CHROM. 4039

A rapid method for the determination of ethylene in the presence of other volatile natural products

Ethylene, which is a gaseous natural product evolved by many plants, acts as a growth regulator at concentrations as low as 0.1–1 p.p.m. (ref. 1). Numerous chromatographic methods are available for its analysis in the presence of saturated or unsaturated hydrocarbons and other gaseous substances[2–5]. McEwen[6] used subtractor columns to remove unsaturated hydrocarbons, and Phan[7] removed ethylene from a gas stream by adsorption in $Hg(ClO_4)_2$ dissolved in $HClO_4$ and supported on pumice, and released it by a column reaction with HCl. All these methods tend to be time-consuming and often cannot be applied to the determination of ethylene in gas samples containing relatively large amounts of water and other compounds of varying polarity.

A rapid and sensitive method was required for the measurement, at frequent intervals, of ethylene produced by slices and by cell-free preparations of fruit tissues. Previous work in this laboratory[8,9] had demonstrated the value of remote precolumns for the removal of interfering substances, particularly water, in headspace gas analysis, and the present paper describes a further application of this technique to the determination of ethylene at very low concentrations in repetitive analyses.

Apparatus

A Series 104 model 4 single flame ionisation detector gas chromatograph (W.G. Pye, Ltd.) fitted with a 5 ft. × 1/4 in. stainless steel column packed with 80–100 mesh Porapak S (Waters Associates Inc.) and operated isothermally at 50° was used. The packing was preconditioned at 100° for 48 h before use.

The remote precolumn[9] used at first to remove interfering substances, consisted of a 6 × 1/4 in. stainless steel tube packed with 20 % diglycerol (May & Baker, Ltd.) on 85–100 mesh siliconised celite and operated isothermally at 20°. This was replaced in later experiments by a 2 × 1/8 in. stainless steel column packed with Porapak S, 80–100 mesh and operated at ambient temperature.

Argon, purified by passage through a tower containing Linde molecular sieve 5A, was used as carrier gas, at a flow rate of 40 ml/min. All pipework was of 1/16 in. I.D. stainless steel and connectors were Edwards R101 unions.

Standard ethylene

Pure ethylene (British Oxygen Co.) was diluted with dry air in calibrated aspirators to suitable concentrations.

Method of injection and procedure

The injection system used (Fig. 1) differs from that described by Grey and Shrimpton[9] by the inclusion of toggle valve T, which allows backflushing of the precolumn to proceed at a faster flow rate while maintaining a constant rate through the analytical column by means of the needle valve N. The valve V is a gas sampling valve (W. G. Pye, Ltd.). The procedure used for sampling and injection of ethylene was as follows.

Stage 1. With toggle valve T closed and valve V in the "fill" position carrier gas

flowed via valves N and V through the column. The small stainless steel concentric tube trap of similar design to that of SWOBODA AND LEA[10] was disconnected from the gas line and cooled by immersion in liquid oxygen, the inner tube of the trap being at the same time ohmically heated (7 A) via a transformer connected across the inlet and outlet tubes of the trap. Gas samples of known volume were then injected into the trap with a gas-tight syringe and the trap reconnected to the gas line.

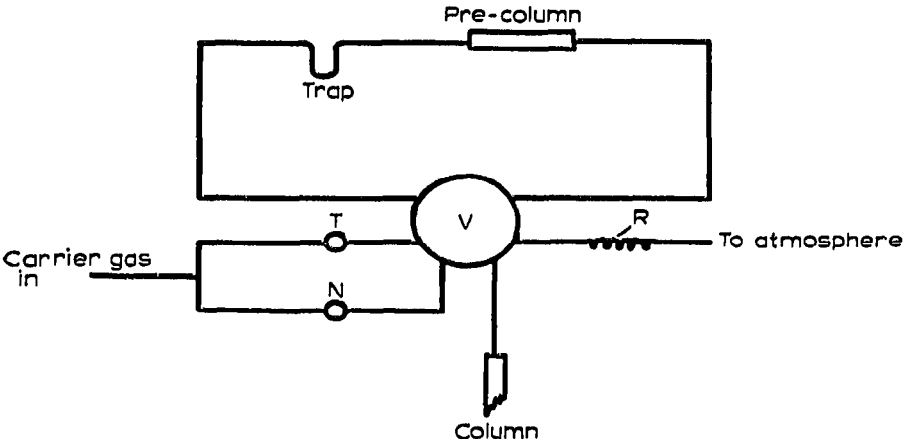

Fig. 1. Injection system for the analysis of ethylene. For description of the apparatus see text.

Stage 2. Valve V was switched to the "inject" position so that carrier gas now flowed through the trap and precolumn before entering the column via V. The liquid oxygen coolant was removed from around the trap, and the condensed gas sample, rapidly volatilised by the ohmic heating, was flushed through the precolumn and on to the main column.

Stage 3. When the volatiles under study had emerged from the column as indicated by the detector response, toggle valve T was opened and valve V switched to the "fill" position. The flow of carrier gas to the column through N and V was maintained and a rapid flow now passed through T, V, the precolumn and the trap, to the atmosphere through V and the restrictor R. Two minutes in this position sufficed to backflush materials from the precolumn and the toggle valve T could then be closed. The apparatus was then ready for the next sample.

Results and discussion

Linearity of detector response to ethylene. Two stock dilutions of ethylene in air were prepared and volumes of these between 0.5 and 10 ml, containing 4×10^{-3} μl to 8.8 μl of ethylene, were put through the above procedure. The relationship between response and concentration is shown in Fig. 2. Because of the wide range of concentration of ethylene studied the $\log_{10}$ of the values taken has been plotted. The response of the flame ionisation detector was found to be linear with respect to ethylene concentration over the whole of the range studied.

Volume of sample. In the calibration of the detector 0.5–10 ml sample volumes were used; however, larger volumes of gas containing lower concentrations of ethylene can be used. The limiting factor was the concentration of water vapour present, but sample volumes of up to 100 ml of water-saturated air have been analysed. However,

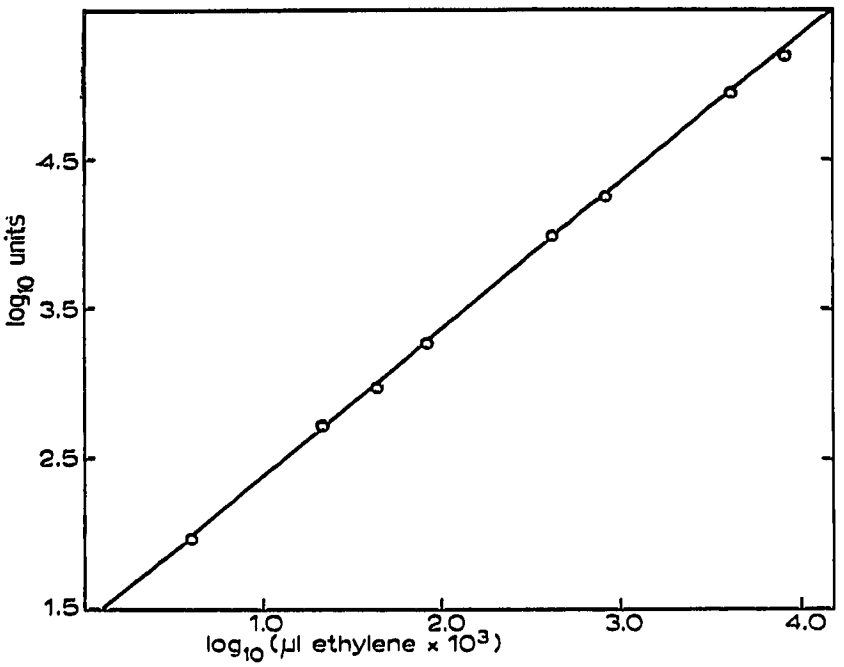

Fig. 2. The relationship between ethylene concentration and flameresponse.

there is a tendency for the free flow of gas through the trap to be restricted when larger volumes are used. In a given sample the lower limit of detection, defined as twice the noise level, was found to be approximately 10^{-10} of ethylene.

Reproducibility of the method. Results were calculated from the equation

$$\frac{\text{peak area} \times \text{attenuation}}{\text{chart speed}} = \text{units}$$

and these units were used as a measure of ethylene concentration.

In a test of reproducibility, eight 5 ml samples containing 0.068 μl of ethylene gave a mean of 1493 units with a standard deviation of 33.6 and coefficient of variation 2.26 % ($n = 8$). Thereafter, duplicate samples were analysed routinely.

Efficiency of precolumn. A study was made of the efficiency of the precolumn for removing more polar, higher molecular weight volatiles produced by small discs of apple peel. Without the precolumn, large peaks were still being eluted from the column for at least 1 h after injection and there was always the possibility that other interfering material would accumulate in the system. With the precolumn, ethylene was eluted after 4.1 min and backflushing commenced 3.5 min after injection, although this time could be shortened to 1 min if necessary. No interfering peaks were obtained when the precolumn was used except that acetaldehyde, present in some samples, was found to be incompletely retained by the diglycerol precolumn, and the peak interfered with subsequent analyses. However, replacement of the diglycerol by a 2 × 1/8 in. Porapak S precolumn completely eliminated the interfering peak, without loss of reproducibility. Ethane, which was present in some samples, was eluted 1 min after the ethylene and, for this reason, the longer delay before backflushing was preferred. An advantage of the present system over that of GREY AND SHRIMPTON[9] was the increased backflush flow resulting from incorporation of the toggle valve T, a constant

flow rate through the column still being maintained. As little as 2 min backflushing was found to purge the system completely.

The precolumn has been found particularly useful in radioactive tracer studies, since it prevents unwanted, labelled volatile material from entering the analytical column and causes increased background radioactivity in the effluent gas stream which was monitored for radioactivity.

Application. GALLIARD *et al.*[11] have used the method here described in studies of the production of ethylene by small amounts of fruit tissue slices. It has also been applied to study the enzymic formation of ethylene by cell-free extracts of apple fruits[12]. The method is now being employed also in tracer studies, the gas chromatograph being coupled in this case to a proportional gas flow counter.

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