Argon Detector: Alternative Detection System for Gas-Liquid Chromatographic Analysis of Short-Chain Organic Acids

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Two detection systems for gas-liquid chromatography were compared for the identification of metabolic end products (short-chain organic acids) from anaerobic bacteria. Argon and flame ionization detectors were connected in series with inert argon as the carrier gas for analysis of 35 stock strains and 148 clinical isolates. There was an excellent correlation between the argon and flame ionization chromatograms, and both were readily comparable to the thermal conductivity detector tracings published in the Virginia Polytechnic Institute manual. The linear response of the argon detector was examined by analyzing twofold serial dilutions of the short-chain organic acids and comparing the results to the very linear flame ionization detector. The argon detector was found to react with sufficient linearity within the organic acid concentration range normally obtained from anaerobic bacterial broth cultures. The argon detector, therefore, appears to be a viable alternative to the flame ionization and thermal conductivity detector systems in the identification of anaerobic organisms.

The analysis of short-chain organic acid (SCOA) metabolic end products by gas-liquid chromatography has been accepted as a vital tool in the identification of anaerobic bacteria (5). The thermal conductivity detector (TC), as originally applied to those taxonomic studies, is a relatively simple and reliable device with good linear range which requires only one gas, helium. The flame ionization (FI) detector, which requires nitrogen, air, and hydrogen, provides both an expanded linear range and a much lower minimal detectable limit than the TC for most organic compounds (2, 7). The FI detector has been applied to the analysis of volatile SCOA end products from anaerobic microorganisms in bacterial culture (2) and in purulent exudates (3). However, its use may entail the additional expense of a hydrogen generator, since the use and storage of bottled hydrogen is closely regulated in hospitals.

The argon detector, which requires a single inert gas (argon), is sensitive to picogram concentrations of nearly all organic compounds (1, 4, 7, 8). Its linear range is somewhat limited when compared with TC and FI systems, and sensitivity may be drastically reduced by contamination with air or water vapor (1, 7). Careful attention to sample preparation techniques, however, can make the argon detector a convenient and practical tool for anaerobic bacteriology.

The aim of this study was to explore the

potential of the argon detector as an alternative to FI and TC detectors in anaerobic microbiology.

MATERIALS AND METHODS

Bacterial cultures. SCOA's were analyzed from 35 well-described anaerobic stock strains and 152 recent clinical isolates. The organisms were identified by methods detailed in the Virginia Polytechnic Institute *Anaerobic Laboratory Manual* (5) and the *Wadsworth Anaerobic Bacteriology Manual* (9), including antibiotic disks (Sensi-discs, Baltimore Biological Laboratory), biochemicals utilizing prereduced anaerobically sterilized media, and gas-liquid chromatography using an FI detector. The 35 stock cultures were previously isolated from clinical specimens at Sepulveda Veterans Administration Hospital, Sepulveda, Calif., and Tufts-New England Medical Center Anaerobic Research Laboratory and were the following generous gifts: *Bacteroides fragilis* and *Peptococcus magnus* from Vera Sutter, Los Angeles, Calif.; *B. fragilis* from William Martin, Los Angeles, Calif.; and *B. fragilis* from Ian Phillips, London, England. *B. fragilis* strain 23745 was obtained from the American Type Culture Collection (ATCC 23745).

The 152 recent clinical isolates were cultured from appropriately obtained clinical specimens from which the normal flora was excluded. The cultures were transported immediately to the Tufts Anaerobe Research Laboratory and processed in the anaerobic chamber, using selective and nonselective media. The organisms were identified according to the methods described above.

All strains were grown in chopped-meat carbohydrate broth (Carr-Scarborough, Stone Mountain, Ga.)

and assayed for SCOAs after 2 to 5 days of incubation. Chopped-meat carbohydrate broth contains glucose, cellobiose, maltose, and starch.

SCOA extraction and preparation. For the volatile SCOAs analysis, 0.1 ml of the internal standard, 2-methylpentanoic acid (10 mmol/100 ml) was added to 2.0 ml of chopped-meat carbohydrate broth or peptone-yeast-glucose (Carr-Scarborough) (6). The sample was acidified with 50% (vol/vol) H_2SO_4 , extracted with 1.0 ml of diethyl ether, mixed by repeated inversion, and centrifuged for phase resolution. The organic phase was collected and dried over anhydrous calcium chloride.

For the nonvolatile SCOAs analysis, 0.2 ml of the internal standard, benzoic acid (10 mmol/100 ml), was added to 1.0 ml of acidified chopped-meat carbohydrate broth or peptone-yeast-glucose (6). The specimen was then supplemented with 1.0 ml of 14% boron trifluoride-methanol (Applied Sciences, State College, Pa.) and incubated for 30 min at 56°C. One milliliter of water was added, and the methylated acids were extracted into 0.5 ml of chloroform. One microliter of each of the organic phases was injected.

Chromatographic instrumentation and conditions. Two dual-channel chromatographs were used for the study. The Kontes series 4000 (Kontes, Vineland, N.J.) was used for all experiments involving the argon detector system and for the culture fluid studies with the argon and FI detectors connected in series. The argon detector's ionizing source was the natural isotope ^{226}Ra . A Shimadzu GC4BMPPF (FI) was utilized in further studies comparing the linearity of the argon detector with the FI detector (Fig. 2).

Columns were Pyrex glass (4 m by 3-mm ID) of spiral configuration for the Kontes chromatograph and of racetrack configuration for the Shimadzu. The columns contained 6% Carbowax 20M-terephthalic

acid coated onto 80- to 100-mesh Gas Chrom Q (Applied Sciences). The column temperature was 165°C, and the detector and injection ports were maintained at 200°C. DC voltage for the argon detector was $1,000 \pm 5$ mV. When the two detectors were connected in series for culture fluid analysis, argon served as the carrier gas for both detectors (35 ml/min). Argon is not the optimum carrier gas for the FI detector. Detector sensitivities were set at 3.3×10^{-8} A for the argon detector and 1.6×10^{-9} A for the FI detector. In the series configuration, single injections of volatile and nonvolatile (methylated) organic acid preparations derived from microbial culture were resolved on one column, the effluent was passed sequentially through the argon and FI detectors, and the detector responses were expressed simultaneously with a dual-pen recorder (Honeywell, Minneapolis, Minn.).

Linearity of argon detector in SCOAs analysis. Experiments were designed to describe partially the linear range of the argon detector for SCOAs and products. Three pools of 0.1% (wt/vol) SCOAs were prepared and twofold serially diluted in peptone-yeast-glucose to 0.00625%. These concentration levels are generally within the range obtained from anaerobic broth cultures. The three replicate dilution series were extracted and chromatographed separately with argon (Kontes) and FI (Shimadzu) detection. Peak areas (A) were measured ($A = H \times W_{1/2H}$), and the results were expressed as area ratios (area of the acid/area of the internal standard). Concentration-response curves were constructed relating the concentration gradient to the area ratios.

RESULTS

The chromatograms of SCOAs mixtures (0.05%, wt/vol) rendered by series-connected argon and FI detectors are shown in Fig. 1. Little

FIG. 1. Chromatograms of SCOAs mixtures as analyzed by series-connected argon and FI detectors.

difference could be observed in the reactivity of most compounds with each of the two detectors. The chromatograms illustrate that the responses of each detector to most of the compounds are visually quite similar.

Concentration-response curves, derived from parallel analyses on separate argon and FI instruments, can more precisely express the relationship between the argon and the FI detectors. Figure 2 contains the graphs for three volatile (acetic, butyric, 4-methylpentanoic) and two nonvolatile (lactic, succinic) acids over the concentration range 0.1 to 0.00625%. These curves

correlate the concentration gradient with an increase in peak area relative to the areas of a fixed amount of the appropriate internal standard (2-methylpentanoic acid or methylated benzoic acid). The recovery slopes and Y intercepts for butyric acid, 4-methylpentanoic acid, and succinyl dimethylester are very similar for both detectors. The argon detector appears to have a proportionally larger unit response to equivalent concentrations of acetic acid and lactyl methylester.

Figure 3 contains chromatograms of SCOA extracted from a broth culture of *Clostridium*

FIG. 2. Comparison of the concentration-response curves of the argon and FI detectors for the gas-liquid chromatography analysis of three volatile ([A] acetic, ■; [Bu] butyric, ●; [4-MP] 4-methylpentanoic, Δ) and two nonvolatile ([L] lactic, ○; [S] succinic, ●) SCOA. Chromatograms were run in parallel on separate argon and FI instruments.

sporogenes. Extracted samples were resolved on one column, and the effluent was passed through the two detectors connected in series. As in the case of standard SCOA mixtures (Fig. 1), the chromatograms were essentially equivalent.

Organic acid extracts from broth cultures of 35 stock anaerobic strains and 152 clinical isolates (Table 1) were similarly assayed in the series detector configuration. In all cases, both the argon and FI chromatograms were readily comparable to the comprehensive library of TC detector tracings presented in the VPI manual (5).

DISCUSSION

The argon detector may be a useful alternative to FI and TC systems for SCOA analysis. Its main advantages are: the use of one inert gas, argon; a lower minimum detectable limit than the TC; and avoidance of the problems of hydrogen storage and generation associated with the FI. The argon detector appears to respond linearly to the organic acid concentration (<0.1%) normally found in microbial broth cultures, and its chromatograms easily correlate with those of the VPI manual (5).

The main disadvantages of the argon detector are its somewhat restricted linear range and a drastic, but temporary, reduction in sensitivity when the system is contaminated with air or

water vapor. Careful sample preparation (e.g., the drying of the sample over anhydrous calcium chloride) will lead to an accurate and practical analysis of SCOA from anaerobic bacterial cultures and other sources. The response characteristics of the detector appear to be linear within the range of interest (<0.1%). However, if quantitation is desired, the relative response of each acid to the appropriate internal standard must be carefully determined.

The ^{226}Ra -ionizing radiation source is a naturally occurring isotope of relatively weak emission characteristics as compared with tritium and ^{63}Ni . The modular detector unit has required one cleaning (7% aqueous KOH) in 2 years of continuous use. The argon detector has

TABLE 1. Anaerobic microorganisms used to compare the argon and FI detectors

Microorganisms	Stock strains (no.)	Recent clinical isolates (no.)
<i>Bacteroides fragilis</i> group	13	34
<i>Bacteroides</i> sp.	1	21
<i>Fusobacterium</i> sp.	0	10
Miscellaneous gram-negative anaerobic bacilli	0	2
<i>Clostridium</i> sp.	10	16
Anaerobic cocci	6	48
Miscellaneous gram-negative nonsporing bacilli	5	21

FIG. 3. Chromatograms of the SCOA extracted from a broth culture of *S. sporogenes* as analyzed by the argon and FI detectors connected in series.

proven, in routine use, to be simple to operate, extremely reliable, and relatively maintenance free.

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