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# A NEW CHROMATOGRAPHIC INSTRUMENT FOR MEASURING TRACE CONCENTRATIONS OF BREATH-HYDROGEN

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## SUMMARY

A new instrument has been developed which offers many advantages over instruments presently utilized for the measurement of breath-hydrogen used to evaluate the intestinal absorption of sugars. The gas analyzer has a solid-state sensor which is more specific for hydrogen than most conventional chromatographic detectors. Air can be used as the carrier gas and can be circulated with a small internal pump, thereby eliminating large carrier gas tanks and pressure regulators. The intersample time is approximately 2 min, allowing rapid serial analysis of breath samples. A unique feature allows a short-term memory circuit to recall the signal and present it on a digital panel meter in parts per million. Recorder terminals on the back permit the generation of a permanent record, if desired. The gas analyzer is small, lightweight and simple to operate. Its application to the serial measurement of hydrogen in alveolar air after ingestion of sugars is demonstrated.

## INTRODUCTION

The intraintestinal production of hydrogen gas results from fermentation of nonabsorbed sugars by intestinal bacteria. Some of this hydrogen is then absorbed and excreted by the lungs [1, 2]. These processes have led to the development of hydrogen breath-analysis tests to diagnose lactose, sucrose, glucose and D(—)-xylose malabsorption, to detect bacterial overgrowth and to measure intestinal transit time.

Gas chromatography (GC) has been most frequently used for the measurement of breath-hydrogen, with the detectors based on thermal conductivity or helium ionization. Instrument development and modifications have permitted sensitive analyses of hydrogen in unconcentrated breath samples from a single exhalation [3, 4], but several disadvantages have been obvious with the application of conventional chromatographic techniques to the measurement: (1) lack

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of portability; (2) requirements for high-pressure carrier gas tanks and regulators; (3) occasional baseline drift; (4) long elution times; and (5) a requirement for technical expertise in instrument operation. In an effort to simplify the measurement of breath-hydrogen, a new instrument has been developed. It is basically a gas chromatograph, but it has been redesigned to eliminate many of the disadvantages of a conventional system applied to the measurement of breath-hydrogen.

*Description of instrument*

The key element in this redesign is the use of a solid-state sensing device [5] for the detection and measurement of breath-hydrogen. This sensor consists of an N-type semiconductor material (sintered $SnO_2$) which exhibits a decrease in electrical resistance when combustible or reducing gases are adsorbed on the sensor surface. These resistance changes are relatively large for samples with low reducing-gas concentrations, thus enabling the sensor to accurately and reliably detect low concentrations of such gases. The sensor exhibits a nonlinear change with varying concentrations of hydrogen; however, because it is a unimodal (exponential) relationship, the nonlinearity is easily corrected with solid-state analogue circuitry utilized in conjunction with the sensor.

Inasmuch as the detector is essentially insensitive to nonreducing gases, the major gases in expired air (oxygen, nitrogen, argon, carbon dioxide) and in room air (oxygen, nitrogen, argon) produce little or no response from the detector. Thus, the system can utilize room air as the carrier gas, and the molecular sieve column need only separate hydrogen from other gases which might interfere with the measurement, such as carbon monoxide. The specificity of the detector allows the molecular sieve column length to be reduced by a factor of approximately 10, thereby shortening the elution time and lowering the intersample interval to approximately 2 min. The shorter column and the ability of the system to utilize room air as the carrier gas permit the use of a small internal air pump and eliminate the need for large tanks of carrier gas and high-pressure regulators. The overall reduction in size of the instrumentation (approximately 23 × 29 × 37 cm) and its light weight (approximately 7 kg) allow it to be transported, when desired, to field laboratories or outpatient clinics. A digital panel meter has been incorporated into the system to display the linearized detector output, thus eliminating the need for a strip-chart recorder. In addition, an analogue track-hold circuit has been included in the circuit to be used with the panel meter for temporary storage of the linearized detector output. These modifications simplify the calibration procedure and allow the meter to display hydrogen concentration directly in parts per million (ppm). In addition, the linearized detector output signal is available as a separate buffered output signal for connecting to a chart recorder if hard copy is desired.

*Gas-flow pattern*

Fundamentally, the gas-flow pattern (Fig. 1) is similar to that of a standard thermal conductivity gas chromatograph. The carrier gas (which is room air that is supplied by an internal pump) is initially dried with a molecular sieve pellet column (to protect the GC column) and carried through the sampling

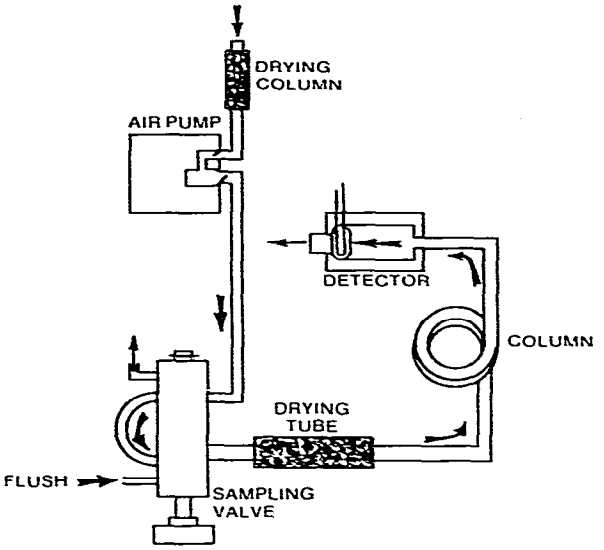


Fig. 1. Gas-flow pattern through the new instrument for measuring breath-hydrogen.

valve and sample loop (8 ml volume). The carrier gas transports the gas sample through an integral drying tube filled with indicating Drierite® to remove moisture from the sample, and then carries it through the GC molecular sieve column. This column separates the hydrogen gas from other combustible gases which might be present in expired air, and allows the hydrogen to be detected without interference. The chromatographic column consists of 5A molecular sieve (60—80 mesh) in 50 cm of 0.635 cm diameter aluminum tubing. The air carrier gas flow-rate is approximately 75 ml/min, delivered at a pressure of about 34 kPa. At this flow-rate, the peak signal for hydrogen is generated at the detector within 11—13 sec after the sample injection. The meter reading returns to baseline (zero) in approximately 100 sec. These time intervals are somewhat variable and depend on column density (packing), system flow-rate and delivery pressure.

*Circuit operation*

The circuit used to detect and process the signal from the sensor is shown in Fig. 2. The entire system, including the digital panel meter, is powered by a network of +12 V, −12 V, and +5 V power supplies. The sensor has a built-in heater which raises its operating temperature to a range of 200—400°C. The power for this heating element, which is buried in the sensor, is provided by the +5 V regulated power supply. It must be well regulated because the sensing material is considerably affected by small temperature changes.

The +5 V supply also provides power to the sensing element and its series dropping resistance supplied by RPI (Fig. 2). The increase in conductance associated with an increasing concentration of hydrogen provides a voltage signal to amplifiers A1—A2. This sensor output signal is then routed to the linearizer circuit which performs the function $V_{out} = V^{R}_{in}$ where $R$ is related to a resistor value selected (for a particular sensor) to produce the most accurate linearizing of the signal. This linearized signal is then summed with a

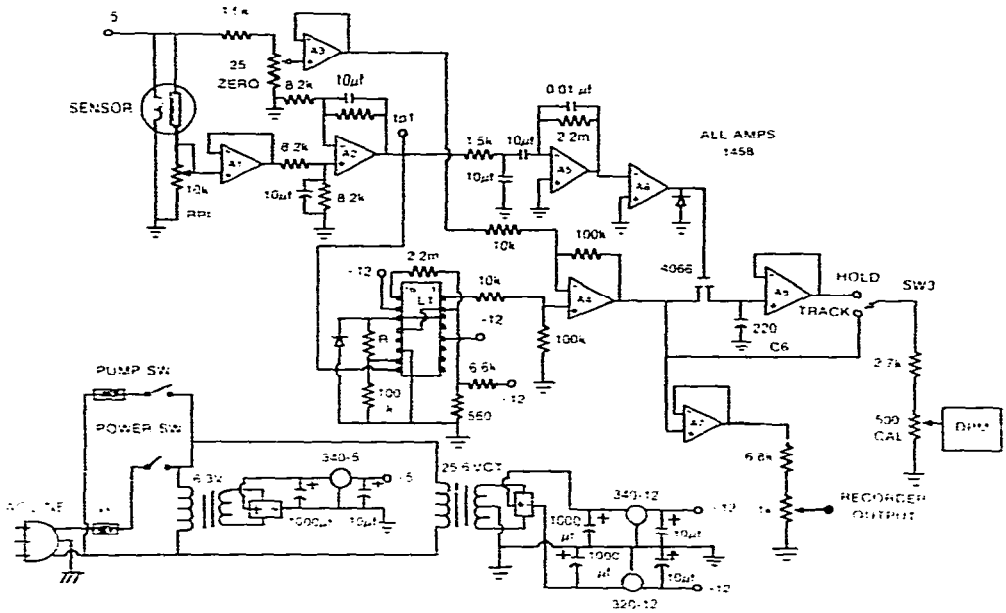


Fig. 2. Electronic circuit diagram for the detector, its amplifier, the linearizing element, and the track-hold system for the new hydrogen-measuring instrument.

zero reference signal (supplied by A3) and amplified A4. The output of A4 is fed into the output buffer amplifier A7 and the track-hold combination of A8—SW3 which supplies the signal to the digital panel meter. The amplifier pair A5—A6 provides a differentiator-driver for the track-hold (short-term memory element). This track-hold element (switch 4066, capacitor C6 and buffer amplifier A8) is used to track the signal to its peak and hold this peak value for the digital panel meter.

This arrangement simplifies the calibration procedure by allowing the digital panel meter to be adjusted to the value of the calibrating gas (by using the 500 Ω calibrate potentiometer) in the hold mode. It will then present the concentration of hydrogen in the unknown sample in ppm.

*Performance of the gas analyzer*

The solid-state gas sensor used in the hydrogen analyzer exhibited a nonlinear relationship between its conductance and the concentration of hydrogen in the sample at the low concentrations measured for the lactose intolerance test (generally below 100 ppm). Although there is some variability among sensors, Fig. 3 demonstrates a typical curve for sensor conductance and hydrogen concentration. The ordinate represents the voltage drop across a fixed resistor in series with the gas sensor. It is proportional to sensor conductance. The abscissa represents hydrogen gas concentration, with the range achieved by proportioning a known calibration gas (118 ppm hydrogen in air) with room air.

Fig. 4 presents a curve based on the output from the linearizer circuit plotted against hydrogen concentration. Six replicate measurements were made during a 4-h period in which the instrument was set at zero with air and at 118

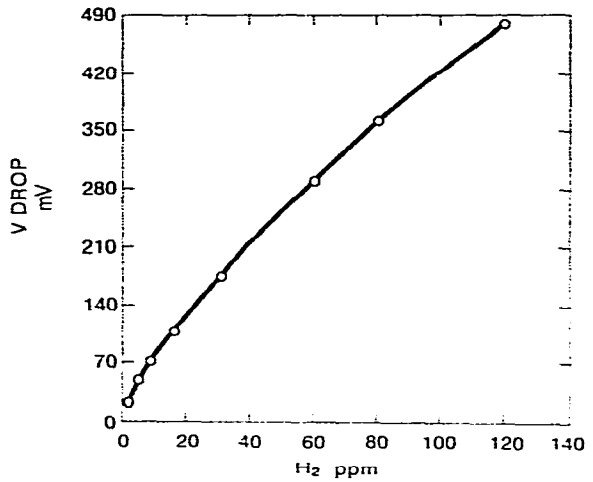


Fig. 3. Conductance change in the sensor (represented by the voltage drop across a fixed resistance in series with the sensor) related to a change in hydrogen concentration in air from 0 to 118 ppm.

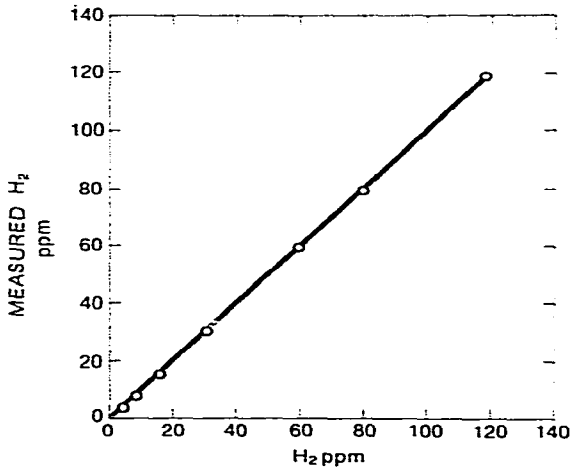


Fig. 4. Relationship between panel meter reading, reflecting the output signal from the linearizer circuit, and the hydrogen concentration in the sample introduced into the instrument. Each point is the mean of six measurements made during a 4-h period.

ppm with the reference gas. The reference gas was then serially diluted with room air to 60, 50, 25, 12.5, 6.25 and 3.12% of its initial hydrogen concentration; the diluted samples were analyzed with the gas analyzer. Both scales on the calibration curve are expressed in ppm hydrogen, with the values on the abscissa calculated to the first place after the decimal from the dilution factor, and the values on the ordinate taken from the digital panel meter display without decimals. The equation for the curve, calculated by the least squares method from the six calibration procedures, was $y = 1.005x - 0.031$. The standard error of estimate for the slope was 0.236 ppm and the standard deviation for the intercept was 0.081 ppm. Thus, the data indicated that the linearity and reproducibility (when calibrated prior to the analysis) were within 0.5 ppm

hydrogen for the range from 0 to 118 ppm. Since the meter readout was in ppm (without decimals), more precision is without meaning.

Because the gas sensor requires a relatively high operating temperature (200—400°C), there is a somewhat extended stabilization period when the instrument is powered up. Fig. 5 shows a typical curve for such stabilization. Conductance decreases with time as the temperature increases. A period of slightly over 2 h is required for equilibration of the system in an average room temperature, as seen in Fig. 5. Linearity will be affected during the stabilization period so that accuracy may be compromised unless adequate warm-up time is allowed. If enough time cannot usually be allowed, it may be more satisfactory to leave the unit on overnight. By using a drying column of molecular sieve

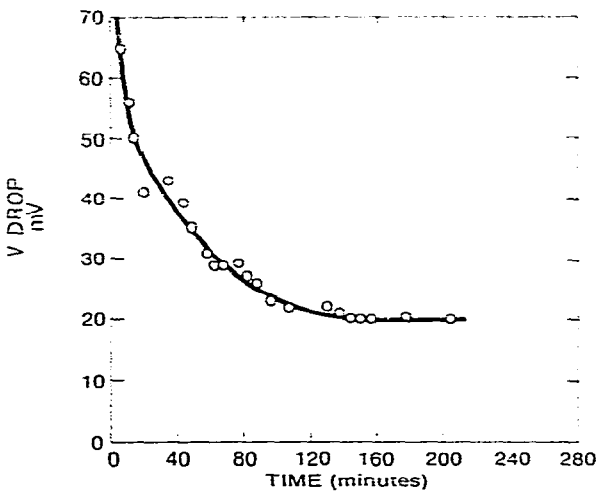


Fig. 5. Changes in conductance of the sensor with time during warm-up, to indicate that a 2-h period is required to assure equilibration and linearity of the system to measure breath-hydrogen.

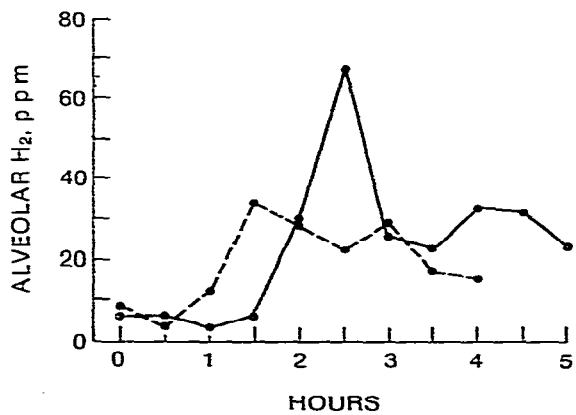


Fig. 6. Serial measurements of breath-hydrogen following sugar ingestion [6]. Solid line is from a lactose-intolerant patient following the ingestion of 18 g lactose (whole cow's milk). Broken line is from a normal subject after the ingestion of 12 g raffinose in 360 ml tomato juice.

pellets on the inlet air supply line, the column will be protected from room air humidity. If it is not protected, the column will be inactivated within a short period of time. The molecular sieve pellets can be reactivated in a drying oven at 300°C for 1—2 h, thereby permitting their use to be cost-effective in allowing continuous availability of the hydrogen gas analyzer.

Fig. 6 shows breath-hydrogen analyses [6] from two subjects to demonstrate the application of the instrument. Alveolar air samples were analyzed in duplicate at 0.5-h intervals following the ingestion of sugar. The broken line represents data following the ingestion of raffinose, an undigestible trisaccharide, which may be used to measure intestinal transit time since it undergoes bacterial fermentation in the large intestine. The solid line represents the hydrogen response following lactose ingestion by a lactose-intolerant patient.

If desired, the output response curves can be recorded with a potentiometric strip-chart recorder. The signal presented to the recorder terminal is from the output of the linearizing circuit and does not represent the track-hold circuit which is accessible to the digital panel meter. The recorded curve can be made a permanent part of the patient's record if needed and, when combined with the response curve from a reference gas, can be used to calculate hydrogen concentration by the conventional chromatographic technique.

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