviewed and approved by the Institutional Review Board of the University of North Dakota.

Hydrogen and methane were determined from expired air samples collected before and after exercise-induced oxidative stress. Prolonged exercise performance tests were conducted using a stationary cycle (ergocycle). Subjects pedaled the ergocycle for 45 min at 70% of maximum heart rate, and 5 min at 90%. Breath samples were collected before and 7 min after exercise. Samples were collected using a one-way breathing valve (Hans Rudolph, Kansas City, MO, USA) connected to 20 l Cali-5-Bond sampling bags (Calibrated Instruments, Hawthorne, NY, USA) and analyzed within 3 h of collection. The one-way breathing valve was attached to the two-way valve of the Cali-5-Bond sampling bag. Subjects were seated and instructed to breath naturally until their expired breath completely filled the sample bag. Two-way valves were opened immediately prior to the subjects' first exhalation and closed immedi-

ately after their final exhalation. Calibration curves were used to quantitate analytes in samples.

3. Results and discussion

3.1. PDHID versus FID selectivity

While the universal nature of the PDHID allows us to analyze *both* methane and hydrogen, it poses potential limitations that must be considered. Separations of compounds in an air matrix, like breath samples, are not as trivial when the PDHID is used as they are when using an FID. Components of air, such as oxygen, nitrogen and water, can interfere with separations detected with the PDHID. These components are essentially invisible to the FID so their retention times are not important. However, since nitrogen and oxygen are present at 78 and 21%, respectively, their presence cannot be neglected in separations detected with a PDHID. The ability of the detector to recover after the introduction of a sample in an air matrix also becomes a concern when separation times are less than 1 or 2 min. With our experimental setup it took approximately 10 s after the air peak to be able to quantify an analyte peak. Recovery of the baseline to initial levels was on the order of minutes. On non-polar columns (e.g. the DB-5 used here), polar compounds such as water produce trailing peaks that interfere with quantitation, especially in HSGC applications. In contrast, the selectivity of the FID allows for the non-detection of compounds that cause interferences with the PDHID.

3.2. PDHID versus FID sensitivity

Reproducibility and sensitivity are major goals in developing any new analytical method. Table 1 shows limits of detection for fast separations detected using the PDHID, FID and TCD (as determined by calibration curves). For all comparisons, all chromatographic conditions were the same (see Section 2); the only difference in the systems was the detector used. Both the PDHID and the FID systems were able to quantitate methane, hexane, benzene and toluene at low ppmv concentrations. For these fast separations, the sensitivity of the PDHID is only equal to that of the FID, as opposed to comparisons with longer separation times in which the PDHID was superior [5,6]. For our fast separations the PDHID measurements were somewhat limited by the coelution of analytes with oxygen and nitrogen. This is illustrated in Fig. 1, which shows chromatograms of 60 ppm (v/v) (ppmv) mixtures detected on both instruments. Specifically, Fig. 1B shows the trailing edge of the air peak in the chromatogram taken with the PDHID. Note that the separation time is not limited by the resolution between analytes in this analysis, but because of the large quantities of oxygen and nitrogen present in the sample, the PDHID baseline does not immediately stabilize. Although it is possible to cut the eluate containing oxygen and nitrogen away from the detector, this strategy is limited for two reasons. First, because of the fast separation times we are trying to achieve, we cannot completely remove all of the oxygen and nitrogen. Second, a small change in flow disrupts the PDHID signal. Note the baseline preceding the analytes in Fig. 1B. Artifacts 4 and 5 are dependent on the time it takes to switch the six-port valve from the load to inject position. The PDHID is responsive to even minute changes in flow, and these artifacts can interfere with quickly eluting analyte peaks, adding to the uncertainty of their integrated peak areas.

Table 1
Comparison of the Limits of detection for selected compounds on the PDHID, FID and TCD when using small volume loop injections (9 μl)

Analyte	Limit of detection (ppmv)		
	PDHID	FID	TCD
Methane	0.3	1	
Hexane	2.3	0.8	
Benzene	1.6	0.9	
Toluene	1.2	0.8	
Hydrogen	0.3		ND
Methane	0.3		2500
Oxygen	1		12000
Nitrogen	1		10000
Carbon monoxide	4		2500

ND: not detected at any concentration.

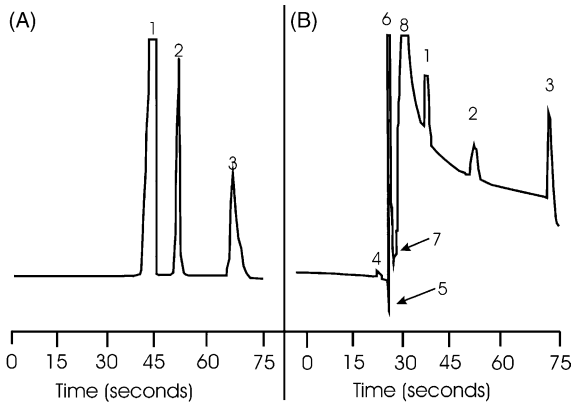

Fig. 1. Separations detected by the FID (A) and PDHID (B). 1: 60 ppmv hexane; 2: 60 ppmv benzene; 3: 60 ppmv toluene; 4: injection artifact; 5: injection artifact; 6: start of air peak; 7: detector overload; 8: end of air peak (2.5 m × 0.25 mm i.d., 0.25 μm, DB-5 column at 7.5 ml/min and 45 °C).

Because of the large background caused by the unresolved air peak, in these fast separations the limits of detection for the PDHID were not better than the FID as reported for GC analyses reported by others [6,7]. However, they were comparable. Depending on the analyte, detection limits for both detectors, as determined by calibration curves, were in the single to sub-parts per million range under the identical conditions used for each system to generate high-speed

separations. The major limitation to quantification with the PDHID was the integration of analyte peaks on the sloping baseline. Same day reproducibility for peak areas of 10 ppmv hexane, benzene and toluene were better for the FID (6, 5 and 6% R.S.D., respectively) as compared to the PDHID (10, 8 and 7% R.S.D., respectively). But, day-to-day reproducibility was better for the PDHID (8, 15 and 11% R.S.D., respectively) than the FID (21, 21 and 19% R.S.D., respectively).

3.3. PDHID versus TCD selectivity and sensitivity

Both the PDHID and the TCD are essentially non-selective detectors, and either could be used to measure both hydrogen and methane in breath samples. One advantage of the PDHID is in its greater sensitivity to light gases. As expected, the TCD showed little or no response to hydrogen, which does not have a significantly different thermal conductivity than the helium carrier gas, while the PDHID was useful for this measurement. Because both the PDHID and the TCD are universal for all other analytes, both are equally affected by the potential interferences from the air matrix.

The TCD was compared to the PDHID for sensitivity and day-to-day reproducibility. Calibration curves were constructed with the PDHID over four orders of magnitude, from 2500 to 2.5 ppmv while calibration curves for the TCD were constructed from 7500 to 2500 ppmv. Table 1 shows the results for calculated limits of detection based on these curves. Peak areas for 25 ppmv oxygen, nitrogen and hydrogen on the PDHID had less than 3% R.S.D. over a 3-day period. The TCD was less stable over the 3-day period; 2500 ppmv oxygen and nitrogen had more than 10% R.S.D. Hydrogen was not detected with the TCD. Carbon monoxide was poorly resolved from nitrogen, which explains both its relatively high limit of detection (4 ppmv) and its poor day-to-day reproducibility on both detectors (>20% R.S.D. at 25 ppmv). Together these data demonstrate that the PDHID is more sensitive and reproducible than the TCD while having a greater linear dynamic range.

Limits of detection for the TCD appear rather high, but they are based on the small injection volumes (9 μl) used in these experiments. Small volume loops help to minimize injection band duration and allow high-speed separations to be made with good resolution. Limits of detection for the TCD have been reported to be 10^{-6} to 10^{-8} g, which are in agreement with our values at these injection volumes [13]. In comparison, the total minimum quantity detected by the PDHID in our experiments was 10^{-10} to 10^{-12} g which is similar to results published by Wentworth et al. [14]. The more sensitive PDHID can detect analytes at levels in the low ppmv range under fast separation conditions using a small volume injection loop, which is not possible with the TCD. This is greatly advantageous for our studies monitoring hydrogen and methane in breath samples.

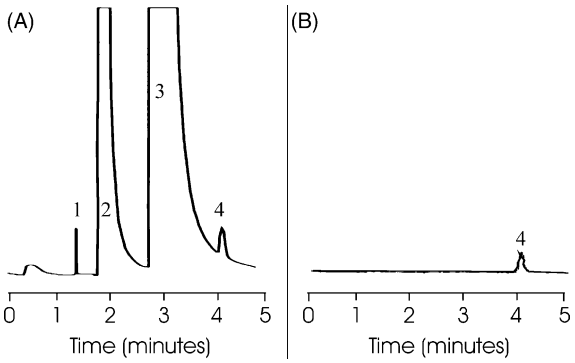

Fig. 2. Air samples spiked with 5 ppmv hydrogen and methane detected with a PDHID (A) and an FID (B). 1: hydrogen; 2: oxygen; 3: nitrogen; 4: methane (30 m × 0.32 mm i.d., RT-Msieve 5A column at 2 ml/min and 30 °C).

3.4. Application to human breath

In the experiments discussed so far in this paper, separations were completed in less than 1 min to determine the suitability of the PDHID for fast GC applications. However, this can limit the sensitivity of the analysis because analyte peaks can be superimposed or lost in larger interfering peaks. The goal of the breath sampling experiments was to reliably monitor methane and hydrogen concentrations at sub-ppmv levels. In order to achieve this goal, some speed was sacrificed. Fig. 2 shows separations of air samples containing 5 ppmv methane and hydrogen detected with the PDHID and the FID. The separation is completed in less than 5 min, which is still useful for monitoring experiments. As Fig. 2 shows, oxygen and nitrogen are not detected by the FID, which simplifies the chromatograms, but does not allow for the detection of hydrogen. In this case, the FID had a limit of detection for methane of 1 ppmv. Limits of detection using the PDHID for hydrogen and methane were both 0.3 ppmv with a linear dynamic range of more than three orders of magnitude (0.3–500 ppmv) when calibration curves were constructed.

It has been reported that approximately half of the human population emits hydrogen and methane in their breath as a product of microbial fermentation in the colon [10]. In the past, separations of human breath were detected using the TCD for hydrogen and an FID for methane, with both methods having a limit of detection of 6 ppmv [12]. As mentioned above, the TCD has poor sensitivity for hydrogen. We were able to use the universal PDHID as a detector for both analytes by using a small volume injection on a capillary column to separate methane from nitrogen.

Fig. 3 shows chromatograms for breath samples from two subjects, classified, respectively, as a ‘non-methane producer’ (Fig. 3A) and a ‘methane producer’ (Fig. 3B) according to the quantities of methane that were exhaled by each. The chromatograms shown correspond to methane concentrations of less than 0.3 ppmv and

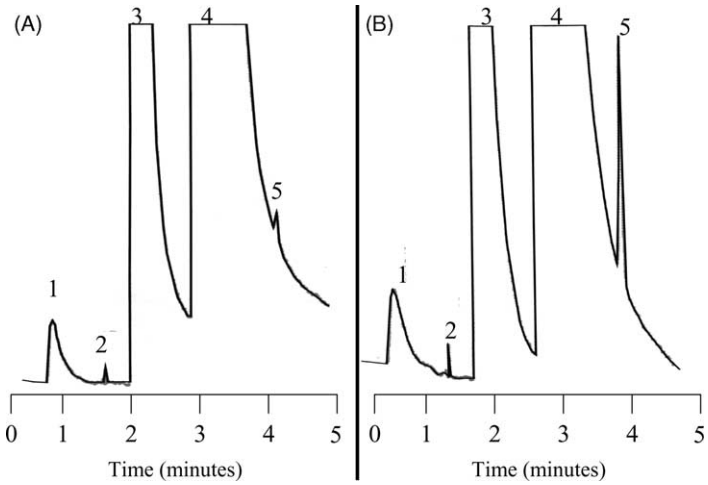

Fig. 3. Separation of breath samples from a 'non-methane producer' (A) and 'methane producer' (B). 1: Injection artifact; 2: hydrogen; 3: oxygen; 4: nitrogen; 5: methane (30 m × 0.32 mm i.d., RT-Msieve 5A column at 2 ml/min and 30 °C).

20.5 ± 0.7 ppmv, respectively. Note that the methane peak is partially resolved from the nitrogen peak, which allows it to be measured with the universal PDHID although it does affect the overall detection limit. The hydrogen peak is well resolved from both the air peaks and from the injection artifact. The chromatograms in Fig. 3A and B correspond to concentrations of 2.0 ± 0.1 ppmv and 8.9 ± 0.7 ppmv hydrogen, respectively. The former of these is below the detection limit for the previously reported method using the TCD, in spite of the fact that the injection volume was two orders of magnitude smaller. The detection limits for hydrogen and methane were both 0.3 ppmv. These detection limits, particularly for hydrogen, allow these metabolic markers to be probed at levels impossible with previous methods. The breath sampling experiments are part of an ongoing study that will be reported at a later time.

The chromatograms shown in Fig. 3 were for samples taken prior to exercising. We were also able to measure samples after exercise (data not shown). Post-exercise hydrogen and methane concentrations were lower than pre-exercise concentrations because of the dilution effects caused by the rapid breathing that followed exercise. When monitoring many subjects, analysis time becomes important, and we were able to keep the separation time to less than 5 min using this method. While the duration of the separation required to separate hydrogen and methane could easily be reduced doing so would cause the injection artifact to interfere with the hydrogen peak, and the methane peak would be lost in the nitrogen peak. This illustrates a limitation of the PDHID that must be considered when using it for fast separations. However, if these factors are taken into consideration, the PDHID is a very powerful detector that can be advantageous compared to an FID or a TCD, or even the combination of the two.

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