A gas chromatographic method for the simultaneous analysis of halothane and nitrous oxide in operating theatre atmospheres has been developed and evaluated. The flame ionization detector is suitable for the quantitative analysis of halothane in concentrations approaching one part per million. The frequency-modulated electron capture detector is highly sensitive to nitrous oxide but we have found it to be non-linear over the range 25–1000 p.p.m. The overall reproducibility of the gas chromatographic method based on the dynamic technique of standard preparation is approximately 4%. Effective exposure of personnel to pollutant anaesthetics is assessed by the analysis of end-expired gas.

For the study of pollution of the operating theatre and its control, an accurate and reproducible method would be of value for the simultaneous measurement of nitrous oxide and volatile anaesthetics such as halothane (Robinson et al., 1976). This paper describes the development of such a method based on the technique of gas chromatography.

MATERIALS AND METHODS

Gas chromatography was performed using a Pye Series 104 gas chromatograph fitted with a heated head containing flame ionization and nickel-63 electron capture detectors. The entire chromatograph together with accompanying electronics, a two-channel potentiometric recorder and size “F” gas cylinders (B.O.C. special gases, Morden) were placed on a trolley designed for mobile use within the operating theatre environment. The dual glass columns used were deactivated with dimethylchlorosilane (B.D.H. Ltd, Poole) according to the method of Eik-Nes and Horning (1968). The column (172 cm × 0.4 cm i.d.) used for analysis of halothane was packed with 15% F.F.A.P. coated on acid-washed silanized diatomite “C” as described by Cole, Salamonsen and Fish (1975). For nitrous oxide analyses, a column (213 cm × 0.4 cm i.d.) was packed with carbosieve B, 120–140 mesh and conditioned for 48 h before use. Twin gas switching valves with sample loops (1 ml for halothane and 0.5 ml for nitrous oxide) were used for the introduction of gas samples into the column: these were kept exclusively for measurements of trace concentrations of the anaesthetics since the internal “O” rings were contaminated easily if exposed to higher concentrations of these agents. Halothane was detected routinely by flame ionization and nitrous oxide was measured using an electron capture detector (Pye Unicam) operating in the recently introduced frequency modulated mode. Nitrogen (“white spot”—B.O.C. Worsley) purified by passage through a molecular sieve was used as carrier gas for both columns and as a “purge” gas for the electron-capture detector. The flame ionization detector was serviced with hydrogen at a flow of 50 ml min⁻¹ and air at 500 ml min⁻¹. Gas samples were introduced consecutively from the same syringe into the gas sampling valves. Recordings were made simultaneously on the two-pen recorder with lead connections made so that the chromatograms of both anaesthetics could be displayed on the same paper record without confusion.

The best compromise of sensitivity, accuracy and convenience was determined for dual column and detector operation. The detector oven temperature was heated stepwise from 50 to 350 °C. Similarly, the column oven temperature was varied in steps between limits of 100 and 200 °C and carrier gas flows were varied at each temperature. Steady states of both temperature and gas flow were achieved before measurements were made.

All standard gas mixtures were prepared in a laboratory where the temperature was controlled to within 22 ±1 °C and the relative humidity and barometric pressure were known. Two methods of preparation were used:

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(1) Dynamic methods. Measured volumes of either halothane or nitrous oxide were mixed with high flows of room air using the apparatus illustrated in figure 1. The flow of room air, produced by a four-stage rotary turbo-blower (Utile Engineering Ltd, Wellingborough), was measured by a series 2000 flow meter (Elliot Process Instruments, Croydon) mounted on the inlet side of the flow generator. The flow rate was controlled by a Saunders valve placed between the flow meter and the turbo-blower to ensure that the flow meter inlet remained at atmospheric pressure irrespective of flow rates. A 24 × glass tube and Duralium “K” float were selected to register flow rates between 100 and 400 litre min⁻¹. The air emerging from the turbo-blower was passed first through a coiled copper tube and a large chamber, both of which were mounted in a thermostatically controlled water bath, and then through wide-bore polypropylene tubing to the mixing chamber. After leaks were eliminated, preliminary testing with a dry gas meter (Parkinson and Cowan Ltd, Manchester) indicated that the apparatus delivered a room air flow in the vicinity of 22 °C accurately and reproducibly.

Nitrous oxide (B.O.C. medical grade, containing a maximum of 200 p.p.m. contaminating oxygen) was fed from the cylinder via a reducing valve (delivery pressure 42 kPa) to a series 1100 flow meter (Elliot Process Instruments, Croydon) containing a 1-A-150 tube and ruby ball float which had been calibrated for nitrous oxide using a bubble flow meter. The flow rate was controlled between 9 and 140 ml min⁻¹ by adjustment of the flow meter needle-valve. The flow meter outlet was connected to a three-way tap directing nitrous oxide either into the mixing chamber or through a separate duct to the gas exhaust flue of the laboratory. At high flow rates of air the increased mixing chamber pressure reduced the apparent flow meter reading. Consequently the flow meter was set before switching to the mixing chamber and any fluctuation was ignored during sampling. After returning the nitrous oxide flow to the drainage flue the flow meter reading was re-checked, and if different from the original value the sample was rejected.

A mixture of halothane in air was prepared by passing medical air (B.O.C., Worsley) through a Dräger vaporizer calibrated under operating conditions using a Rayleigh interferometer (Carl Zeiss, Jena). The vapour mixture was connected via a three-way tap to the Series 1100 flow meter calibrated for air using a bubble flow meter. At the extremely low flow rates used, the calibration scale provided with the vaporizer was inappropriate. However, it was found that after a period of stabilization (about 20 min), the concentrations delivered by the vaporizer were reproducible provided that flow and pressure remained approximately constant. Accordingly, medical air at 138 kPa was passed first through a needle-valve adjusted to give a flow rate of 90–95 ml min⁻¹, and then through the vaporizer to the flow meter where fine adjustment of the flow to 87 ml min⁻¹ was made. Under these conditions the vaporizer produced 0.8% at the 1% setting and 2.35% at the 3.0% setting. By adjustment of the room air flow through the mixing chamber, all halothane in air standards were produced with the vaporizer at these two settings.

The gas mixture was passed from the mixing chamber to the laboratory drainage flue. The first few feet of ducting contained a loose copper mesh (to ensure complete mixing) followed by a sampling port through which the samples could be aspirated into 50-ml glass syringes fitted with polypropylene stopcocks.

(2) Static methods. A single "concentrate" gas mixture containing either nitrous oxide 1000 p.p.m. or halothane 100 p.p.m. in air was prepared using the dynamic method and checked with a Rayleigh interferometer containing a 1-m cell before storage in a gas-tight 200-ml glass syringe fitted with a polypropylene stopcock. Each standard mixture was prepared separately from an aliquot of this concentrate to avoid the error propagation inherent in serial dilution techniques.
EVALUATION OF THE METHOD

All studies on the method itself were conducted using the gas chromatograph conditions found to be optimum for this application.

Linearity of the detector response. Series of standards prepared by both the static and dynamic methods were used, each individual standard being injected into the chromatograph immediately after preparation to avoid any loss of anaesthetic during storage in the glass syringe. Since good resolution of both nitrous oxide and halothane gas chromatographic peaks was obtained, peak heights were used as the criterion of the response of the detector.

Reproducibility. The study of the reproducibility of the method was conducted in three stages. In each stage a number of injection sequences, each consisting of 10 injections, were carried out for both nitrous oxide and halothane standards. The inherent variability in response of the gas chromatograph was assessed first using a single calibrating standard. Then the cumulative variability of chromatograph response and standard preparation using the dynamic method was assessed. Each standard sample was prepared separately, commencing with the control valve of the flow generator closed and the turbo-blower turned off. Finally, variability of the chromatograph response together with standard preparation using the static method, was measured using standards prepared separately by 1:10 syringe dilutions of freshly prepared concentrate.

Sensitivity. Sensitivity studies were conducted on the flame ionization detector for halothane and on the electron capture detector for nitrous oxide and halothane. Samples of very low anaesthetic concentrations were prepared using syringe dilution as outlined previously.

Operating room studies. Pilot studies were conducted to test the application of the method for analysis of nitrous oxide and halothane concentrations in operating room air and in end-expired gas of the anaesthetist. The gas chromatograph complete with gas cylinders and recorder was wheeled on the mobile trolley to the anaesthetic room adjoining the operating theatre late in the day before the studies. The machine was allowed to stabilize overnight. Theatre air within the vicinity of the anaesthetist’s face, and end-expired gas were aspirated into thoroughly cleaned ground glass syringes and analysed within 10 min. The chromatograph was calibrated either immediately before or after sample analysis using freshly prepared standards obtained by the dynamic method. The anaesthetic concentrations were calculated with reference to the calibration curves reported in this paper.

One of these studies was selected for detailed presentation. Halothane and nitrous oxide were administered in a well-ventilated operating theatre...
Initially through a Magill circuit, but subsequently through a circle absorber system. The change in circuit was made to determine if the high anaesthetic concentration in the region of the anaesthetist's face could be reduced by the simple expedient of using another circuit containing the expiratory valve further away from the anaesthetist.

**RESULTS AND DISCUSSION**

**Detector temperature.** The sensitivity of the electron capture detector (e.c.d.) depends largely on temperature. Table I shows the effect of detector temperature on the measurement of nitrous oxide.

<table>
<thead>
<tr>
<th>Detector temperature (°C)</th>
<th>Relative peak height (%)</th>
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<tbody>
<tr>
<td>50</td>
<td>0.9</td>
</tr>
<tr>
<td>150</td>
<td>1.45</td>
</tr>
<tr>
<td>250</td>
<td>16.5</td>
</tr>
<tr>
<td>350</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Increasing the temperature from 50 to 350 °C caused a thousand-fold increase in sensitivity. This temperature dependence of e.c.d. sensitivity to nitrous oxide was reported also by Wentworth and Freeman (1973) and may be explained by easier attachment of low energy electrons to the nitrous oxide molecule when its normal geometric arrangement is distorted by thermal agitation (Wentworth, Chen and Freeman, 1971). The detector oven temperature was set at 300 °C for routine use since at this lower temperature the nickel isotope is more stable and detector sensitivity, being 40% of its value at 350 °C, was adequate for the application.

**Column oven temperature and carrier gas flows.** The conditions selected to give the best compromise between convenience and quality of the chromatogram were a column oven temperature of 125 °C and carrier gas flows of 60 ml min⁻¹ for nitrous oxide and 40 ml min⁻¹ for halothane. The short retention time of 1.6 min for halothane allowed a second analysis to be made at a more suitable flame ionization detector (f.i.d.) attenuator setting within the time of one nitrous oxide analysis if the first halothane peak was off scale or inconveniently small (fig. 2). In practice the e.c.d. attenuator needed little adjustment. At the column temperature used, the carbosieve B stationary phase simplified analysis of nitrous oxide as it trapped halothane contaminants completely in the sample, thus preventing their access to the detector.

**Linearity of response.** The calibration curve for halothane (fig. 3) indicates a linear response over the range 5–100 p.p.m. (v/v) of f.i.d. operating with a negative jet polarizing voltage. The peak height response of the detector to the same standards was slightly less (about 4.8%) with the polarity reversed, which is in agreement with the predictions of McWilliam (1961). However, the difference is functionally insignificant and since both responses were linear it was concluded that detector polarity is unimportant for analysis of halothane in the low p.p.m. range. The linear response allows quantitative analysis involving the use of only one calibrating gas standard.

In figures 3 and 4 the peak heights are expressed as percentages of the peak height of the most concentrated standard (100 p.p.m. for halothane and 1000 p.p.m. for nitrous oxide) to facilitate precise comparison of standards prepared by static and dynamic methods. The good fit of the points to the curve in figure 3 suggests that both methods of preparation were satisfactory. Equally good agreement between the two methods of preparation is seen for nitrous oxide (fig. 4) but the curve suggests that the response of e.c.d. is not linear despite its frequency.
SIMULTANEOUS ANALYSIS OF N₂O AND HALOTHANE

modulated mode of operation. This does not compare well with the response of an identical detector system for dieldrin reported originally by Maggs and others (1971). When a regression analysis was conducted on the data of figure 4, the correlation coefficient of log peak height vs. log sample size (mole) was 0.9989 over a range of 40–1, compared with 0.9997 for log peak area vs. log sample size over a range of $5 \times 10^4$ to 1. However, other workers have since reported linearity, albeit different in pattern for a number of substances (Sullivan and Burgett, 1975). In practical terms this means that, for precise quantitative analysis, results must be obtained by reading them from the standard curve.

Sensitivity. This study indicates that f.i.d. is quantitative down to 1 p.p.m. of halothane. E.c.d. at 300 °C is highly sensitive to nitrous oxide. Its quantitative limit for this gas was not determined since standards below 0.5 p.p.m. could not be prepared accurately. Although Wentworth (1973) suggests that it can be used to measure nitrous oxide in concentrations approaching the parts per billion range, this sensitivity is not required since normal atmospheric concentrations of nitrous oxide vary from 0.2 to 0.6 p.p.m. (Israel, 1967). For precise studies of concentrations of halothane less than 1 p.p.m. the greater sensitivity of the e.c.d. would be required.

Reproducibility. Reproducibility of the method for halothane is shown in table II. The coefficient of variation of gas chromatography alone was less than 1%, indicating the accuracy of the gas sampling valve. When separate standards were prepared for each analysis, an increase in the coefficient of variation was observed although the dynamic method appeared to be the more precise of the two. With nitrous oxide (table III) a similar increase in variation was observed when each standard was prepared separately, but here the precision of the two methods was comparable. Inferior performance of the dynamic method for nitrous oxide is possibly a result of the lower driving pressure of the nitrous oxide supply which could make it more susceptible to turbulence in the mixing chamber. Despite the low variance attributable solely to gas chromatography, the routine application of the method is affected by inaccuracies in standard preparation and hence figures for gas chromatography plus the dynamic method of standard preparation are perhaps the most significant.

Operating room studies. Figure 5 shows the results of a theatre study in which halothane and nitrous oxide concentrations in the vicinity of the anaesthetist’s face were compared with values in the

<table>
<thead>
<tr>
<th>Set standard concentration (p.p.m., v/v)</th>
<th>Coefficient of variation (n = 10)</th>
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<tbody>
<tr>
<td></td>
<td>G.C. alone (%)</td>
</tr>
<tr>
<td>100</td>
<td>0.56</td>
</tr>
<tr>
<td>50</td>
<td>0.28</td>
</tr>
<tr>
<td>10</td>
<td>0.25</td>
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G.C. = gas chromatograph; D = dynamic method of standard preparation; S = static method of standard preparation.

TABLE III. Reproducibility of the method for nitrous oxide. (The figures for variance of the gas chromatograph alone were taken from analyses of a single standard. Otherwise a separate standard was prepared for each analysis)

<table>
<thead>
<tr>
<th>Set standard concentration (p.p.m., v/v)</th>
<th>Coefficient of variation (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G.C. alone (%)</td>
</tr>
<tr>
<td>1000</td>
<td>0.71</td>
</tr>
<tr>
<td>100</td>
<td>0.71</td>
</tr>
<tr>
<td>25</td>
<td>0.6</td>
</tr>
</tbody>
</table>

G.C. = gas chromatograph; D = dynamic method of standard preparation; S = static method of standard preparation.

Fig. 4. Calibration curve of electron capture detector for nitrous oxide. O—O₂ standards prepared by dynamic method: •—• standards prepared by static method.
Fig. 5. Halothane and nitrous oxide concentrations in a well-ventilated operating theatre. \(\text{o}--\text{o}\), sampled from within vicinity of anaesthetist's face; \(\bullet--\bullet\), sampled from end-expired gas of the anaesthetist. The arrow indicates the point at which a circle absorber system was substituted for the Magill circuit without adjustment of fresh gas flow.

The availability of the gas chromatograph for on-site analysis largely removes errors resulting from loss of halothane and nitrous oxide from samples during storage. A loss of up to 6% halothane from the glass syringes used for this study has been detected within 3 h although no significant loss of nitrous oxide was detected within a similar period.

ACKNOWLEDGEMENTS

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REFERENCES


de l'halothane en concentrations d'environ 1 p.p.m. Le détecteur de capture d'électrons à modulation de fréquence est fortement sensible au protoxyde d'azote, mais nous avons trouvé qu'il est non linéaire dans la plage de 25–1000 p.p.m. La reproductibilité d'ensemble de la méthode par chromatographie en phase gazeuse basée sur la technique dynamique de la préparation standard est d'environ 4%. L'exposition effective du personnel aux agents anesthésiques polluants est évaluée par l'analyse du gaz d'expiration résiduel.

ANALISIS SIMULTANEO DE TRAZAS DE OXIDO NITROSO Y HALOTANO EN EL AIRE

Se ha desarrollado y evaluado un método cromatográfico de gas para el análisis simultáneo de halotano y óxido nitroso en la atmósfera de salas de operaciones. El detector por ionización de llama es apropiado para el análisis cuantitativo en concentraciones que se aproximan a una parte por millón. El detector de captura de electrones modulado por frecuencia es sumamente sensitivo al óxido nitroso pero lo encontramos no-lineal en el ámbito de 25–1000 p.p.m. La reproducibilidad del método cromatográfico de gas basado en la técnica dinámica de preparación normalizada es de aproximadamente 4%. La exposición efectiva del personal a las anestesias contaminantes se evalúa mediante el análisis del gas final expirado.