APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1979, p. 897-899
0099-2240/79/05-0897/03$02.00/0
Vol. 37, No. 5

Measuring Radioactive Methane with the Liquid Scintillation Counter

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Received for publication 5 February 1979

Although a gas proportional counter is the most convenient method of measuring the radioactivity of fixed gases such as methane, it cannot be used when high nonradioactive concentrations of methane are present in the gas phase, due to quenching. If only methane and carbon dioxide are present in radioactive form in the gas phase, a liquid scintillation method for measuring these substances can be used. The procedure is described in detail, and the solubility of methane in liquid scintillation cocktails is determined.

Nelson and Zeikus (4) have described the use of a gas proportion counter for determining the radioactivity of methane and other gases. Although this method is quick and convenient, the efficiency is low and variable, and it cannot be used if high concentrations of nonradioactive methane are present, due to quenching. McBride and Wolfe (3) and Ferry and Wolfe (2) used a liquid scintillation counter for measuring radioactive methane. Our method is based on theirs but has been calibrated and quantified so that exact amounts of [^{14}C]methane can be determined. Nelson and Zeikus (4) compared gas proportional and liquid scintillation counting. For many purposes, a gas proportional counter is ideal, but it is not routinely available. Also, as described here a gas proportional counter cannot be used when large amounts of nonradioactive methane are present.

METHODS

Chemicals. The methane and CO_2 used were obtained from Matheson Co., Joliet, Ill. and were of the highest purity. $NaH^{14}CO_3$ was purchased from New England Nuclear Corp., Boston, Mass. Radioactive methane was prepared from $NaH^{14}CO_3$ by using a culture of *Methanobacterium thermoautotrophicum*, as described by Zehnder and Brock (5). All other chemicals were reagent grade. Two different liquid scintillation cocktails were used. The toluene-based cocktail contained 0.375 g of 2,5-diphenyloxazole and 0.1 g of dimethyl-POPOP [1,4-bis-2(methyl-5-phenoxazolyl)-benzene] per liter of toluene (scintillation grade). The Bray solution was composed of 60 g of naphthalene (scintanalyzed), 4 g of 2,5-diphenyloxazole, 0.2 g of POPOP, 100 ml of methanol, 20 ml of ethylene glycol, and 1,4-dioxane (scintillation grade) to make 1 liter (1).

Radioactive counting. The gas proportional counter used was that described by Nelson and Zeikus (4). Quantification of recorder tracings used peak heights. The liquid scintillation counter was a Packard Tri-Carb counter, operating at 4°C. Its efficiency was determined by using internal standards in the test solutions, and quench corrections were made by the channels ratio method.

Gas chromatograph. Nonradioactive methane was assayed with a Packard model 419 gas chromatograph equipped with a flame ionization detector. The column (1.5 m long by 2.5-mm ID) was packed with Poropack QS (80/100 mesh) and held isothermally at 180°C. Nitrogen as the carrier gas had a flow rate of 45 ml/min. The size of the sample injected was 20 μl. With these settings the detection limit was 4.5 pmol/sample.

RESULTS

Quenching in the gas proportional counter. During a study to determine whether methane would inhibit its own formation, it was discovered that nonradioactive methane strongly suppressed the ability of the gas proportional counter to measure radioactive methane (Table 1). Even a pressure as low as 1 atmosphere of methane significantly reduced the ability of the gas proportional counter to measure the radioactive methane present. Nonradioactive methane reduces the sensitivity of the gas proportional counter to radioactive methane because of quenching in the gas proportional detector. Thus, the gas proportional counter cannot be used to accurately measure radioactive methane if there are significant amounts of non-

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TABLE 1. *Effect of nonradioactive methane on the sensitivity of the gas proportional counter to radioactive methane*[a]

Radioactivity recorded by gas proportional counter (cpm)	Sp act (μCi/mmol)	Gas phase injected	
		Gas	Pressure (atmospheres)
4,400	0.73	N_2	1
4,000	0.73	N_2	9.3
2,900	0.39	CH_4	1
2,300	0.24	CH_4	2.3
1,150	0.15	CH_4	4.3
450	0.08	CH_4	9.3

[a] The gas volume injected was 0.4 ml at the indicated pressure. All samples contained identical amounts of $^{14}CH_4$. The efficiency of the counter was 13% (based on radioactive and nonradioactive standards). The proportional counter was operated as described by Nelson and Zeikus (4).

radioactive methane present. Since sewage sludge and many cultures produce methane pressures of 1 atmosphere or more in closed systems, it is clear that the gas proportional counter cannot be used in many experiments involving radioactive methane.

Procedure for liquid scintillation counting of radioactive methane. When methane is the only radioactive gas present in the system, it can be counted simply by injecting a gas sample into a liquid scintillation vial which has been modified to hold a septum. McBride and Wolfe (3) used septa manufactured by Packard (catalog no. 5396730). We used septa manufactured by Pierce Chemical Co., Rockford, Ill. (described as Discs, Tuf-Bond, Teflon-Silicone, 20 mm [black]; catalog no. 12720). These disks were used with either the Teflon or the silicone side toward the scintillation fluid. The screw cap of the scintillation vial was modified by drilling a small hole for the entry of a hypodermic needle. The septum could be reused several times if the hole was drilled off-center so that the septum could be rotated after each use. The scintillation vial was filled with 20 ml of scintillation cocktail, and the headspace remaining was measured (our headspace was 3.5 ml). The vials were first equilibrated at 4°C. Using a Pressure-Lok syringe (Precision Sampling Co., Baton Rouge, La.), 1 ml of gas sample was injected through the septum into the scintillation vial. The vial was shaken vigorously, and the methane was allowed to equilibrate at 4°C for 1 h. The radioactivity was then determined by conventional liquid scintillation spectrometry. The counting efficiency was reduced by about 4% when 20 ml of scintillation fluid was used instead of 10 ml. However, when 10 ml was used, most of the $^{14}CH_4$ was in the headspace and was not counted.

No decrease in radioactivity was found when scintillation vials containing $^{14}CH_4$ were stored overnight at 4°C.

Removal of $^{14}CO_2$. If the gas sample contains $^{14}CO_2$, it is necessary to remove this gas before injecting it into a scintillation vial. For this purpose, an 8-ml serum vial was entirely filled with 1 N NaOH, closed with a rubber septum, and capped with an aluminum seal. Special attention was given to the exclusion of any air bubbles. Subsequently, 1 ml of the alkali was removed with a syringe. The liquid was replaced by air which entered through an inserted needle while the alkali was taken out. One milliliter of gas was injected into the headspace, and the vial was shaken vigorously and allowed to equilibrate at 25°C for 1 h. (Removal of $^{14}CO_2$ into the alkali was virtually instantaneous; the 1-h incubation was merely a precaution.) Controls showed that there was no radioactivity in the headspace over the 1 N NaOH even if as much as 750,000 dpm of $^{14}CO_2$ was injected into the vial. To count $^{14}CH_4$, 1 ml of headspace was removed from the alkali-containing 8-ml serum vial for injection into a scintillation vial, as described above. It was important when gas was removed from the alkali-containing vial that the needle remained completely uncontaminated with alkali, as the liquid would have had radioactive CO_2 which would have been transferred to the scintillation vial and counted.

Solubility of methane in scintillation cocktails. The solubility of methane in conventional toluene-based scintillation cocktail was determined by injecting known amounts of nonradioactive methane into scintillation vials by the procedure outlined above and then assaying the headspace for methane, after equilibration. Table 2 shows that when 20-ml volumes were used, about 80% of the methane dissolved in the scintillation cocktail, with 20% remaining in the headspace. The size of the headspace influenced considerably the efficiency of counting, and with only 10 ml of scintillation fluid in the vial, much more of the methane was in the headspace, and less was in the toluene. The use of 20 ml of scintillation fluid was a compromise, permitting injection of large amounts of gas without any buildup of excessive headspace pressure.

Bunsen absorption coefficients. The Bunsen absorption coefficients (α_B) for methane were determined in 1 N NaOH, in toluene-based scintillation cocktail, and in Bray solution (Table 3). Because of the relatively low coefficient of methane for 1 N NaOH, only a small amount of methane was absorbed by the CO_2 trapping

TABLE 2. *Solubility of CH_4 in toluene-based scintillation cocktail*[a]

Amt of CH_4 injected (μl)	Trial no.	Amt in headspace (μl)	Amt in toluene (μl)[b]	Ratio of amt in toluene to amt in headspace
20	1	4.03	15.97	3.96
	2	3.98	16.02	4.02
100	1	19.8	80.2	4.05
	2	19.7	80.3	4.08
400	1	76.2	323.8	4.24
	2	78.7	322.3	4.09
800	1	154	646	4.18
	2	154	646	4.18

[a] One milliliter of gas containing the stated amount of methane was injected. Headspace was sampled after equilibration for 1 h at 4°C and analyzed by gas chromatography.

[b] Calculated by difference.

TABLE 3. *Bunsen absorption coefficient (α_B) for methane in various solvents*[a]

Temp (°C)	α_B (atmosphere^{-1}) in:		
	1 N NaOH	Toluene-based scintillation cocktail	Bray solution
4		0.650 ± 0.012	0.414 ± 0.02
10		0.638 ± 0.013	0.395 ± 0.008
25	0.0137 ± 0.00066	0.603 ± 0.009	0.396 ± 0.018

[a] Means of five different experiments ± standard deviations.

solution. Since only the dissolved radioactive gas was counted, the toluene-based scintillation cocktail, with its higher α_B, was more suitable for the determination of the radioactive methane than Bray solution.

Radioactive methanol. In some experiments with methanogenic bacteria, [^{14}C]methanol may be used. Because of its volatility, there was the possibility that some [^{14}C]methanol might appear in the headspace and be measured mistakenly as $^{14}CH_4$. Vials (36.5 ml) were filled with 20-ml methanol-water mixtures containing 10 to 100 mM nonradioactive methanol. To all vials, 10 μCi of ^{14}C-labeled CH_3OH was added. The vials were shaken vigorously and allowed to equilibrate at 25°C for 1 day, and 1 ml of headspace was removed and analyzed by injecting into liquid scintillation cocktail. Even when the specific activity of the radioactive methanol was very high (50 μCi/mmol), only negligible radioactivity was found in the headspace (13.5 cpm/ml). Thus, because of its high solubility in water, radioactive methanol should not present any problem in headspace analysis.

DISCUSSION

When compared with the gas proportional counter, the efficiency of counting with liquid scintillation is generally higher. If CO_2 is removed by alkali trapping, the gas to be analyzed is diluted by only 50% (if a 1-ml sample is used). However, the efficiency of liquid scintillation counting for ^{14}C is high (generally around 80 to 90%). A sample size even larger than 1 ml could be used to increase the sensitivity of the method, whereas with the gas proportional counter the sample size is limited, to avoid overloading the column.

As Nelson and Zeikus (4) point out, the gas proportional counter has a number of advantages, including the simultaneous analysis of both methane and CO_2 and the direct determination of specific radioactivity. If specific radioactivity is to be determined by using liquid scintillation counting, then a separate analysis of the headspace for nonradioactive methane and CO_2 is necessary, using the gas chromatograph. However, the present method has the distinct advantage that it is not affected by the presence of even large amounts of nonradioactive methane (we have successfully used the method even when the headspace contained 10 atmospheres of nonradioactive methane) and does not require expensive or complicated equipment. The overall efficiency of counting (including the CO_2 trapping step) is at least as high by liquid scintillation counting as by gas proportional counting and is less subject to day-to-day fluctuations.

ACKNOWLEDGMENTS

This work was supported by the College of Agricultural and Life Sciences and by a contract from the Department of Energy (COO-2161).

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