Notes

Irreproducibility in the quantitative calibration of a flame ionisation detector

Since 1960 an increasing amount of work has been done on the optimisation of the working parameters of the flame ionisation detector in order to achieve the maximum linear range and sensitivity[1–4]. It has been demonstrated that in a nitrogen carrier gas system, with a hydrogen–compressed air flame, sensitivity increases with carrier gas flow rate[5] and that for maximum ionisation current for a given sample an optimum hydrogen and compressed air flow rate exist. Where routine daily analyses are concerned, it is convenient to operate the flame ionisation detector under such conditions that the sensitivity is relatively unaltered by the slight irreproducibilities in hydrogen and compressed air flow rates occasioned by the use of diaphragm pressure gauges rather than precision flowmeters, and it was to determine these conditions that the present work was undertaken.

In connection with some other work it was necessary to determine ethane, propylene and isobutane present in concentrations of 0.1 % to 1 % in propane ($\sim$25 μmoles). The instrument used was a Perkin-Elmer model 452 G.L.C., using hydrogen dried by passing through a Linde 5A molecular sieve (B.D.H.) and fitted with a thermistor katharometer and a Perkin Elmer flame ionisation detector (F.I.D.) similar to that described by CONDON *et al.*[3]. A sample splitter ratio of 1:5.6 was used. Compressed air was dried by passing it through a column of daily regenerated Linde molecular sieve 5A (B.D.H.), although moisture has little effect[6] on the detector's sensitivity. A Perkin Elmer 2 m "J" (silica gel) column was used at 150° and the gas sample volume was 25 ml. The chromatograph was recorded on a Honeywell-Brown chart recorder (full scale deflection 1 mV) fitted with an offset zero.

Gases used were obtained from Matheson and were fractionally distilled under high vacuum. The mixtures were made up in a conventional gas burette of maximum volume 60 ml, each component being expanded at room temperature to minimise the effect of impurities. Impurities (in every case < 2 %) would not, however, affect the slope of the calibration graphs, but would only appear as an intercept. Each measured component was distilled, by means of liquid nitrogen, into a glass sample bulb, thirty minutes at room temperature being allowed for mixing. The estimated error in each component varied from $\pm$ 2 % to $\pm$ 0.5 % at molar ratios of 0.003 and 0.1, respectively.

From a series of calibrations using 10 p.s.i. of compressed air supplied to the flame ionisation detector, a cathode voltage of —200 V and 15 p.s.i. of carrier gas (no separate supply of hydrogen to the flame was necessary) it was observed that repeated injection of the same sample gave peak height ratios reproducible only to $\pm$14 % even for the completely resolved ethane peak.

However, as can be seen from Fig. 1a the peaks had good shapes.

If sample pressures greater than 3 mm Hg were used "peak inversion" occurred, particularly with propane, in agreement with NOVÁK AND JANÁK[7], as shown in Fig. 1b. These effects could not be attributed to the gas injection system as the

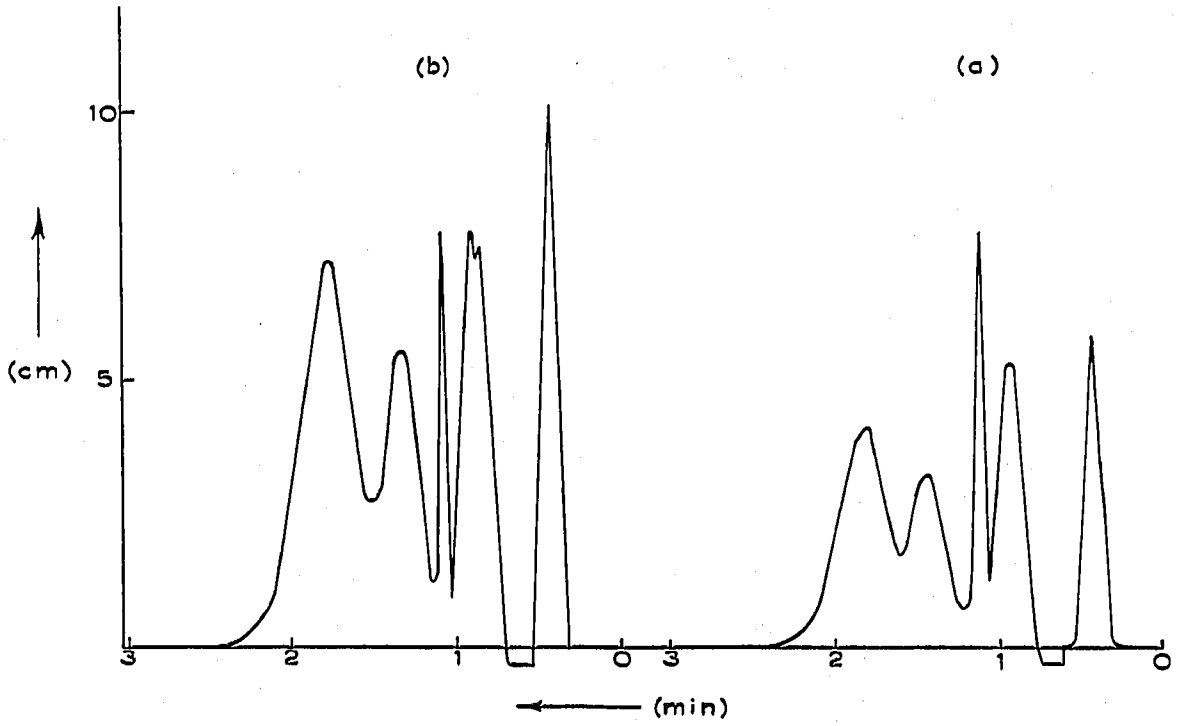

Fig. 1. Chromatogram at compressed air pressure 10 p.s.i. (a) Sample pressure 3 mm Hg. (b) Same sample but sample pressure 4 mm Hg. Peaks are eluted in the order: ethane, propane, propylene, and isobutane. The central sharp peak is due to attenuation switching.

thermistor detector, using even greater samples, was completely reproducible. With aging (600 h use), the erratic behaviour shown in Fig. 2a resulted. This was similar to that reported for air starvation with a micro F.I.D.[2].

Accordingly a second 2 m "J" column was calibrated under the same conditions and peak shape was again good at sample pressures less than 3 mm Hg. Above this "peak inversion" again occurred. From Fig. 3 it is obvious that the G.L.C.

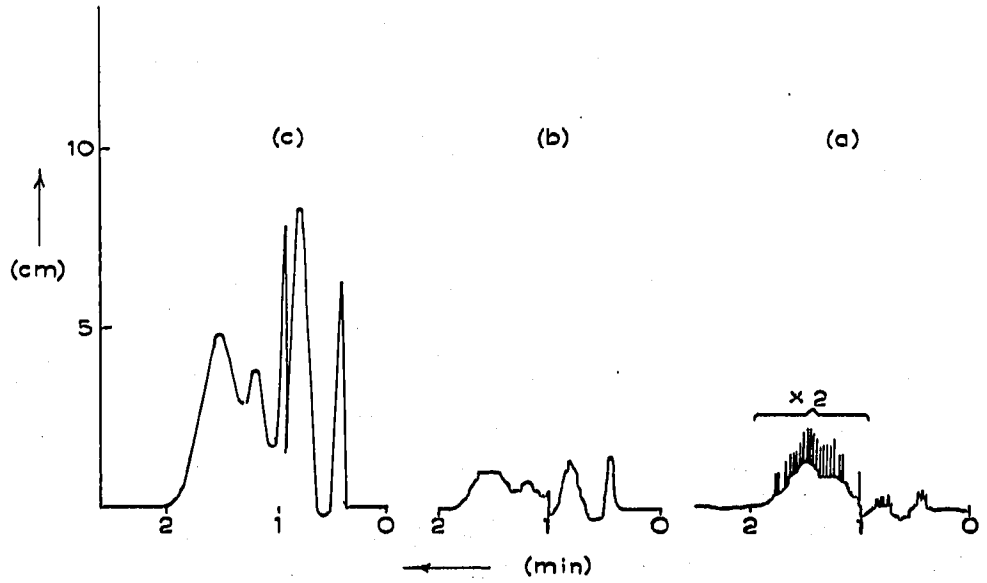

Fig. 2. Chromatograms of same sample injected at 3 mm Hg pressure using an aged column and compressed air pressures of (a) 10 p.s.i., (b) 12 p.s.i., and (c) 20 p.s.i.

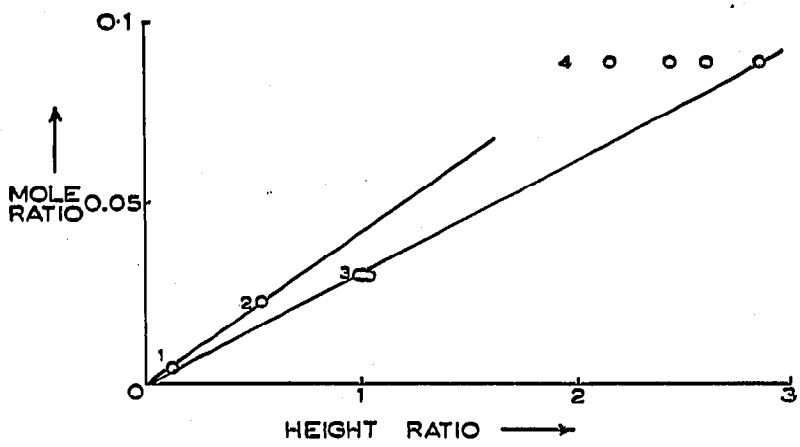

Fig. 3. Calibration curve for ethane–propane. Points 1 and 2 (averages of several runs) plotted on one day. Points 3 and 4 (individual values for each run shown) plotted on another day.

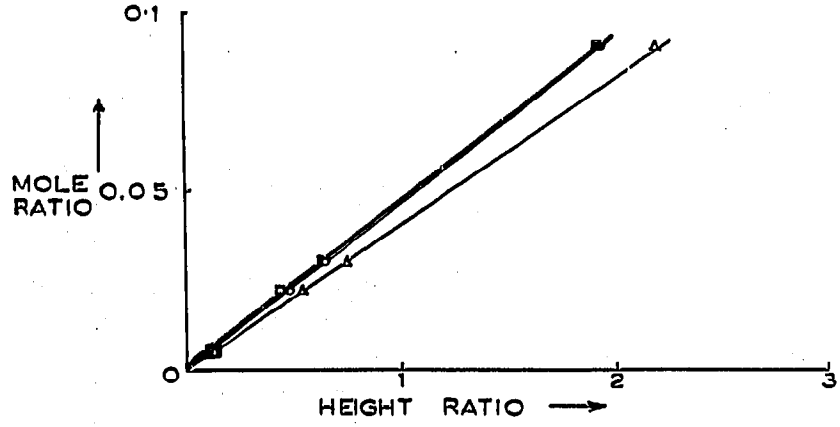

Fig. 4. Calibration graphs for ethane–propane at (△) 20 p.s.i., (□) 30 p.s.i., and (○) 40 p.s.i. compressed air.

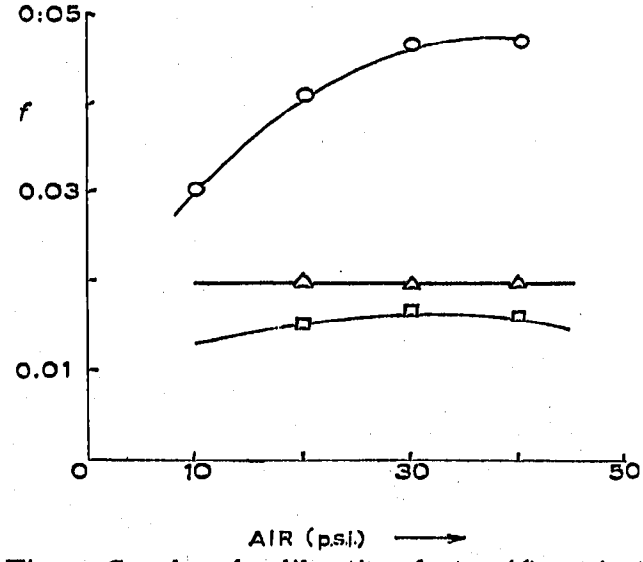

Fig. 5. Graphs of calibration factor (f) against compressed air pressure for (○) ethane–propane, (△) isobutane–propane, and (□) propylene–propane.

setting was irreproducible from day to day and that there was considerable irreproducibility of peak height ratios for any one sample.

The behaviour of the original column at compressed air pressures of 20 to 40 p.s.i. was therefore examined and specimen calibration graphs for ethane–propane are shown in Fig. 4. As shown in Figs. 2a, 2b and 2c peak shape and sensitivity of the detector improve as the compressed air pressure is increased. Each calibration graph of Fig. 4 was reproduced over a two-day period and it can be seen that linearity and reproducibility of analyses are good. No variation of calibration factor with total sample pressure as has been reported for 1,4-dimethylcyclobutene and *trans*-3-methylpenta-1,3-diene[8] was observed. Fig. 5 shows that the sensitivity towards propane increases more quickly than that towards ethane with increasing compressed air pressure. The sensitivity for the other gases remains essentially constant, except that it is lower by a factor of about ten at 10 p.s.i. than at 20 p.s.i. This could be because air starvation of the flame will produce considerably more artificial peak attenuation of compounds with a high carbon content than for low molecular weight compounds such as ethane. That the shape of the curves in Fig. 5 is not a result of decrease in absolute sensitivity with increasing compressed air pressure is evident from the curves in Fig. 6 which show, in agreement with KAISER[2] that sensitivity increases with increasing compressed air pressure.

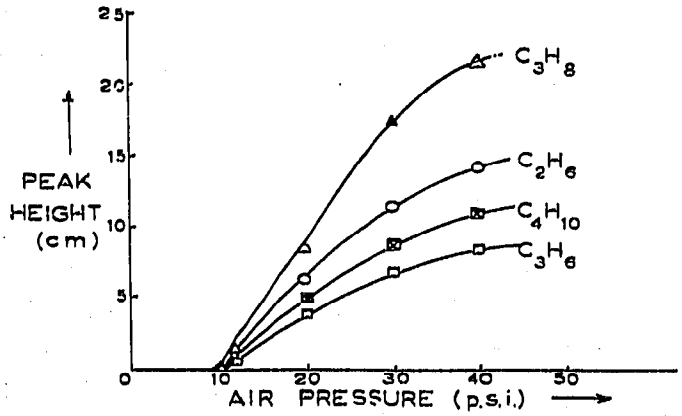

Fig. 6. Same sample at 3 mm Hg injected at various compressed air pressures.

From the above results it would seem that, in a system using hydrogen as carrier gas a fraction of which is burned in a flame ionisation detector, an extremely high flow rate of compressed air is necessary for flame stability, optimum sensitivity and reproducibility of analyses. Irreproducible results can arise through shortage of compressed air even if this does not always give bad peak shapes. High molecular weight hydrocarbons are more susceptible to air shortage and this may explain the variation of calibration factor with sample pressure as reported by FREY *et al.*[8]. Optimum sensitivity is obtained at different pressures for different compounds as can be seen from the different gradients of the curves of Fig. 6. There would appear to be five distinct phases in the response of a flame ionisation detector to increase compressed air flow rates, *viz.*

(1) erratic behaviour (Fig. 2a);
(2) peak flattening (Fig. 2b);
(3) "peak inversion" (Fig. 1b) which can be cured by increased air flow rate;

(4) good peak shape but giving irreproducibility of analyses from day to day due to detector response being excessively critical to the G.L.C. setting;

(5) good peak shape, reproducibility and high sensitivity at high air flow rate—obtained at the expense of slightly increased baseline noise and background current.

FRANK R. CRUICKSHANK gratefully acknowledges the receipt of a Carnegie Scholarship from the Carnegie Trust for the Universities of Scotland.

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Received August 19th, 1965

J. Chromatog., 21 (1966) 296–300

Gas chromatography of hexosaminitols

The cleavage of oligosaccharide side chains from the protein cores of several glycoproteins has been accomplished by the use of alkaline solutions containing sodium borohydride[1–3]. Under these conditions, the sugar linked to the amino acid is reduced to the corresponding sugar alcohol. Hydrolysates of the cleaved reduced oligosaccharides may contain reducing sugars, sugar alcohols, or amino sugar alcohols. PERRY[4] reported that amino sugars can be separated from an amino sugar alcohol, and SWEELEY *et al.*[5] have shown that amino sugars can be separated from reducing sugars and reducing sugar alcohols by gas–liquid chromatography of their trimethylsilyl (TMS) derivatives; however, resolution of the amino sugar alcohols was not achieved. This report will show that the TMS derivatives of 2-acetamido-2-deoxy-D-glucitol and 2-acetamido-2-deoxy-D-galactitol can be resolved from one another and from a mixture of reducing sugars, sugar alcohols and, amino sugars.

L-Fucose, L-fucitol, D-galactose, D-galactitol, 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-galactose were obtained from Mann Research Laboratories, New York City, and were used without further purification. 2-Acetamido-2-deoxy-D-glucitol was prepared as described by CRIMMINS[6]; 2-acetamido-2-deoxy-D-galactitol was prepared from 2-amino-2-deoxy-D-galactose by reduction with sodium borohydride. The borohydride was decomposed with mineral acid, and the borate