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Note

Gas chromatographic determination of C_2–C_5 fatty acids in aqueous media with a Porapak N column

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Saturated C_2–C_5 fatty acids are metabolic products of numerous microorganisms, and gas chromatographic analysis of these acids has been used to differentiate between certain microorganisms[1–4]. A simple gas chromatographic procedure for fatty acid determinations at concentrations normally found in microbial culture media would therefore be valuable. The direct quantitation of volatile fatty acids in aqueous media is possible with Porapak N (a porous polymer) as column packing, *i.e.*, no extraction or concentration procedures are required[5].

In the qualitative or quantitative determination of volatile fatty acids in culture media, the phenomenon known as "ghosting" is an important source of error[6], yet surprisingly few workers have mentioned ghosting in their reports[7,8]. Henkel[5] experienced ghosting with a Porapak N column, but only when cells or cell fragments remained in the sample. This phenomenon has, however, been reported even with pure aqueous solutions containing no biological material[7]. In this paper the ghosting effect, using a Porapak N column, is examined.

EXPERIMENTAL

Apparatus

A Beckman GC-5 dual-column gas chromatograph with hydrogen flame ionization detectors and a 1-mV Beckman potentiometric recorder was used.

The columns used were of stainless steel, 150 cm × 1/6 mm (1/16 in.) I.D., packed with Porapak N, 80–100 mesh (Waters Assoc., Milford, Mass., U.S.A.), in the normal manner using vacuum and vibration. Both ends of each column were plugged with the minimum amount of glass-wool. The following conditions were used. Gas flows: nitrogen, 50 ml/min; hydrogen, 35 ml/min; and dry air, 300 ml/min. Temperatures: on-column inlet, 210°; column compartment, 185°; and detector, 240°.

Sample preparation

Standard aqueous acidic mixtures were made up containing acetic (B. Owen Jones, Johannesburg, South Africa; purity > 99.7%), propionic, isobutyric, *n*-butyric, isovaleric and *n*-valeric acids (BDH, Poole, Great Britain; purity > 99%) at concentrations ranging from 0.05 to 6 mg/ml. These standard solutions were

treated by either (a) adjusting to *ca.* pH 1 with 2 *N* HCl, or (b) adding 1.0 ml of formic acid (Merck, Darmstadt, G.F.R.; purity > 98%) to 4.0 ml of the standard solution immediately prior to injection, giving a final pH of *ca.* 1. The use of formic acid to minimise ghosting has been discussed previously[9].

Procedure

On-column injections of 5-μl volumes of the standard solution were made with a microsyringe of 10-μl capacity. The syringe was cleaned after each injection by rinsing it several times with distilled water and placing it in a Hamilton syringe cleaner. Ghosting was detected by subsequent injection of 5 μl of an aqueous 20% formic acid solution with a second syringe used only for this purpose.

RESULTS AND DISCUSSION

The pH of the fatty acid sample was a major factor influencing the appearance of ghost peaks. Very little of the fatty acids was eluted when a sample at an alkaline pH was injected, and these acids appeared as ghost peaks during subsequent injections of 20% formic acid or even distilled water. This observation was similar to that

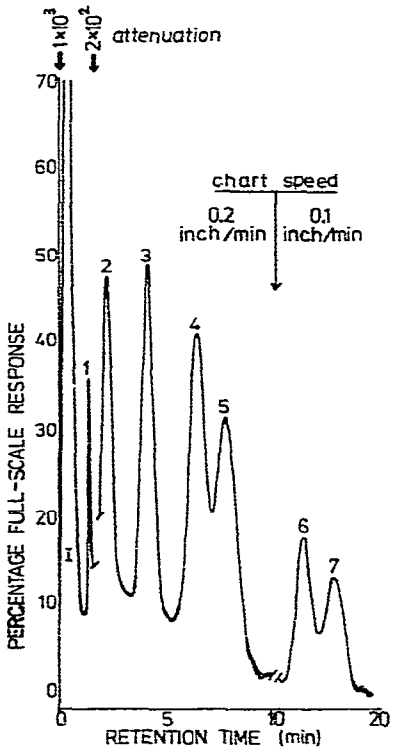

Fig. 1. Gas chromatographic separation of volatile fatty acids in aqueous solution on a Porapak N column. 1 = Formic acid (20%); 2 = acetic acid (0.042 mg/ml); 3 = propionic acid (0.04 mg/ml); 4 = isobutyric acid (0.038 mg/ml); 5 = *n*-butyric acid (0.038 mg/ml); 6 = isovaleric acid (0.037 mg/ml); 7 = *n*-valeric acid (0.038 mg/ml). I = injection peak.

previously reported[10]. Even at a pH of about 3 the aqueous standard solutions (containing no biological material) gave rise to ghosting. By lowering the pH of each sample to *ca.* pH 1 with either HCl or formic acid, ghosting was virtually eliminated and linear calibration charts of peak height *versus* fatty acid concentration were obtained. However, despite the lowered pH, ghosting tended to increase with the number of fatty acid samples injected, and it was therefore necessary to purge the column with 5 μl of a 20% aqueous formic acid solution after every 4–5 injections so as to ensure column cleanliness. Column stability was excellent and the same column was used for more than 300 injections of aqueous fatty acid mixtures without impairing the column efficiency.

A peak was produced by 20% formic acid at a retention time of 80 sec (Fig. 1). At greater formic acid concentrations, the magnitude of this and the injection peak increased, but the retention time of the former decreased to 70 sec for 50% formic acid and to 57 sec for 100% formic acid. The peak height and retention time of the 20% formic acid peak were consistent. The fact that peaks are obtained with formic acid contradicts observations by various workers[5,9,11,12], who have stated that formic acid does not give a response in the hydrogen flame ionization detector (HFID), but confirms other reports[13–15] that formic acid can be detected with the HFID, even in aqueous solutions. This discrepancy can be attributed to two factors, namely (i) the HFID in less sensitive to formic acid in comparison with the higher monocarboxylic acids[14], and (ii) evidence exists that adsorption phenomena may prevent the detection of formic acid with certain columns[15].

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