Journal of Chromatography, 299 (1984) 432–435
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,955

Note

Determination of carbon dioxide at the ppm level: a statistical comparison of a single-filament and a four-filament thermal conductivity detector

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(Received April 19th, 1984)

Carbon dioxide is an important component in many biological reactions and a simple sensitive method of analysis is needed. Methods using chemical reactions[1] with sodium hydroxide are simple but slow and of low sensitivity. Gas chromatographic methods[2] using four filament thermal conductivity (TC) detectors are simple, fast and sensitive to 1.79 μg carbon dioxide. However, for the determination of carbon dioxide at the ng level, a more sensitive method is required.

In this investigation, a method using a single-filament TC detector was developed and employed to measure the carbon dioxide produced by a single *Sitophilus granarius* (L) insect. Also, weighed least squares regression analysis was performed on both single- and four-filament detector calibration data, in order to statistically assess the performance of these detectors.

EXPERIMENTAL

A Hewlett-Packard 5880 A gas chromatograph with a single-filament TC detector was used along with a nickel column, 2 m × 3 mm O.D. and filled with Porapak N, 50–80 mesh. A column temperature of 30°C and carrier gas (helium) flow-rate of 20 ml/min resulted in retention times of 0.32 min for oxygen, 0.36 min for nitrogen and 1.07 min for carbon dioxide.

For the four-filament TC detector analysis, a Bendix 2200 gas chromatograph was used. The column was the same type, however the column temperature was 60°C, the helium flow-rate was 15 ml/min and the bridge current was 200 mA. Under these conditions the retention time was 0.33 min for air and 1.2 min for carbon dioxide.

For sampling, 100-μl and 1000-μl gas-tight syringes and a 10-μl liquid syringe, made gas-tight by the method previously described[3], were used.

A calibration standard was prepared, for low levels, by injecting 100 μl of high purity carbon dioxide into a 216-ml flask, fitted with a sampling port. The flask was previously filled with air, from a compressed gas cylinder, after being passed through a barium hydroxide solution trap to remove carbon dioxide. For calibration, 5–1000-μl samples of standard were drawn by a gas syringe and injected into the gas chromatograph. Before each sample was taken, the syringe was flushed with helium to eliminate carbon dioxide adsorbed on the syringe barrel. To avoid contamination

of the sample by air during syringe transfer to the gas chromatograph, the tip of the syringe needle was blocked with a silicone septum (6 mm in diameter and 10 mm long). The silicone septum was slipped over the needle before sampling and then slid to the needle tip, while the syringe was withdrawn from the sampling flask, thus preventing exposure to air.

In order to analyze carbon dioxide produced by a single *Sitophilus granarius* (L) insect, the insect was placed into a 1-ml glass tube fitted with sampling ports. At selected intervals, 10-μl samples were withdrawn by a syringe and injected into the gas chromatograph. Occasionally baseline drift occurred, likely as a result of water elution. This was corrected by conditioning the column at 160°C for 2 min.

RESULTS AND DISCUSSION

Tables I and II show calibration data obtained for single-filament and four-filament detectors, respectively. Five replicate determinations were performed for each mass of carbon dioxide and this allowed calculation of pure error variance estimates for each mass of carbon dioxide analyzed. When identical sample sizes were analyzed, the single-filament result was more precise. For example, using a 41.4-ng carbon dioxide sample, the coefficient of variation was 1.7% for the single-filament detector, compared with 8.9% for the four-filament.

The pure error variance could not be considered constant within the calibration region. The standard deviation ranged from 2.1 to 5.7 counts (Table I) with the single-filament detector and from 13 to 56 counts, (Table II) with the four-filament detector. Thus ordinary least squares regression analysis could not be performed. Instead, weighted least squares regression analysis was applied, whereby each data point was weighted inversely proportional to its pure error variance. The resulting calibration parameter estimates are given in Table III. Calibration curve slope estimates indicate higher sensitivity and better precision for the single-filament detector, 6.84 $\pm$ 0.09 counts/ng (or $\pm$ 1.3%), as compared with 3.1 $\pm$ 0.1 counts/ng (or $\pm$ 3%) for the four-filament detector. A higher correlation coefficient for the single-filament detector (0.9997 compared to 0.9971) suggests better linearity. Also, the single-filament calibration curve passed closer to the origin than the four-filament, as indicated by intercept values of -2 ± 3 and 16 $\pm$ 16 counts, respectively.

TABLE I

SINGLE-FILAMENT DETECTOR CARBON DIOXIDE CALIBRATION DATA

Sample volume (μl)	*Amount of CO_2 (ng)*	*Detector* response (counts)*	*S.D. (counts)*	*C.V. (%)*	*Calculated amount of CO_2** (ng)*
5	4.14	24.3	2.1	8.6	3.85
10	8.28	55.6	3.7	6.7	8.42
30	24.9	173	3.2	1.8	25.6
50	41.4	248	4.9	1.7	41.8
100	82.8	558	5.7	1.0	81.9

* Mean of five determinations.

** Calculated using regression parameters given in Table III.

TABLE II

FOUR-FILAMENT DETECTOR CARBON DIOXIDE CALIBRATION DATA

Sample volume (μl)	*Amount of CO_2 (ng)*	*Detector* response (counts)*	*S.D. (counts)*	*C.V. (%)*	*Calculated amount of CO_2** (ng)*
50	41.4	157	14	8.9	45.5
100	82.8	255	13	4.9	77.1
300	248.6	793	33	4.1	250.6
500	414.0	1257	56	4.4	400.3

* Mean of five determinations.
** Calculated using regression parameters given in Table III.

TABLE III

CARBON DIOXIDE CALIBRATION PARAMETERS OBTAINED USING WEIGHTED LEAST SQUARES REGRESSION ANALYSIS

Detector type	*Intercept (counts)*	*Slope (counts/ng)*	*Correlation coefficient*
Single-filament	-2 ± 3*	6.84 ± 0.09*	0.9997
Four-filament	16 ± 16*	3.1 ± 0.1*	0.9971

* 95% Confidence level.

The limit of detection, in practice, is lower with the single-filament detector as a result of (1) greater precision for determinations and calibration parameters, (2) higher sensitivity and (3) an intercept value closer to zero.

Therefore, this method is very useful for samples, where low concentration must be analyzed. For example, Tables IV and V show the results of an application of this method to determine carbon dioxide production by single *Sitophilus granarius* (L) insects, each placed in a 1-ml space. In the past, the carbon dioxide produced collectively by a group of insects had to be analyzed because existing methods of

TABLE IV

CARBON DIOXIDE PRODUCTION FOR SINGLE SUSCEPTIBLE INSECT, IN AIR AND IN METHYL BROMIDE (19 mg/l)

Time (min)	*Amount of CO_2 (μg/g)*					
	3-mg Insect		*3.8-mg Insect*		*4.2-mg Insect*	
	Air	*Methyl bromide*	*Air*	*Methyl bromide*	*Air*	*Methyl bromide*
0	0	0	0	0	0	0
10	121	373	165	369	231	399
25	413	645	612	959	824	815
40	702	1090	945	1331	1272	1091
60	1075	1501	1767	1760	1988	1762

TABLE V

CARBON DIOXIDE PRODUCTION FOR SINGLE METHYL BROMIDE-RESISTANT INSECT, IN AIR, AND IN METHYL BROMIDE (19 mg/l)

Time (min)	*Amount of CO_2 (μg/g)*					
	3.8-mg Insect		*4.6-mg Insect*		*5.1-mg Insect*	
	Air	*Methyl bromide*	*Air*	*Methyl bromide*	*Air*	*Methyl bromide*
0	0	0	0	0	0	0
10	845	164	197	122	349	106
25	1478	595	612	369	783	572
40	1871	1006	908	591	1228	882
60	2490	2163	1604	991	2202	1630

analysis were not sensitive enough to determine small quantities of carbon dioxide produced by single insects. Table IV shows a roughly two-fold increase in carbon dioxide production by the susceptible insects at the 10-min interval, when exposed to methyl bromide. In Table V, this effect is reversed, for the resistant insects, where the carbon dioxide production is reduced to approximately 50%, in the presence of methyl bromide.

In the future, by exposing an insect to various conditions of temperature, humidity or fumigants, the resulting respiratory response can be investigated. Similarly, the carbon dioxide production by fruits and plants can be analyzed using this method.

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