EVALUATION OF INSTRUMENTATION AND CERTAIN METHODOLOGY TO QUANTITATE EXPIRED HYDROGEN

By

ASEGASH TSEGAYE

Bachelor of Science in Home Economics

Oklahoma State University

Stillwater, Oklahoma

1976

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
July, 1977

EVALUATION OF INSTRUMENTATION AND CERTAIN

METHODOLOGY TO QUANTITATE

EXPIRED HYDROGEN

Thesis Approved:

Donna Payne Bose
Thesis Adviser

Esther Winterfeldt

Robert D Morrison

Norman N Durham
Dean of the Graduate College

ACKNOWLEDGMENTS

The writer wishes to express her sincere appreciation to Dr. Donna P. Bose, major adviser, for her many hours of concern, encouragement and constructive criticism in the preparation of the thesis. The opportunity to work on this project was made possible by her.

Grateful acknowledgments are also extended to Dr. R. D. Morrison, Professor of Statistics, for serving on the advisory committee and for his valuable assistance and for arranging the statistical design and analysis.

Special appreciation is extended to Dr. Esther Winterfeldt, a committee member, for her willingness to provide guidance by reading the thesis manuscript.

Special gratitude is due to Marlece Ebbesen, the Nutrition Laboratory technician, for her great help in running the experiments.

Gratitude is also extended to Mary Ann Nichols, a graduate student, and Marsha Storjohann for their assistance.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

As interest in breath tests and their application is increasing, information on methodology should become more readily available. If the method is to be used extensively in clinical situations, an important factor to consider is equipment that is relatively easy to maintain, has good precision, and has a method of sampling and analysis that is both simple and reliable. Data on such precision and reliability need to be accessible.

Several various techniques are currently being used to quantitate H_2 is expired air in studies of carbohydrate malabsorption. The variations include sampling, analysis and expression of results.

Since the TCD apparently is generally believed unable to quantitate H_2 at sufficiently low concentrations (Levitt, 1969; Gearhart et al., 1976) gas chromatographs equipped with a helium ionization detector (HID) have been used to analyze whole breath (Bose et al., 1977; Caskey et al., 1976; Gearhart et al., 1976; Calloway and Murphy, 1968; Calloway, Murphy and Bauer, 1969; Hickey, Murphy and Calloway, 1972; Murphy, 1972). The HID, however, has some radiation hazard and is difficult to maintain. The TCD is easy to maintain and has no radiation hazard.

Others who have used the TCD have either used a rebreathing technique in a closed circuit to concentrate the sample rather than tidal volume collection (Levitt, 1969; Levitt and Donaldson, 1970; Bond and

Levitt, 1972; Bond and Levitt, 1970, Levitt and Ingelfinger, 1968; Newcomer et al., 1977; Newcomer et al., 1975) or an end expiratory sampling technique (Metz et al., 1976; Mafei et al., 1976; Metz and Jenkins, 1975; Metz, Newman and Jenkins, 1976; Mafei, Metz and Jenkins, 1976). However, data relative to instrument preference is lacking. One previous study has used a TCD to analyze whole breath samples, but a different column was used than reported here, the oven temperature was lower (-20° C) and a different carrier gas (N_2) was used (Calloway, 1966). There is no data on precision and reliability of analysis.

This report demonstrates a method by which a gas chromatograph equipped with a TCD is used to analyze samples of tidal volume without concentrating the sample. Typical chromatograms for H_2, reproduced in actual size are shown for low levels of H_2 such as might be found in a fasting tidal volume breath sample so that the detector response can be seen. Such raw results need to be available to those who are considering use of the breath H_2 test. This is of special concern where the peak area is determined by hand measurements. The peak areas for H_2 reported in this study were not determined by hand triangulation but byyy an electronic integrator which was a part of the gas chromatograph. The precision and reliability of the method using standard gas mixtures is demonstrated. An illustration of the method as used to detect malabsorption of a low level of a poorly absorbed sugar (raffinose) is presented in one subject.

The Hewlett-Packard Model 5830A gas chromatograph has a built-in digital processor that operates throughout the analytical system. The keyboard at the terminal is used to control the plotter, the integration parameters, the calibration and computation.

Automation of gas chromatography has been used for accurate quantitative analysis of several different gases. It makes analysis easier and provides better knowledge of errors. Automation should allow long-term repeatability of results (Charrier et al., 1972). Each automatic instrument should be calibrated individually.

> Accurate quantitative results, within the limits of the repeatability of the instrument, may be obtained only by calibration of each chromatograph with a synthetic mixture of known composition covering the range of composition expected in the samples and being as near in composition to the samples as possible (Deans, 1968, p. 192).

The Hewlett-Packard Model 5830A gas chromatograph was evaluated for precision by using standard H_2 in Ar mixtures and standard H_2 in N_2 mixtures. The instrument allows the analysis of gaseous mixtures by injecting the gas sample being analyzed into a column having a carrier gas circulating through it. As the gases arrive separately at the column exit, they must be detected. The TCD employs a register which is electrically heated. Carrier gas dissipates heat from the element at a constant rate, but when a sample component passes through the detector, the rate is altered and the temperature of the resistor changes. The change is transmitted to the recorder, appearing as a peak. The automatic integrator prints the retention time in minutes of each gas and area count in arbitrary units. Argon was used as the carrier gas in this particular gas chromatograph. The carrier gas is obtained from a high pressure cylinder which then flows through a pressure regulator. The carrier gas was left flowing even when the instrument was not in use in order to prevent atmospheric moisture from entering the columns. Moisture will deactivate the molecular seive.

Need for Research

The problem of detection and determination of small concentrations of permanent gases such as H_2, O_2, N_2, CO_2, Ar, and methane was difficult before the development of gas chromatography.

The continuing quest for identifying gas-producing carbohydrate foods which are malabsorbed by the human small intestine has stimulated interest in methods to detect these gases. "Gas chromatography with its advantage of rapid analysis of one sample for several gases is one of the possible analytical methods favored" (Brouke, Dawson and Denton, 1964, p. 387).

Since there is a growing need for a rapid and a reliable method for quantitative analysis of respiratory gases, the use of gas chromatography for the analysis and separation of complex mixtures of volatile substances has become important. Breath tests have been used to study intestinal malabsorption and the effect of gastrointestinal bacteria on exogenous compounds. Application of these tests become more important since they are simple, reliable and safe to the subject.

Intestinal carbohydrate malabsorption, especially that of lactose has been traditionally studied by the lactose tolerance test (LTT) which requires a large dose of lactose. Such a large dose might create discomfort and cramps for those people who are malabsorbers (Calloway, 1966; Calloway, Hickey and Murphy, 1971; Murphy and Calloway, 1972).

Undoubtedly many various techniques and methodologies can serve the purpose of detecting carbohydrate malabsorption. Some methods may be better suited for particular studies than others. Since results rest entirely on the area of the H_2 peak, comparisons and evaluations should

be more objective if an automatic integrator is used instead of hand measurements. The automization also saves a technician's time.

Purpose of the Study

The general purpose of this study was to evaluate instrument precision of the Hewlett Packard Model 5830A Gas Chromatograph and its application in breath H_2 analysis. The specific purpose of this study was to:

(1) determine instrument precision and drift,

(2) determine whether standard gas mixtures can be stored for three days,

(3) determine the repeatability of H_2 analysis,

(4) determine which of the two types of standard gas mixtures are better to use, and

(5) determine what the relationship is between the standard curves for H_2 in the two types of standard gas mixtures.

Definition of Terms

Ar--argon gas.

CO_2--carbon dioxide gas.

HID--helium ionization detector.

H_2--hydrogen gas.

N_2--nitrogen gas

TCD--thermal conductivity detector.

ppm--parts per million.

O_2--oxygen gas.

CHAPTER II

LITERATURE REVIEW

Gas chromatography has permitted the isolation of substances which would have been extremely difficult to separate by older methods. Hamilton (1959) used a modified Model 25 Fisher Gas Partitioner in the determination of air and blood gases. This gas partitioner was modified to adjust the column composition and length to permit better separation of different gases.

Dressler, Mastio and Allbritton (1960) used a Beckman Gas Chromatograph Model No. 178 to analyze gaseous mixtures by injecting the gas into the column in which the carrier gas was helium. They concluded that the gas chromatograph offers a rapid reliable method for the analysis of respiratory gases. In addition, a small amount of the sample is needed for analysis. The error in repeated analysis of CO_2 did not exceed 0.1 volume percent and the maximum error in O_2 determination was 0.8 volume percent in higher concentrations of the gases. Hartman and Dimick (1966) also used a helium detector and after measuring H_2 for 11 consecutive times found a standard deviation of 1.7%.

Analysis of small amounts of gases is particularly prone to errors. Its systematic error often exceeds the random error by several orders of magnitude. This is of special concern where the peak area is determined by hand measurement (Kaiser, 1970). The method of measuring

peaks and calculating the results can have a marked effect on the error characteristics of the quantitative results (Deans, 1968; King and Dupre, 1969).

According to Deans (1968) and Ball and Harris (1967), for most accurate analysis calibration of the chromatograph with synthetic mixtures is required. The prime requirements for accuracy are:

(1) The measurement should have good repeatability.

(2) The measurements should increase linearly with the increase in component concentration.

(3) The measurement should tolerate a certain amount of interference from neighboring peaks without loss of repeatability.

Repeatability is an essential requirement for accurate quantitative analysis. No detector investigated is truly linear over an appreciable range of concentrations.

Dupis, Charrier and Lutz (1972) stated that variety of analysis and achievement of the highest possible precision is needed along with speed of the various separations. The separation should be extremely efficient to avoid peak overlapping. Precision and sensitivity are very compatible with automatic control. This study supports the work done by Guiochon, Goedert and Jacob (1970). They stated that the technician and/or instrument errors cannot be eliminated, but can often be reduced by improving the equipment.

It is practically very easy to analyze a mixture of few samples at 10% precision level. The 1% level of precision requires a good control of temperature, flow rate and voltage. The necessary quality is not often reached since in most cases a precision of only 2 to 3% is observed.

CHAPTER III

METHODS AND MATERIALS

Eleven collection bags were filled in random order with 11 different standard mixtures of H_2/Ar or H_2/N_2. The collection bags held about six liters of sample and were constructed of double layers of a 3-ml multilaminar material of polyester, aluminum and polyethylene. A Teflon male tubing connector was attached to each bag by using hot glue and glass gauze as reinforcement, and the bags were heat sealed. A 3-cm length of tygon tubing with a screw clamp was attached to the tubing connector. The standard gas mixtures were 13, 24, 40, 77, 122 and 166 ppm H_2 in Ar and 10, 37, 72, 122 and 165 ppm H_2 in N_2. All of the H_2 in nitrogen standard mixtures and the 13 ppm H_2 in Ar were obtained from Linde Speciality Gases. The remaining five standard mixtures of H_2 in Ar were obtained from Matheson Gas Products. They had certified analysis accuracy.

Duplicate samples were drawn from the 11 standard mixtures contained in the sample bags on each of three consecutive days and analyzed by gas chromatography. The 11 duplicate samples were analyzed in random order each day disregarding whether the H_2 was in argon or in nitrogen.

Three sets of 11 bags were filled and analyzed as described above; therefore, nine test days were needed to complete all the analysis. This gave a total of 198 samples withdrawn and analyzed. The study was done within a period of 10 days. No data was discarded on the basis of

chromatographs or reported results.

A Hewlett-Packard Model 5830A Reporting Gas Chromatograph equipped with a thermal conductivity detector (TCD) was used with the following parameters: oven temperature of 50° C, TCD temperature of 165° C, chart speed of 1 cm/min, peak attenuation of 2^2, slope sensitivity of 0.1 for H_2/Ar standard mixtures and 0.15 for the H_2/N_2 standard mixtures, area rejection of one, carrier gas flow through dual columns of 13.5 to 13.7 ml/min.

Argon was used as the carrier gas, and flow through the columns was determined by a soap film flowmeter. The chromatograph was equipped with a flow sensor having two independent channels calibrated for various carrier gases, but not for argon. Since it was not equipped with a flow sensor for argon carrier gas, the circuit for nitrogen carrier gas was used and calibrated for the desired flow rate.

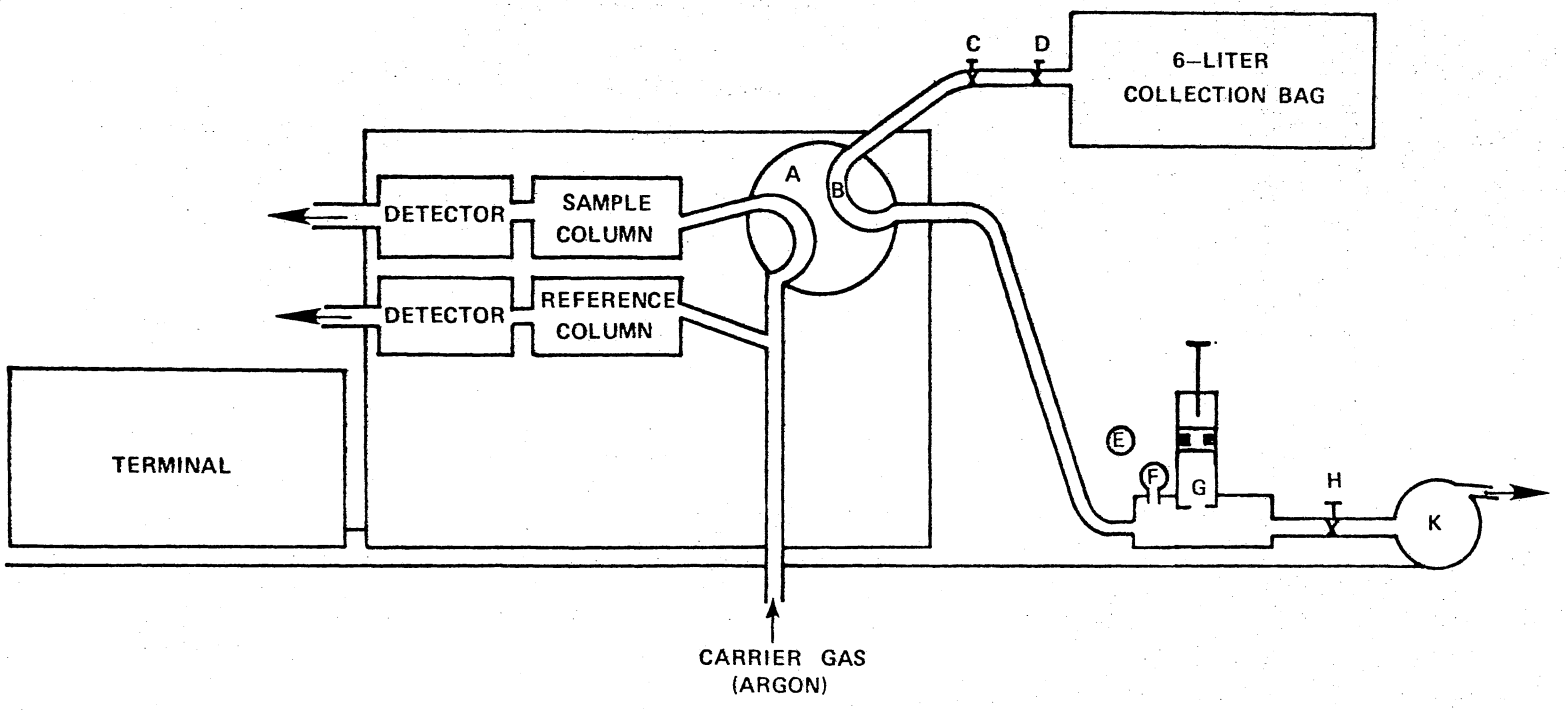
The dual columns were 1/8-inch o.d. x 10 ft stainless steel packed with 5A molecular seive. The detector was balanced at the beginning of each day. Columns were conditioned for 1 hr at 325° C and the detector baked out at 350° C for 6 hr on the day preceeding each three-day set, and columns were conditioned for 1 hr at 325° C at the start of each day. The following procedure was used for sampling (Figure 1).

(1) The tygon tubing of the collection bag was slipped onto the tubing leading to the sample loop and wrapped with parafilm.

(2) The vacuum pump (K) was turned on.

(3) The on/off valve (H) nearest the pump was opened.

(4) The plunger on the sample pressurizing pump (G) was pressed fully down.



Note: A = sample valve, B = 0.5 ml sample loop, C = on/off toggle valve; D = screw clamp, E = remote control button to actuate sample valve, F = psig pressure guage, G = sample pressurizing pump, H = on/off toggle valve, K = vacuum pump.

Figure 1. Schematic Representation of Sampling and Analysis

(5) The on/off valve (D) closing the bag was opened.

(6) The screw clamp (D) closing the bag was opened, and the sample was pulled through the system for 2 min.

(7) After this period of time the on/off valve (H) nearest the vacuum pump was closed.

(8) The pump (K) was turned off.

(9) The plunger of the sample pressuring pump (G) was then pulled fully up and after 1 min, the plunger was depressed until the sample was pressurized to 30 psig as shown by the pressure guage (F).

(10) After a short pause to be certain the pressure had equilibrated and to detect gross leaks by watching the pressure guage, the automatic sampling value (A) was actuated.

The actuator button at the terminal had been moved to a remote control position (E) near the sample pressurizing pump so that only one person was required to carry out the analysis.

The sample pressurizing pump had a volume of approximately 50 ml, and a rubber O-ring was attached to the Teflon plunger. The sampling system must be leak free from the sampling bag through the final connection to the vacuum pump to prevent contamination from room air. It was necessary to wait for 4 min between sampling to allow O_2 and N_2 to elute from the columns, and their presence was used as a check for contamination of the samples or a leak into the system.

The data was statistically analyzed as split plot in which a set was considered as a block, concentration as main plots, succession of days in a set as subplots. Two successive samples withdrawn from each bag on each day were sampling units for each bag.

CHAPTER IV

RESULTS AND DISCUSSION

The statistical analysis of the data for the H_2/Ar and H_2/N_2 standard gas mixtures and the appropriate means are given in Tables I, II and III. The variance between duplicates was found to be very small (= 334.6) with respect to the overall mean response (= 1735 ppm), giving a C.V. of only 1.0%. The H_2/N_2 standard gas mixture showed a smaller variance between duplicates (= 135.0) with an overall mean response of 1940 ppm, giving a C.V. of only 0.6%. The mean squares for day-to-day variation were small for each mixture indicating very little or no change in the H_2 concentration when the breath samples are stored in the plastic bags over a period of three days.

When the tests were repeated, to give three sets of trials, it was found the set-to-set variation was also relatively small for each of the gas mixtures.

The relationship between peak areas and concentration of H_2 for the H_2/Ar mixtures and for the H_2/N_2 mixtures were not parallel (Figure 2). For example, a 1 ppm increase in H_2 in the H_2/Ar mixture would increase the reported area count 25.5 units, but when H_2/N_2 mixtures were used, a 1 ppm increase of H_2 would increase the reported area count 23.2 units.

Peak areas for H_2 at the lower H_2 concentration were greater when H_2/N_2 standard gas mixtures were used than when H_2/Ar standard gas mixtures were used. For example, reported peak areas were greater for

TABLE I

ANALYSIS OF VARIANCE FOR VARIABLE H_2/Ar AND H_2/N_2

Source	H_2/Ar			H_2/N_2		
	df	Mean Square	Values of F	df	Mean Square	Values of F
Total	107			89		
Sets (S)	2	5532.6	0.068	2	18444.0	0.034
Concentration (C)	5	42619627.8	524.900	4	39307050.7	76.100
Linear	1	212726990.0	2620.400	1	156066511.8	302.200
Quadriatic	1	127607.0	1.572	1	128726.6	0.249
Residual	3	81180.4		2	516482.2	
S*C (error a)	10	31491.0	18.900	8	2070.6	1.444
S*C (linear)	2	6999.0	4.190	2	5371.8	3.746
S*C (quadriatic)	2	3737.4	2.380	2	43.2	0.030
S*C (residual)	6	1669.6		4	1433.6	
Days (D)	2	11074.7	0.682	2	1021.4	0.476
D*C	8	1303.0	0.345	8	3186.5	1.480
D*C (linear)	2	5597.3	0.016	2	4021.1	1.872
D*C (quadriatic)	2	261.1	0.014	2	4428.9	2.060
D*C (residual)	6	218.9		4	2147.9	
S*D	4	16243.1		4	371.5	
S*D + S*D*C (error b)	24	4799.9		20	2229.4	
Duplicate (S.C.D.) (sampling error)	54	334.6		45	135.0	

TABLE II

PEAK AREAS AND AVERAGE FOR H_2/Ar STANDARD GAS MIXTURES

Concentration of H_2 (ppm)	Set 1				Set 2				Set 3			
	Day				Day				Day			
	1	2	3	Avg.	1	2	3	Avg.	1	2	3	Avg.
13	178	181	175	178	164	172	192	175	188	187	220	198
24	483	501	503	496	481	474	527	494	509	506	497	504
40	894	896	881	890	873	865	910	883	877	895	869	880
77	1843	1840	1833	1839	1809	1777	1876	1821	1809	1823	1822	1817
122	2906	2922	2907	2912	2804	2765	2952	2840	2800	2813	2798	2804
166	4228	4154	4167	4183	4117	4061	4331	4169	4135	4187	4140	4154
Average	1755	1749	1744	1750	1708	1685	1797	1730	1719	1735	1724	1726

TABLE III

PEAK AREAS AND AVERAGE FOR H_2/N_2 STANDARD GAS MIXTURES

	Set 1				Set 2				Set 3			
	Day				Day				Day			
Concentration	1	2	3	Avg.	1	2	3	Avg.	1	2	3	Avg.
10	246	255	254	251	247	243	241	243	246	240	264	250
37	990	971	963	974	920	946	911	925	977	948	946	957
72	1777	1787	1769	1778	1718	1716	1846	1760	1776	1772	1764	1770
125	2777	2800	2769	2782	2687	2699	2681	2689	2723	2762	2741	2742
165	4065	4029	4010	4034	4043	3977	3850	3957	4000	3989	4013	4001
Average	1971	1968	1952	1964	1922	1916	1905	1915	1944	1941	1945	1944

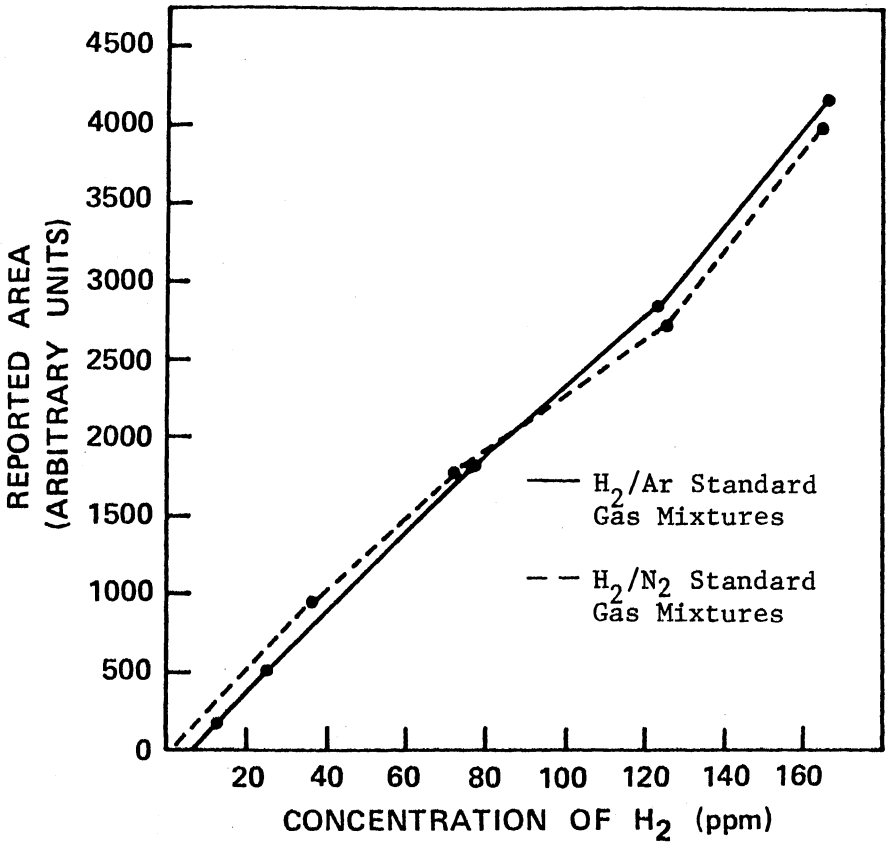


Figure 2. Reported Area (Arbitrary Units) of H_2 Peak Versus H_2 Concentration

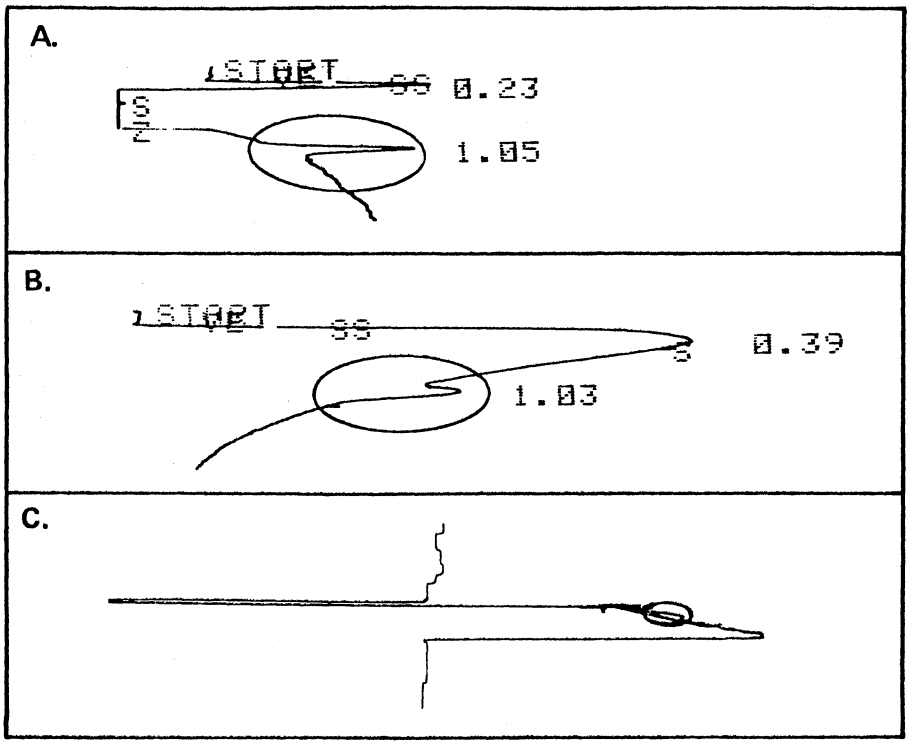
10, 37, and 72 ppm of H_2/N_2, respectively, than for 13, 40 and 77 ppm of H_2/Ar, respectively (Tables II and III).

The H_2 bases below the peaks were broader when the H_2/Ar mixtures were used than when the H_2/N_2 mixtures were used. Figure 3 is a reproduction of typical chromatograms in actual size showing the H_2 peak obtained when (A) 10 ppm H_2/N_2 standard mixtures were used and (B) 13 ppm H_2/Ar standard mixtures were used. The last chromatogram (C) is from a previous study in which a helium ionization detector was used and demonstrates 9 ppm H_2.

Samples could be injected at intervals of c.a. 5 min which the time required for other components of the sample (O_2, CO_2, if present, and N_2) to elute from the column.

Data resulting from the application of the method on a human subject is shown in Figure 4 and Tables IV and V. After the subject consumed 5 g raffinose, the breath H_2 concentration rose to 48 ppm within three and one-half hours. There were no symptoms other than minor borborygmus once. There were only about 6 ppm H_2 difference between tidal volume (31 ppm H_2, area count 829) and end expiratory volume (37 ppm H_2, area count 988).

If the gas concentrations reported by the suppliers are correct, and the gas chromatograph technique properly detects the H_2 concentration, there should be a linear relationship between the H_2 concentration reported by the suppliers and the H_2 ppm area given by the chromatograph. The analysis of this data shows a definite linear effect due to reported concentration. However, it should be noted that the quadriatic and the higher degree polynomial effects are present. Although these effects are statistically present, they are so small with respect to ppm



Note: H_2 peak is circled. A = 10 ppm H_2 in N_2 standard gas mixture, B = 13 ppm H_2 in Ar standard gas mixture, C = 9 ppm H_2 in He standard gas mixture.

Figure 3. Typical Chromatograms of H_2 Analysis, Actual Size

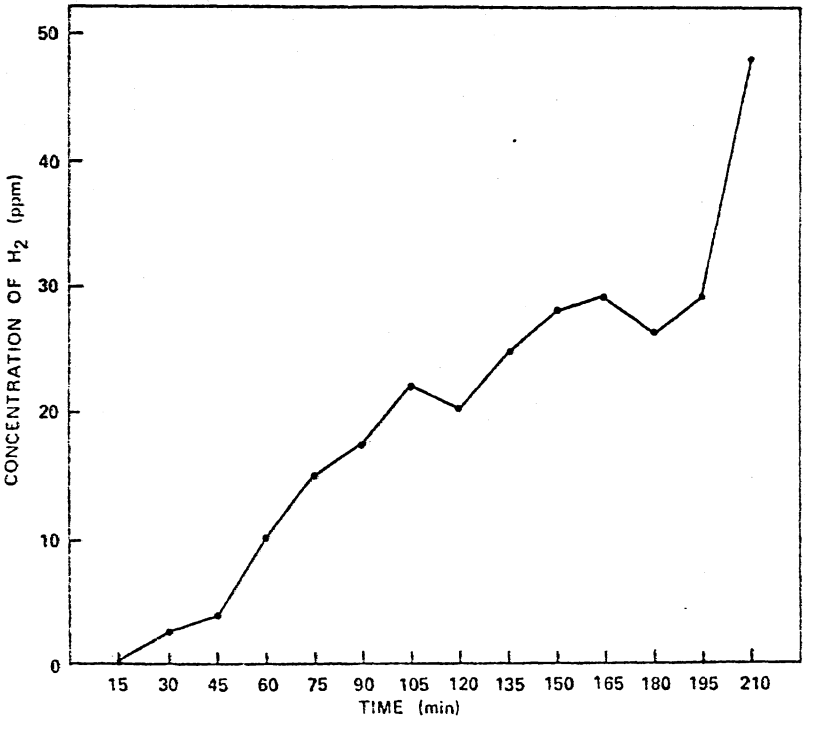


Figure 4. Breath H_2 Concentration for a Subject Versus Time After Consumption of Five Grams of Raffinose

TABLE IV

BREATH H_2 RESPONSE AFTER INGESTION
OF FIVE GRAMS RAFFINOSE

Time	Peak Area	H_2 Concentration (ppm)
0	no area count	---
15	no area count	---
30	72	2.5
45	101	4.0
60	265	10.0
75	397	15.0
90	473	17.5
105	585	22.0
120	524	20.0
135	662	25.0
150	743	28.0
165	775	29.0
180	703	26.0
195	779	29.0
210	1224	47.5

TABLE V

BREATH H_2 RESPONSE FOR TIDAL AND END
EXPIRATORY PARTS OF THE BREATH

Time	Breath	Peak Area	H_2 Concentration (ppm)
160	Tidal	820	31.0
160	End Expiratory	988	37.0

of H_2 present that they might be ignored in clinical trials. These curvature effects could be due to incorrect ppm reported by the suppliers. They state that their concentrations may have a small variation from the concentration given on the gas cylinder.

Discussion

This study demonstrates that a gas chromatograph equipped with a TCD is sufficiently sensitive and reliable to quantitate H_2 in breath for the demonstration of carbohydrate malabsorption. The H_2 peak areas were obtained by the instrument and not by hand measurements (triangulation or peak height) and, therefore, should give more subjective results. Peak retention times and areas are recorded by an electronic integrator. For example, the retention time of H_2 in Figure 3 (A) was 1.03.

Usually, the standard gas mixture would be composed of the gas being analyzed (H_2) in the gas used as a carrier (Ar). However, this writer has demonstrated that for test procedure, a standard mixture of H_2/N_2 is desirable. Not only is there better H_2 peak resolution, but N_2 is the main component of breath and this type of standard mixture gives a chromatogram appearing like that obtained from a breath sample.

The collection containers in this study were adequate for standard gas mixture storage over a three-day period, though it must be realized that for routine testing this may not be advisable, as the collection bags used were relatively new. Since c.a. 5 min is required for analysis, it would rarely be necessary to store samples. Although the variance between duplicates was very small, duplicates may be done at intervals to assure that consistent procedures are being used and to

alert the technician to any methodology or equipment problem.

Pressurization of the sample is necessary to minimize the deflection due to pressure change within the detector which is caused by turning of the automatic sampling valve. As demonstrated in Figure 2, the apparent peak with a retention time of 0.39 min is in reality the deflection due to the pressure change. Unfortunately, H_2 elutes from the column so rapidly that the baseline upset which occurs after injection of the sample is not complete and the best alternative is to minimize the upset and make conditions such that H_2 elutes as late as possible. For the procedure described here, the gas sampling valve, injection of the sample is a better choice than the syringe injection for several reasons. The pressure change upset would be far greater and more difficult to control, it would be necessary to put septums in each collection bag making construction of the bags more difficult and introducing a potential leak source.

There is some indication that the end expiratory breath has slightly greater H_2 concentration than the tidal breath, but the difference may not be great enough to be of concern. In fact, sampling may be more uniform if the subjects are told to breath in a normal, relaxed fashion since there would be variation among individuals on the volumes of end expirations.

Finally, it was shown that a rise in breath H_2 can be detected when as little as 5 gm of raffinose is ingested. One-third of the raffinose molecule would be expected to be absorbed and, therefore, the response demonstrated may have been produced from as little as about 3.3 gm of sugar not absorbed.

CHAPTER V

SUMMARY

A method of quantiating expired H_2, using relatively easy to maintain instrumentation, for studying carbohydrate malabsorption was evaluated for quantitative precision and reliability. The study demonstrates that the method is adequate to determine H_2 concentration at levels found in whole breath without concentrating the sample or without using end expiratory samples.

Two different types of standard gas mixtures (H_2/Ar and H_2/N_2) with 11 various H_2 concentrations were used. Samples were drawn in duplicate from three sets of 11 collection bags on three consecutive days for a total of nine days and analyzed by gas chromatography. Details of the method and performance data on human subjects are presented.

The variance between duplicate peak areas were very small, being 334.6 (C.V. = 1.0%) for H_2/Ar standard gas mixtures and 135.0 (C.V. = 0.6%) for H_2/N_2 standard gas mixtures. Day-to-day differences were insignificant and differences among the three sets were insignificant. There was a linear relationship between peak areas and concentration of H_2 as reported by suppliers for each gas mixture (the H_2/Ar standard gas mixtures and the H_2/N_2 standard gas mixtures.

SELECTED BIBLIOGRAPHY

Ball, D. L. and Harris: Errors in manual integration techniques for chromatographic peaks. Journal of Gas Chromatography. 5:613, 1967.

Bond, J. H. and Levitt, M. D.: Use of pulmonary hydrogen (H_2) measurements to quantitate carbohydrate absorption. Journal of Clinical Investment. 51:1219, 1972.

Bond, J. H. and Levitt, M. D.: Investigation of small bowel transit time in man utilizing pulmonary hydrogen (H_2) measurements. Journal of Laboratory and Clinical Medicine. 85:546, 1975.

Bourke, P. J., Dawson, R. W. and Benton, W. H.: Detection of volume parts per million of permanent gases in helium. Journal of Chromatography. 14:387, 1964.

Calloway, D. H.: Respiratory hydrogen and methane as affected by consumption of gas forming foods. Gastroenterology. 51:383, 1966.

Calloway, D. H., Hickey, C. A. and Murphy, E. L.: Reduction of intestinal gas forming properties of legumes by traditional and experimental food processing methods. Journal of Food Science. 36:251, 1971.

Calloway, D. H. and Murphy, E. L.: Use of expired air to measure intestinal gas formation. Annals of the New York Academy of Sciences. 150:82, 1968.

Calloway, D. H., Murphy, E. L. and Bauer, D.: Determination of lactose intolerance by breath analysis. American Journal of Digestive Diseases. 14:811, 1969.

Caskey, D. A., Payne-Bose, D., Welsh, J. D., Gearhart, H. L., Nance, M. K. and Morrison, R. D.: Effects of age on lactose malabsorption in Oklahoma Native Americans as determined by breath H_2 analysis. American Journal of Clinical Nutrition. 22:113, 1976.

Charrier, G., Dupis, M. C., Merllvat, J. C., Pons and Sigelle, R.: Automation of gas analysis with a computerized gas chromatograph system. Charomatographia. 5:119, 1972.

Chromatography Symposium of the University of Houston, Proceedings of the Sixth International Symposium on Advances on Gas Chromatography (Miami Beach, Florida: Zlatkis, A., June 2-5, 1970), p. 21.

Deans, D. R.: Accurate quantitative gas chromatographic analysis part 1: method of calculating results. Chromatographia. 1:187, 1968.

Dressler, D. P., Mastio, G. J. and Allbritton, F. F.: The clinical application of gas chromatography to analysis of respiratory gases. Journal of Laboratory and Clinical Medicine. 55:144, 1960.

Gas Chromatography Discussion Group of the Institute of Petroleum, Proceedings of the Eighth International Symposium on Gas Chromatography (Dublin, Royal Dublin Society: Stock, R., September 28-October 2, 1970), p. 169185.

Gas Chromatography Discussion Group of the Institute of Petroleum, Proceedings of the Ninth International Symposium on Gas Chromatography (Montreux, Switzerland: Perry, S. G., October 9-13, 1972), p. 209.

Gearhart, H. L., Bose, P. D., Smith, C. A., Morrison, R. D., Welsh, J. D. and Smalley, T. K.: Determination of lactose malabsorption by breath analysis with gas chromatography. Analytical Chemistry. 48:393, 1976.

Hamilton, L. H.: Application of gas chromatography to respiratory and blood gases determination. Physiologist. 2:52, 1959.

Hartman, C. H. and Dimick, R. P.: Helium detector for permanent gases. Journal of Gas Chromatography. 4:63, 1966.

Hickey, C. A., Murphy, E. L. and Calloway, D. H.: Intestinal gas formation following ingestion of fruit and fruit juices. American Journal of Digestive Diseases. 17:383, 1972.

King, W. H. and Dupre, G. D.: Critique of some conventional evaluation methods and continuous flow, steady-state blender for evaluation of gas chromatography detector linearity. Analytical Chemistry. 41:1936, 1969.

Kinney, T. D. and Melville, R. S. (Eds.): Mechanization, automation and increased effectiveness of clinical laboratory. A status report by the automation in mechanical laboratory science. Review Committee of the National Institute of General Medical Sciences, National Institute of Health. DHEW Pub. No. (NIH) 77-145, 1976.

Levitt, M. D.: Production and excretion of hydrogen gas in man. New England Journal of Medicine. 281:122, 1969.

Levitt, M. D. and Donaldson, R. M.: Use of respiratory hydrogen (H_2) excretion to detect carbohydrate malabsorption. Journal of Laboratory and Clinical Medicine. 75:937, 1970.

Levitt, M. D. and Ingelfinger, F. J.: Hydrogen and methane production in man. Annals of the New York Academy of Sciences. 150:75, 1968.

Mafie, H. V. L., Metz, G. L. and Jenkins, D. J. A.: Hydrogen breath test: adaption of simple technique to infants and children. Lancet. 1:1110, 1976.

Metz, G. L., Gassull, M. A., Drassar, B. S., Jenkins, D. A. J. and Blendis, L. M.: Breath hydrogen test for small intestinal bacterial colonization. Lancet. 1:668, 1976.

Metz, G. L., Gassull, M. A., Leeds, A. R., Blendis, L. M. and Jenkins, D. A. J.: A simple method of measuring breath hydrogen in carbohydrate malabsorption by end expiratory sampling. Clinical Science Molecular Medicine. 50:237, 1975.

Metz, G. L., Jenkins, D. J. A., Peters, T. J., Newman, A. and Blendis, L. M.: Breath hydrogen as diagnostic method for hypolactasia. Lancet. 1:1155, 1975.

Metz, G. L., Newman, A., Jenkins, D. J. A. and Blendis, L. M.: Breath hydrogen in hyposucrasia. Lancet. 1:119, 1976.

Murphy, E. L. and Calloway, D. H.: The effect of antibiotic drugs on the whole volume and consumption of intestinal gas from beans. American Journal of Digestive Diseases. 17:639, 1972.

Newcomer, A. D., McGill, D. B., Thomas, P. J. and Hoffman, A. F.: Prospective comparison of indirect methods for detecting lactase deficiency. New England Journal of Medicine. 298:1232, 1975.

Newcomer, A. D., Thomas, P. J., McGill, D. B. and Hoffman, A. F.: Lactase deficiency: a common genetic trait of the American Indian. Gastroenterology. 72:234, 1977.

Payne-Bose, D., Welsh, J. D., Gearhart, H. L. and Morrison, R. D.: Milk and lactose hydrolyzed milk. American Journal of Clinical Nutrition. 30:695, 1977.

APPENDIXES

APPENDIX A

RANDOMIZATION FOR FILLING SAMPLE BAGS WITH STANDARD GAS MIXTURES OF H_2/Ar AND H_2/N_2 FOR ANALYSIS OF THE STANDARDS

TABLE VI

RANDOMIZATION FOR FILLING SAMPLE BAGS WITH STANDARD GAS MIXTURES OF H_2/Ar AND H_2/N_2 FOR ANALYSIS OF THE STANDARDS

					Set 1--Bag Numbers[a]						
Day 1 Order of Filling Sample Bags	1	2	3	4	5	6	7	8	9	10	11
Order of Analysis	4	2	10	5	1	9	7	11	3	8	6
Day 2 Order of Analysis	7	1	5	8	3	9	10	2	4	6	11
Day 3 Order of Analysis	1	3	9	11	5	6	7	4	8	2	10
					Set 2--Bag Numbers[b]						
Day 1 Order of Filling Sample Bags	1	2	3	4	5	6	7	8	9	10	11
Order of Analysis	7	9	5	11	6	8	3	2	4	10	1
Day 2 Order of Analysis	1	2	10	11	3	4	9	5	6	8	7
Day 3 Order of Analysis	2	9	6	8	11	7	3	10	4	1	5
					Set 3--Bag Numbers[c]						
Day 1 Order of Filling Sample Bags	1	2	3	4	5	6	7	8	9	10	11
Order of Analysis	11	10	5	3	4	1	2	8	6	9	7
Day 2 Order of Analysis	7	9	4	2	5	8	10	1	11	3	6
Day 3 Order of Analysis	6	1	10	7	11	2	4	9	8	3	5

[a]Bag numbers and contents: 1 = 165 ppm H_2/N_2, 2 = 77 ppm H_2/Ar, 3 = 72 ppm H_2/N_2, 4 = 40 ppm H_2/Ar, 5 = 24 ppm H_2/Ar, 6 = 125 ppm H_2/N_2, 7 = 10 ppm H_2/N_2, 8 = 122 ppm H_2/Ar, 9 = 37 ppm H_2/N_2, 10 = 13 ppm H_2/Ar,

11 = 166 ppm H_2/Ar.

[b]Bag numbers and contents: 1 = 166 ppm H_2/Ar, 2 = 37 ppm H_2/N_2, 3 = 40 ppm H_2/Ar, 4 = 77 ppm H_2/Ar, 5 = 125 ppm H_2/N_2, 6 = 24 ppm H_2/Ar, 7 = 10 ppm H_2/N_2, 8 = 165 ppm H_2/N_2, 9 = 122 ppm H_2/Ar, 10 = 13 ppm H_2/Ar, 11 = 72 ppm H_2/N_2.

[c]Bag numbers and contents: 1 = 122 ppm H_2/Ar, 2 = 72 ppm H_2/N_2, 3 = 125 ppm H_2/N_2, 4 = 37 ppm H_2/N_2, 5 = 13 ppm H_2/Ar, 6 = 77 ppm H_2/Ar, 7 = 40 ppm H_2/Ar, 8 = 10 ppm H_2/N_2, 9 = 165 ppm H_2/N_2, 10 = 24 ppm H_2/Ar, 11 = 166 ppm H_2/Ar.

APPENDIX B

INDIVIDUAL VALUES FOR RETENTION TIME (MINUTES) AND REPORTED AREA (ARBITRARY UNITS) OF H_2 PEAK USING STANDARD H_2/Ar AND STANDARD H_2/N_2 GAS MIXTURES

TABLE VII

INDIVIDUAL VALUES FOR RETENTION TIME (MINUTES) AND REPORTED AREA (ARBITRARY UNITS) OF H_2 PEAK USING STANDARD H_2/N_2 GAS MIXTURES

Set	Day	Duplicates	Retention Time/Min.	H_2 Concentrations (ppm)	Reported H_2 Peak Area (Arbitrary Units)
			A. H_2/Ar Standard Gas Mixtures		
1	1	1	1.03	13	183
1	1	2	1.03	13	173
1	1	1	1.05	24	486
1	1	2	1.04	24	480
1	1	1	1.05	40	896
1	1	2	1.05	40	892
1	1	1	1.06	77	1841
1	1	2	1.06	77	1845
1	1	1	1.05	122	2906
1	1	2	1.05	122	2906
1	1	1	1.06	166	4208
1	1	2	1.06	166	4249
1	2	1	1.02	13	174
1	2	2	1.01	13	188
1	2	1	1.03	24	507
1	2	2	1.02	24	409
1	2	1	1.03	40	890
1	2	2	1.03	40	901
1	2	1	1.03	77	1845
1	2	2	1.04	77	1834
1	2	1	1.04	122	2912
1	2	2	1.04	122	2731
1	2	1	1.04	166	4143
1	2	2	1.06	166	4164
1	3	1	1.01	13	170
1	3	2	1.00	13	180
1	3	1	1.01	24	498
1	3	2	1.02	24	508
1	3	1	1.03	40	871
1	3	2	1.02	40	891
1	3	1	1.03	77	1820
1	3	2	1.03	77	1846
1	3	1	1.03	122	2920
1	3	2	1.03	122	2894
1	3	1	1.03	166	4191
1	3	2	1.04	166	4142

TABLE VII (Continued)

Set	Day	Duplicates	Retention Time/Min.	H_2 Concentrations (ppm)	Reported H_2 Peak Area (Arbitrary Units)
		A. H_2/Ar Standard Gas Mixtures (Continued)			
2	1	1	0.99	13	154
2	1	2	0.99	13	173
2	1	1	1.01	24	487
2	1	2	1.01	24	475
2	1	1	1.03	40	873
2	1	2	1.04	40	873
2	1	1	1.03	77	1769
2	1	2	1.03	77	1821
2	1	1	1.02	122	2818
2	1	2	1.02	122	2790
2	1	1	1.03	166	4174
2	1	2	1.03	166	4060
2	2	1	0.99	13	158
2	2	2	0.99	13	185
2	2	1	1.01	24	472
2	2	2	1.01	24	476
2	2	1	1.02	40	862
2	2	2	1.02	40	868
2	2	1	1.02	77	1769
2	2	2	1.02	77	1785
2	2	1	1.02	122	2796
2	2	2	1.02	122	2733
2	2	1	1.02	166	4043
2	2	2	1.02	166	4078
2	3	1	1.03	13	190
2	3	2	1.05	13	194
2	3	1	1.07	24	524
2	3	2	1.08	24	529
2	3	1	1.08	40	908
2	3	2	1.01	40	911
2	3	1	1.07	77	1880
2	3	2	1.07	77	1872
2	3	1	1.07	122	2953
2	3	2	1.07	122	2951
2	3	1	1.00	166	4347
2	3	2	1.03	166	4314

TABLE VII (Continued)

Set	Day	Duplicates	Retention Time/Min.	H_2 Concentrations (ppm)	Reported H_2 Peak Area (Arbitrary Units)
		A. H_2/Ar Standard Gas Mixtures (Continued)			
3	1	1	1.02	13	189
3	1	2	1.02	13	186
3	1	1	1.03	24	502
3	1	2	1.03	24	515
3	1	1	1.03	40	878
3	1	2	1.03	40	876
3	1	1	1.05	77	1796
3	1	2	1.03	77	1821
3	1	1	1.04	122	2800
3	1	2	1.04	122	2800
3	1	1	1.04	166	4111
3	1	2	1.04	166	4159
3	2	1	1.03	13	173
3	2	2	1.04	13	200
3	2	1	1.02	24	516
3	2	2	1.03	24	496
3	2	1	1.04	40	904
3	2	2	1.05	40	885
3	2	1	1.04	77	1825
3	2	2	1.04	77	1820
3	2	1	1.03	122	2801
3	2	2	1.02	122	2825
3	2	1	1.04	166	4191
3	2	2	1.03	166	4183
3	3	1	1.01	13	215
3	3	2	1.01	13	224
3	3	1	1.03	24	510
3	3	2	1.02	24	484
3	3	1	1.03	40	896
3	3	2	1.03	40	868
3	3	1	1.03	77	1821
3	3	2	1.03	77	1823
3	3	1	1.03	122	2791
3	3	2	1.03	122	2805
3	3	1	1.03	166	4136
3	3	2	1.03	166	4144

TABLE VII (Continued)

Set	Day	Duplicates	Retention Time/Min.	H_2 Concentrations (ppm)	Reported H_2 Peak Area (Arbitrary Units)
			B. H_2/N_2 Standard Gas Mixtures		
1	1	1	1.07	10	256
1	1	2	1.07	10	236
1	1	1	1.08	37	991
1	1	2	1.08	37	988
1	1	1	1.07	72	1793
1	1	2	1.07	72	1761
1	1	1	1.07	125	2778
1	1	2	1.07	125	2780
1	1	1	1.08	165	4053
1	1	2	1.08	165	4077
2	1	1	1.05	10	248
2	1	2	1.05	10	245
2	1	1	1.05	37	916
2	1	2	1.05	37	923
2	1	1	1.05	72	1705
2	1	2	1.05	72	1730
2	1	1	1.04	125	2684
2	1	2	1.04	125	2689
2	1	1	1.06	165	4048
2	1	2	1.06	165	4938
2	2	1	1.05	10	249
2	2	2	1.05	10	237
2	2	1	1.05	37	943
2	2	2	1.05	37	948
2	2	1	1.05	72	1719
2	2	2	1.05	72	1713
2	2	1	1.05	125	2708
2	2	2	1.05	125	2689
2	2	1	1.05	165	3989
2	2	2	1.05	165	3965
1	2	1	1.06	10	249
1	2	2	1.07	10	260
1	2	1	1.06	37	988
1	2	2	1.06	37	978
1	2	1	1.06	72	1775
1	2	2	1.06	72	1799
1	2	1	1.06	125	2803
1	2	2	1.06	125	2796
1	2	1	1.06	165	4015
1	2	2	1.06	165	4042

TABLE VII (Continued)

Set	Day	Duplicates	Retention Time/Min.	H_2 Concentrations (ppm)	Reported H_2 Peak Area (Arbitrary Units)
1	3	1	1.06	10	256
1	3	2	1.05	10	251
1	3	1	1.05	37	961
1	3	2	1.05	37	965
1	3	1	1.05	72	1770
1	3	2	1.06	72	1768
1	3	1	1.06	125	2765
1	3	2	1.06	125	2772
1	3	1	1.05	165	4001
1	3	2	1.06	165	4018
2	3	1	1.05	10	240
2	3	2	1.05	10	241
2	3	1	1.04	37	910
2	3	2	1.04	37	912
2	3	1	1.11	72	1855
2	3	2	1.11	72	1837
2	3	1	1.05	125	2678
2	3	2	1.05	125	2683
2	3	1	1.04	165	3844
2	3	2	1.05	165	3855
3	1	1	1.07	10	243
3	1	2	1.06	10	248
3	1	1	1.07	37	974
3	1	2	1.07	37	980
3	1	1	1.07	72	1787
3	1	2	1.07	72	1764
3	1	1	1.07	125	2716
3	1	2	1.06	125	2729
3	1	1	1.07	165	3974
3	1	2	1.07	165	4026
3	2	1	1.05	10	234
3	2	2	1.05	10	246
3	2	1	1.06	37	950
3	2	2	1.06	37	945
3	2	1	1.06	72	1770
3	2	2	1.06	72	1773
3	2	1	1.07	125	2763
3	2	2	1.07	125	2760
3	2	1	1.05	165	3969
3	2	2	1.05	165	4008

TABLE VII (Continued)

Set	Day	Duplicates	Retention Time/Min.	H_2 Concentrations (ppm)	Reported H_2 Peak Area (Arbitrary Units)
		B. H_2/N_2 Standard Gas Mixtures (Continued)			
3	3	1	1.05	10	266
3	3	2	1.05	10	262
3	3	1	1.05	37	944
3	3	2	1.05	37	947
3	3	1	1.05	72	1758
3	3	2	1.05	72	1769
3	3	1	1.06	125	2736
3	3	2	1.05	125	2745
3	3	1	1.05	165	4021
3	3	2	1.05	165	4005

VITA

Asegash Tsegaye

Candidate for the Degree of

Master of Science

Thesis: EVALUATION OF INSTRUMENTATION AND CERTAIN METHODOLOGY TO QUANTITATE EXPIRED HYDROGEN

Major Field: Food, Nutrition and Institution Administration

Biographical:

Personal Data: Born in Asella, Ethiopia, April 19, 1952, daughter of Mr. Tsegaye Desta and Mrs. Belainesh Tilahun.

Education: Received high school diploma from Ras Dargay School, Asella, Ethiopia; received a diploma from College of Agriculture, Alemaya, Ethiopia, June, 1972; received Bachelor of Science in Home Economics degree with a major in Food, Nutrition and Institution Administration from Oklahoma State University, Stillwater, Oklahoma, 1976; completed the requirements for the Master of Science degree with a major in Food, Nutrition and Institution Administration at Oklahoma State University, Stillwater, Oklahoma, July, 1977.

Professional Experience: Served as a graduate technical Assistant in the Home Economics Department, College of Agriculture, Ethiopia, September, 1972 to December, 1973.