Journal of Chromatography, 416 (1987) 119–124
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3547

Note

Gas chromatographic evaluation of hydrogen in the breath as a diagnostic method for the study of intestinal digestion and absorption of dietary carbohydrates in man

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(First received August 5th, 1986; revised manuscript received December 8th, 1986)

Hydrogen breath testing is used to evaluate the concentration of this gas in the breath after oral administration of carbohydrates. This technique is based on Levitt's observation that hydrogen is produced in the colon when carbohydrates are fermented by colonic bacteria, with subsequent rapid excretion through the lungs [1, 2].

Several gas chromatographic (GC) techniques have been used for this purpose [2–13]. Comparison of the results is difficult, mainly because often the sensitivity limit and linearity of the various techniques were not clearly reported [2, 3, 7, 13]. The aim of this study was therefore to evaluate and compare different detectors and columns in order to establish the technique and sensitivity that are suitable for different applications (clinical purposes, research, etc.). The detection methods examined were thermal conductivity (TCD, two types of detector) and a helium ionization (HeD).

EXPERIMENTAL

Sample collection

The breath tests were carried out in the following manner. During the day before test, the patient was asked to follow a starch-free diet (avoiding bread,

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pasta, rice, potatoes and beans). The test was then performed after an overnight fast, at least 1 h after waking. First, a basal sample of breath was collected. Next, the test substance (lactose, lactulose, or pasta) was administered orally in a dose of 20, 13 or 100 g and breath samples were collected at fixed intervals for a variable time period (ranging from 2 to 7 h). The patient was asked to expire a single and deep breath into a latex balloon. During the test period, the patient was not allowed to eat or smoke.

Apparatus

The analyses were carried out by connecting the latex balloon or the syringe to the sampling loop (0.5 cm^3) of the gas chromatograph.

Two gas chromatographs, both produced by Varian (Palo Alto, CA, U.S.A.), were used for evaluation of the performance of different TCD instruments in routine analysis: a Model 1420 (dual-column, equipped with a four-filament, tungsten–rhenium WX, hot-wire, constant-current TCD instrument) and a Model 3760 (dual-column, equipped with a four-filament, tungsten–rhenium WX, hot-wire, constant-mean-temperature TCD instrument, 10- and 100-fold signal output amplification). The separation of hydrogen was carried out by using columns filled with porous polymer beads (PPB) or active solid molecular sieves. Two PPB columns connected in series were used (2.4 mm I.D., stainless steel): 1.5 m, filled with Porapak Q (100–120 mesh) (Waters Assoc. Milford, MA, U.S.A.), followed by 3 m, filled with Porapak S (100–120 mesh). By using this column hydrogen and carbon dioxide were separated, while oxygen and nitrogen were eluted as a unique peak.

Nitrogen was used as the carrier gas at a flow-rate of 25 cm^3 min^{-1}, in order to enhance the response of hydrogen, due to the difference in their thermal conductivities (44.4 and 6.2 cal s^{-1} cm^{-1} $°C^{-1}$ for hydrogen and nitrogen,respectively at 25°C).

Greater sensitivity could be achieved by using argon as the carrier gas (thermal conductivity 4.2 cal s^{-1} cm^{-1} $°C^{-1}$) or by increasing the TCD filament current. Not more than 170 mA were used in these experiments, in order to reduce the background noise and to avoid overheating of the filaments, which would reduce the long-term performance of the instrument. Although the maximum filament temperature suggested by the manufacturer for extended operation with nitrogen as the carrier gas was 350°C, in this application the filament temperature was maintained below 280°C, as the high oxygen content of the samples could produce rapid oxidation of the filament.

A type 5A molecular sieve column (3 m×2.4 mm I.D., stainless steel) was also used at 60°C with a carrier gas flow-rate of 30 cm^3 min^{-1}. Hydrogen, oxygen, nitrogen and carbon monoxide peaks were separated, whereas the carbon dioxide was retained by the column.

RESULTS AND DISCUSSION

Some experiments (Fig. 1) showed that the hydrogen concentration in the latex sampling bags decreased as a function of time and that about 5% of the

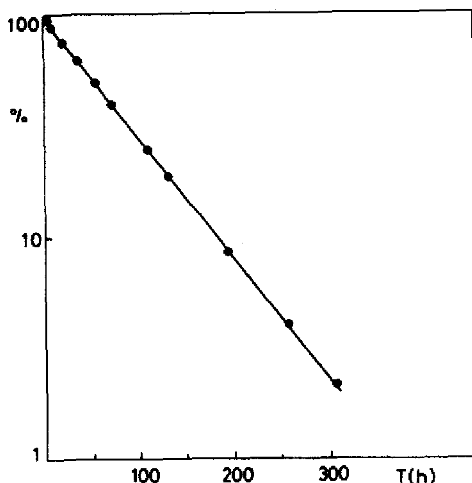

Fig. 1. Effect of long-term storage of the samples in latex bags. A decrease in hydrogen concentration as a function of time is evident.

initial hydrogen amount was lost within 2 h of sample collection. Exponential decay and reproducible results were found. As an example, by using three latex balloons of the same type and by starting from different initial hydrogen concentrations, the least-squares method applied to the data (triplicate determinations) using the equation

$$\ln C_t = t \cdot \ln C_0 + b$$

where C_t is the percentage hydrogen concentration at time t (min) and C_0 the initial concentration, gave $C_0 = 99.16\%$ and $b = 1.01$, with a correlation coefficient of 0.9935.

A suitable correction factor may therefore be applied for storage times up to 6 h, but for longer intervals between the sampling and the GC analysis glass ampoules or gas-tight syringes should be used.

Table I gives the retention times and Fig. 2 shows typical chromatograms obtained on the two columns used.

The calibration of the hydrogen response in the two systems (PPB column and Model 1420 chromatograph, or molecular sieve column and Model 3760 chromatograph) was obtained by using an exponential dilution flask [14, 15] and by injecting a sufficient amount of hydrogen to ensure an initial concentration of 10^4 ppm. The dependence of the hydrogen peak height on the concentration is shown in Fig. 3. As the detector response was linear over a wide concentration range, peak heights could be used for quantitative analysis. This is convenient mainly for manual evaluation of the chromatogram. The elution of hydrogen is so fast that an electronic integrator would be necessary for the correct evaluation of the peak areas, without a significant improvement in accuracy.

A minimum detectable amount (MDQ) of 20 ppm of hydrogen was found with the PPB column and Model 1420 chromatograph (not amplified TCD) at a signal-to-noise ratio (S/N) of 2. With amplified constant-mean-temperature TCD

TABLE I

RETENTION TIMES OF BREATH COMPONENTS ON PORAPAK AND MOLECULAR SIEVE COLUMNS

Gas	Retention time (min)	
	1.5 m Porapak Q+ 3 m Porapak S columns	3 m Molecular sieves 5A column
Hydrogen	1.85	0.80
Oxygen	2.20	1.57
Nitrogen		3.20*
Carbon dioxide	9.50	Absorbed by column

*Measured using helium as the carrier gas.

instrument (Model 3760 chromatograph) with PPB or molecular sieve columns, a ten-fold increase in sensitivity can be achieved with the same S/N, but with the molecular sieve column the baseline deviation due to the change of the column pressure during sample injection can impair the correct determination of the peak height. The use of longer molecular sieve columns (up to 6 m) resulted in increased retention times of the hydrogen peak, thus avoiding its interference with the baseline disturbance due to the sample injection; this permitted the determination of smaller amounts of hydrogen, but increased the time needed for each determination.

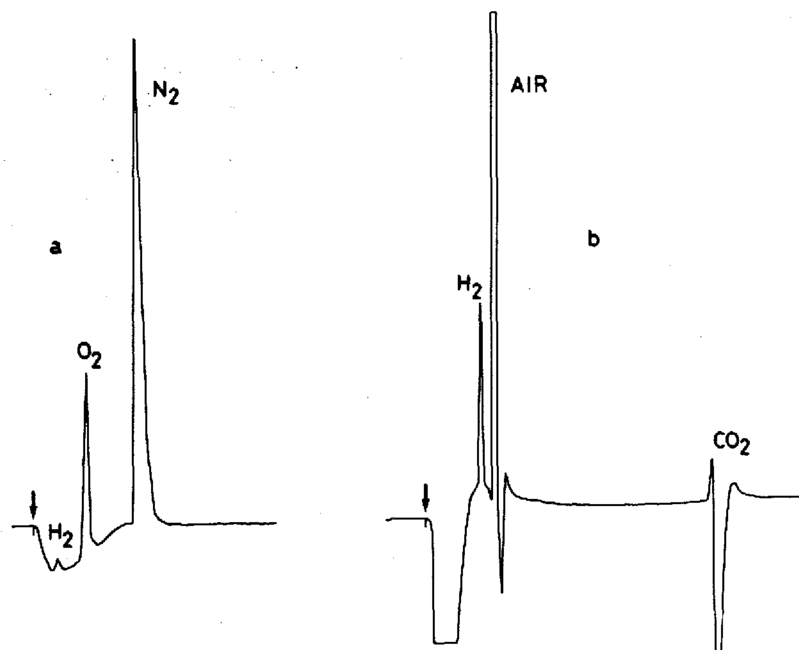

Fig. 2. Chromatograms of light gases analysed using: (a) 5A molecular sieve column (3 m×2.4 mm I.D.); carrier gas (helium) flow-rate, 30 cm^3 min^{-1}; temperature, 63°C; (b) 5 m Porapak Q +3 m Porapak S columns, both 2.4 mm I.D.; carrier gas (nitrogen) flow-rate, 25 cm^3 min^{-1}; temperature, ambient.

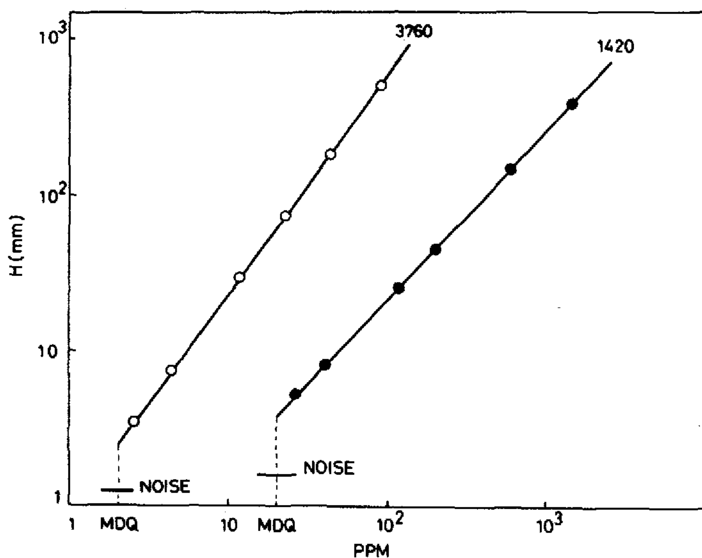

Fig. 3. Exponential dilution flask calibration of hydrogen: sensitivity and linearity in ppm range. (○) Varian 3760, amplified TCD; 170 mA; molecular sieve column; MDQ at 2 ppm; (●) Varian 1420, TCD; 150 mA; PPB column; MDQ at 20 ppm. For other conditions see text.

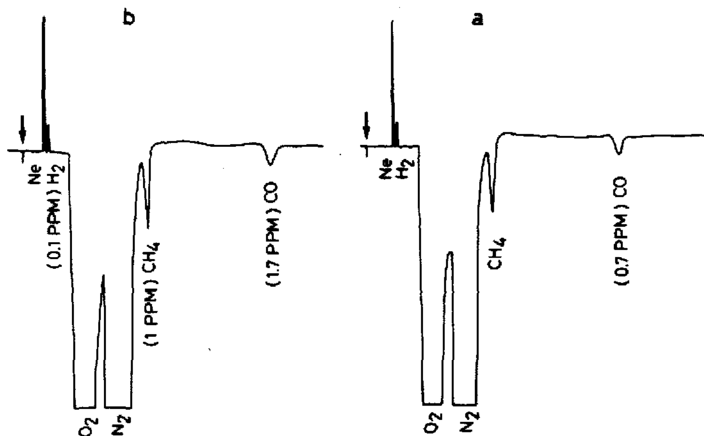

Fig. 4. Analysis of expired breath of healthy subject (b) compared with the inhaled air (a). Sample, 0.5 cm^3; helium ionization detector; 5A molecular sieve column (6 m×2.4 mm I.D.); temperature, 105°C; carrier gas (helium) flow-rate, 60 cm^3 min^{-1}; sensitivity, $16 \cdot 10^{-9}$ A mV^{-1}.

Table I shows the retention times on the two column systems used for routine analysis.

The comparison of the results obtained with the two TCD methods shows that a non-ampified detector routinely gives a sensitivity that is high enough to permit the determination of 20 ppm hydrogen in breath. This MDQ is acceptable for clinical tests, as reported by many workers [5, 13, 16, 17], but for research purposes it is too high and therefore the use of amplified TCD is convenient when amounts below 20 ppm of hydrogen have to be measured.

Further improvements in the sensitivity could be achieved by using a helium

ionization detector [18–24], with an MDQ of about 20 ppb of hydrogen and a linear dynamic range between 20 ppb and 100 ppm. The high purity required for helium used as the carrier gas and the extreme sensitivity of this detector to contamination are severe drawbacks to its use in routine analysis. Fig. 4b shows the analysis of approximately 0.1 ppm of hydrogen in the breath of a healthy subject, which corresponds to the concentration of the same gas in the inhaled air (Fig. 4a).

Methane and carbon monoxide are also separated on the 6 m$\times$2.4 mm I.D. 5A molecular sieve column at 105°C, with a flow-rate of purified helium of 60 cm^3 min^{-1}. The concentration of carbon monoxide in the expired air of the non-smoker is higher than that in the inspired air owing to the presence of this gas in the urban atmosphere and to its slow release due to decomposition of carboxy haemoglobin in blood cells. Much higher concentrations of carbon monoxide (up to 18 ppm) were detected in the breath of cigarette smokers.

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