Journal of Chromatography, 207 (1981) 365–372
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,519

METHOD FOR THE ANALYSIS OF [^{13}C]CARBON MONOXIDE BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

ADRIAN R. SWANSON* and M. W. ANDERS

Department of Pharmacology, University of Minnesota, 435 Delaware St. S.E., Minneapolis, MN 55455 (U.S.A.)

(Received November 24th, 1980)

SUMMARY

A procedure is described for the isotopic analysis of [^{13}C]carbon monoxide in air at levels as low as 10 ppm. A conventional gas chromatograph–mass spectrometer equipped with a gas-sampling valve, a jet-orifice separator and multiple ion monitoring capability is used. As the carbon monoxide component of an air sample is eluted from a molecular sieve (5 Å) column, it is catalytically converted to carbon dioxide using Hopcalite as the reactant medium. Enhanced performance is obtained as the mass spectrometric measurement is shifted from masses 28 and 29 to masses 44 and 45 to take advantage of reduced background signals and more stable chromatographic baselines. Typical applications involve the analysis of nanomole quantities of biosynthetic carbon monoxide. The [^{13}C]carbon monoxide mole percent of an air sample containing 100 ppm carbon monoxide can be measured with a precision of ±0.02 at the 0.20 atom-percent excess level. No prior sample concentration procedures are required.

INTRODUCTION

The isotopic analysis of [^{12}C]- and [^{13}C]carbon monoxide (CO) as a constituent of air by mass spectrometry (MS) is hampered by spectrometer background ion currents at masses 28 and 29 which are large and noisy relative to the signal produced by the CO in the sample. Such impediments to the satisfactory trace analysis of CO may be reduced by its conversion to carbon dioxide (CO_2) and the subsequent monitoring of the resultant ion current signals at masses 44 and 45. This is because much smaller and more stable background ion currents are usually found at these masses. The method described by Stevens and Krout[1] isolates the naturally occurring CO in a sample of air by a cryogenic process involving the oxidation of the CO with Schutze reagent (I_2O_2) to produce CO_2. Although excellent results are obtained a very large air sample (300 l) is required and the scheme is not easily scaled down to meet the needs encountered in investigations of biosynthetically produced CO.

We report herein the use of combined gas chromatography (GC)–MS to accomplish a one-step assay in which the components of a CO containing air sample are separated chromatographically and passed through a simple post-column reactor which quantitatively converts the CO to CO_2 for the subsequent, on-line mass spectral analysis. Hopcalite, a proprietary mixture of metallic oxides and superoxides[2,3], is used alone to effect the conversion.

EXPERIMENTAL

Apparatus

A Hewlett-Packard (Palo Alto, CA, U.S.A.) Model 5992A and a Finnigan (Sunnyvale, CA, U.S.A.), Model 3200E, equipped with a PROMIM multiple ion monitoring device, gas chromatograph–mass spectrometers were used. Both were fitted with a 6-port gas-sampling valve having a 1-ml loop (catalog number 57-000034-00; Varian, Palo Alto, CA, U.S.A.). The GC–MS interface must be of a type which has reasonable transmission efficiencies with respect to CO; therefore, a jet-orifice separator was used. A stainless-steel (12 ft. × 0.118 in. I.D.) column packed with 100–120 mesh molecular sieve 5 Å was employed. The column was conditioned by heating at 250–300°C with the carrier gas flowing overnight or until the elution of absorbed water diminishes as indicated by a minimum and constant mass 18 ion current signal. It was not necessary to remove the Hopcalite reactor for the conditioning process. The post-column reactor is constructed as shown in Fig. 1. It consists of a 1/8-in. to 1/8-in. reducing unit (Swagelok, part number SS-200-R-2; Crawford Fitting, Solon, OH, U.S.A.) filled with 100 mg of Hopcalite (part number 41566; Mine Safety Appliances, Pittsburgh, PA, U.S.A.) as received. A 1/4-in. to 1/8-in. Vespel reducing ferrule (part number RF-400/200-V; Alltech, Arlington Heights, IL, U.S.A.) and a 1/4-in. nut are used to allow the reactor to also serve as an adaptor between the 1/8-in. column and the 1/4-in. inlet fitting of the mass spectrometer interface. Plugs of glass wool are used to retain the Hopcalite.

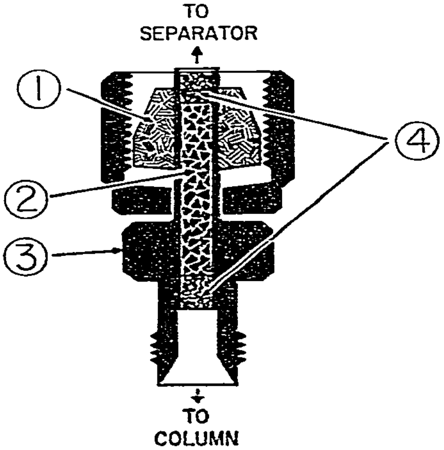

Fig. 1. Cross section of reactor assembly as used with 1/8 in. metal GC columns. 1 = Vespel reducing ferrule, 1/4 in. O.D. to 1/8 in. I.D.; 2 = Hopcalite, *ca.* 100 mg; 3 = stainless-steel reducing union, 1/8 in. to 1/8 in.; 4 = glass wool.

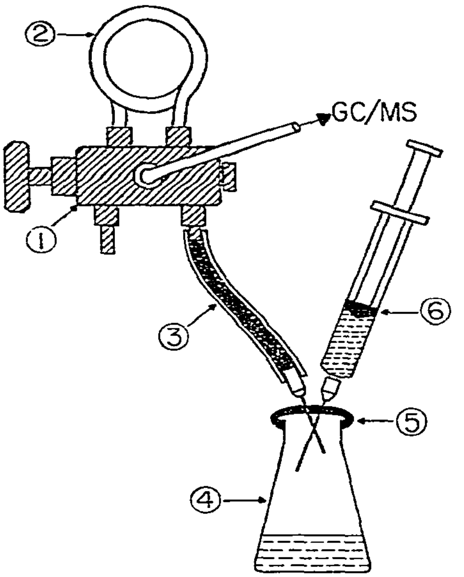

Fig. 2. Typical headspace sampling scheme. 1 = 6-Port gas sampling valve; 2 = 1-ml sampling loop; 3 = Tygon tubing filled with 10–20 mesh Drierite; 4 = 10-ml erlenmeyer flask reaction vessel; 5 = rubber serum stopper; 6 = water-filled syringe. The headspace gas is displaced through the sampling loop by injecting water into the headspace of the reaction flask.

Helium (high purity grade; Matheson Gas Products, Joliet, IL, U.S.A.) at 40 p.s.i.g. served as the carrier gas; the oven temperature was held at 160°C. These conditions produced a flow-rate of approximately 15 ml/min, which is nearly optimal for the jet orifice separator; a well resolved CO peak with a retention time of 5 min and a full width at half maximum of 12 sec was obtained. Because the gas-sampling valve was not pressure compensated it was necessary to bypass the chromatograph flow controller to minimize baseline disturbances due to pressure transients produced upon sample introduction.

Procedures

Any convenient method may be used to introduce gas samples. In applications requiring the sampling of headspace volumes, a gas sampling valve was used (Fig. 2). The flexible tubing connecting the gas-sampling valve inlet port and the hypodermic needle was filled with a drying agent (Indicating Drierite, 10–20 mesh, Hammond, Xenia, OH, U.S.A.) to retard the rate of column deterioration due to water absorption as well as to minimize the dead volume. When using a 1-ml sample loop, the headspace gases were displaced into the valve by injecting 2 ml of water through the septum into the flask. Calibration gases were introduced directly from their pressurized containers or via gas-tight syringes into the sampling valve inlet.

RESULTS AND DISCUSSION

The primary advantage of Hopcalite as the catalyst is that it is evidently constituted to provide its own source of oxygen to support the oxidation of CO to CO_2

(refs. 2 and 3). This leads to a simplifed apparatus since no provision need be made to supply oxygen. No difference in the conversion efficiency of CO to CO_2 was observed when up to 5% oxygen was added to the carrier gas or when the carrier gas was scrubbed of oxygen ("Oxy-Trap", Catalog Number 4001; Alltech) (data not shown). Although it may be expected that the reactant will be consumed with use, the system described here has been used to assay approximately 100 samples of CO at the 1 to 10,000 ppm level in air with no noticable loss or change in yield. The catalytic action of the Hopcalite was inhibited for low level CO samples by exposing it to several repeated injections of neat CO. The number of apparent theoretical plates exhibited by the overall system remained unchanged at *ca.* 3260 ± 50 with the reactor connected or disconnected.

To establish the conversion efficiency of the method, a gas mixture containing 10% CO, 5% air and the balance helium was analyzed without (case 1) and with (case 2) the reactor in place. The chromatograms produced by monitoring the molecular ions of CO and CO_2 are shown in the upper trace of Fig. 3. The area under the CO peak, measured as CO_2 (peak 3b, Fig. 3B) relative to that measured as CO (peak 3a, Fig. 3A) yielded a value of 1.2 ± 0.2 (mean ± S.D.) and was consistent with the expected value of 1.4 ± 0.1 predicted by the relative ionization cross sections of CO_2 and CO (ref. 4). The residual peak observed in case 2 at mass 28 (peak 4, Fig. 3B) was 3.3% of the peak at mass 44 and was consistent with the value of 4.0% obtained from the fragmentation pattern of CO_2 on the GC–MS employed (data not shown).

The linearity of the conversion process was studied. Commercial gas standards containing 1 ppm to 10% CO in nitrogen or air were used (Scotty-1, mixtures: 28,

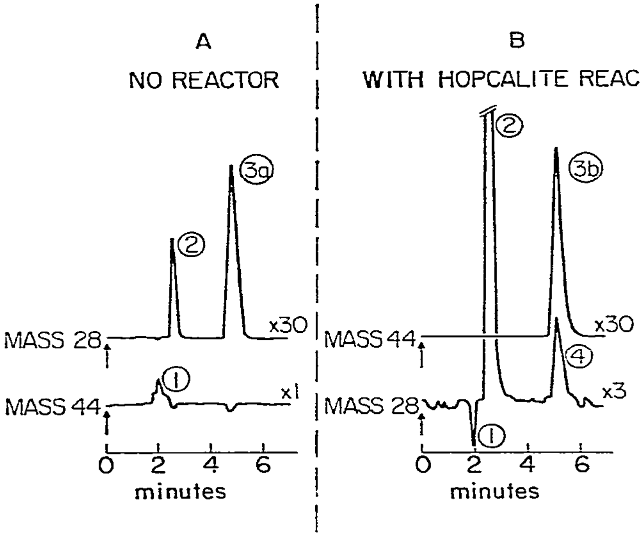

Fig. 3. Typical multiple ion chromatograms of a mixture of 10% CO, 5% air and the balance helium. A, no reactor, case 1; B, with reactor, case 2. The individual peaks are: 1 = oxygen; 2 = nitrogen; 3a = carbon monoxide; 3b = carbon monoxide as converted to carbon dioxide; 4 = mass 28 fragment due to carbon dioxide. These data were obtained with the Finnigan 3000 instrument.

45, 49, 51, 52, 59, 62, 65, 69 and 214; Scott Speciality Gases, Plumsteadville, PA, U.S.A.). Masses 44 and 45, with the reactor in place (case 2), or masses 28 and 29 with no reactor (case 1), were monitored. A plot of the GC–MS response (area under the CO chromatographic peak) *versus* CO concentration is shown in Fig. 4. Since the automatic peak area integration facility of the HP5992A data system becomes unreliable at signal-to-background ratios of less than 0.1, the concentration at which this occurs at each mass is also indicated in Fig. 4 and serves to define the lower limit of CO concentration which can be analyzed. Considering only the absolute detectability of CO, the use of Hopcalite as the reactant medium for the conversion of CO to CO_2 led to a lowering of the threshold of detectability by a factor of about 100 to a value of 0.4 ppm. By this same measure the ability to measure the $^{13}C/^{12}C$ isotope ratio was limited to a 10-fold improvement on this system.

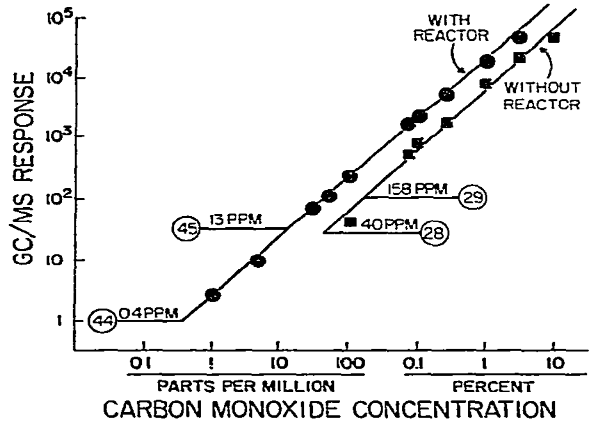

Fig. 4. GC–MS response *vs*. CO concentration. The response is the sum of the ^{12}CO and ^{13}CO peak areas. ●, Masses 44 and 45, with reactor, $r = 0.9985$; ■, masses 28 and 29, no reaction, $r = 0.9923$. The horizontal lines indicate the concentration below which the signal-to-background ratio is less than 0.01 for the ion signal at a given mass. These data were obtained with the HP5992A instrument.

As a second measure of the linearity of the GC–MS response each CO standard was compared against the response obtained by the method of Porter and Volman[5]. In this scheme, the column effluent is mixed with hydrogen and passed through a nickel catalyst which converts the CO to methane for detection by flame ionization. A plot of the GC–MS response *versus* this GC response is shown in Fig. 5 and is essentially identical with Fig. 4. On the basis of total CO response both methods exhibited comparable linearity. When fitted to a regression of the form:

$$(\text{response}) = A + B\cdot(\text{CO concentration})$$

a correlation coefficient of 0.9985 (−0.0026, +0.0013, $p = 0.05$, $n = 10$) was found for this GC–MS method which compares well with the value 0.9993 (−0.0020, +0.0005, $p = 0.05$, $n = 11$) obtained by the method of Porter and Volman.

The relationship between CO concentration and the precision of measurement, at low ^{13}CO enrichments, was examined (Table I). Three standards having an 0.181

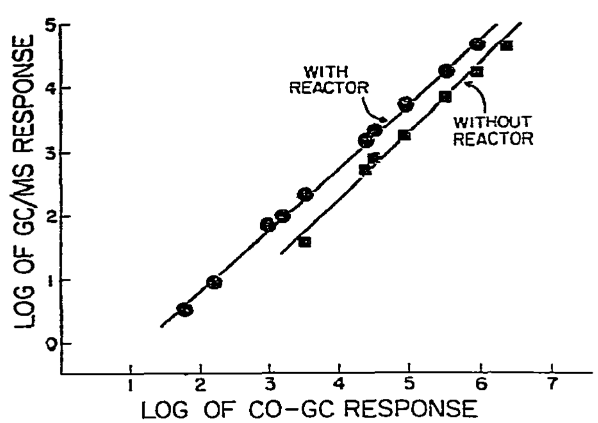

Fig. 5. GC–MS response *vs.* GC response. The data are presented similarly to that in Fig. 4 except that the GC–MS response of each sample is compared against its response as measured by the GC method of Porter and Volman[5]. ●, $r = 0.9991$; ■, $r = 0.9951$.

(±0.005) atom-percent excess ^{13}CO isotopic reference were prepared (see below) at 900, 90 and 9 ppm levels in air. Four replicates of each were assayed for their ^{13}C mole fraction with and without the Hopcalite reactor in place and the standard deviation was used as a measure of the instrumental precision. For the GC–MS system employed (HP5992A), satisfactory repeatability was obtained at signal-to-background ratios as low as 0.4. Although the absolute isotope ratios obtained were significantly and variably lower than "normal" the absolute differences between samples were well preserved.

TABLE I

RELATIONSHIP BETWEEN CO CONCENTRATION AND PRECISION OF MEASUREMENT

Expected atom-percent excess: 0.181 ± 0.005. Values are shown as mean ± S.D., $n = 4$. N.M. = Not measurable.

Hopcalite reactor	*CO concn. (ppm)*	*Observed atom-percent excess*	*Typical signal-to-background ratio*
No	900	0.196 ± 0.010	4.7
	90	0.223 ± 0.038	0.5
	9	N.M.	0.07
Yes	900	0.171 ± 0.007	45
	90	0.158 ± 0.012	1.2
	9	0.217 ± 0.130	0.4

The suitability of the method with respect to conservation of isotopic abundance and potential interferences arising from memory effects was also investigated. A series of ^{13}CO samples were prepared having gravimetrically determined isotopic abundances. The CO was generated in closed 10-ml flasks by the reaction of concentrated sulfuric acid with dilute aqueous solutions of [^{13}C]sodium formate (90 atom-percent; Merck, Rahway, NJ, U.S.A.) mixed with unlabeled sodium formate. A final concentration of about 700 ppm CO in air was produced with ^{13}C enrichments ranging from natural abundance to about 90 atom-percent. Each preparation was assayed at least twice in a random order. Fig. 6 shows a plot of the % deviation between the observed and expected ^{13}C enrichments *versus* the ^{13}C percent abundance. The deviations, which are shown for individual measurements, appeared to be randomly distributed and were typically less than 1 % over the entire range of concentrations studied.

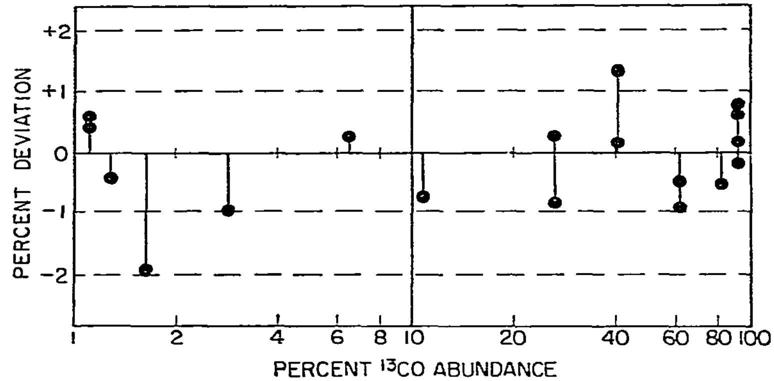

Fig. 6. Deviation between the expected and observed ^{13}C enrichment *vs.* total ^{13}C abundance. All quantities are expressed as percentages. Percent deviation is calculated as: 100%·([^{13}C]$_{obs}$ — [^{13}C])/[^{13}C]$_{exp}$.

The utility of this method has been demonstrated in metabolic experiments in which ^{13}CO was formed as a metabolite of xenobiotics. For example, the isotopic enrichment of the ^{13}CO formed metabolically from [^{13}C]bromoform has been quantified, both *in vivo*[6] and *in vitro*[7], using this method.

The advantage of this method has varied with the GC–MS system employed, the major factor being the relative background at masses 44 and 45 compared to masses 28 and 29. The efficiency of the jet orifice separator at low molecular weights is also a factor. When the HP5992A instrument was used, the signal-to-noise limit of detectability was improved by only a factor of 10 because of its generally higher level of background contributions. However, its specific response to CO_2 derived from CO was approximately 30% greater than that due to CO itself (Fig. 4), whereas on the Finnigan instrument, the responses were nearly equal. The most likely explanation is that the ion source optics on the Finnigan instruments are operator adjusted to give equal source efficiencies at masses 28–29 and 44–45, whereas on the Hewlett-Packard instrument the operator has no manual control over the source optics. However, both instruments generally were of equal usefulness in the analysis of biosynthetically produced CO.

CONCLUSIONS

A new method for the isotopic analysis of CO present in a matrix of air has been described. At concentrations as low as 10 ppm ^{13}C enrichments relative to a standard can be measured with a precision of better than 1%. Sample volumes as small as 10 ml can be assayed without prior sample preparation by direct introduction into a easily modified GC–MS system having a suitable data handling capacity.

This technique may find application involving ^{13}C isotopic tracers in a wide variety of research projects involving CO production.

ACKNOWLEDGEMENTS

The authors thank Ms. Joan Sunram for her excellent technical assistance. This research was supported by National Institutes of Health grant ES 01082 and National Cancer Institute grant CA 21899.

REFERENCES

1 C. M. Stevens and L. Krout, *Int. J. Mass Spectrom. Ion Phys.*, 8 (1972) 265.
2 A. B. Lamb, C. C. Scalione and G. Edgar, *J. Amer. Chem. Soc.*, 44 (1922) 738.
3 R. E. Coburn, in R. E. (Editor), *Carbon Monoxide*, National Academy of Sciences, Washington, DC, 1977, Ch. 2.
4 F. W. Lampe, F. H. Field, J. L. Franklin, *J. Amer. Chem. Soc.*, 79 (1957) 6129.
5 K. Porter and D. H. Volman, *Anal. Chem.*, 34 (1963) 748.
6 M. W. Anders, J. L. Stevens, R. W. Sprague, Z. Shaath and A. E. Ahmed, *Drug Metab. Disp.*, 6 (1978) 556.
7 J. L. Stevens and M. W. Anders, *Biochem. Pharmacol.*, 28 (1979) 3189.