GAS CHROMATOGRAPHIC DETERMINATION OF OSTWALD SOLUBILITY COEFFICIENTS FOR CYCLOPROPANE, HALOTHANE AND TRICHLOROETHENE (TRICHLOROETHYLENE)

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SUMMARY

Gas chromatographic methods using solvent extraction for the analysis of cyclopropane and tri-chloroethene (trichloroethylene) are described and evaluated; cyclopropane was extracted into carbon tetrachloride and trichloroethene into carbon disulphide, using chloroform and toluene respectively as the internal standards. Ostwald solubility coefficients were measured for cyclopropane, halothane and trichloroethene in Krebs solution: at 310 K the respective values $\pm$ SEM of the Ostwald coefficients are 0.181 ± 0.009, 0.78 ± 0.02 and 1.54 ± 0.02; over the temperature range 295–310 K the respective temperature coefficients of solubility are -2.27, -4.18 and -3.81 in units of per cent/K at 310 K.

Krebs solution is used widely in pharmacological studies, but little information is available in the literature on concentrations of anaesthetic agents and solubility coefficients in this medium. The present work is part of an investigation into the effects of anaesthetics on neuromuscular transmission *in vitro* and provides the quantitative chemical information necessary to establish the concentration of anaesthetic acting on isolated tissues.

There have been a number of reports of gas chromatographic analysis of inhalation anaesthetic agents in blood and other biological media using equilibration techniques (Yamamura et al., 1966; Fink and Morikawa, 1970), direct injection of blood samples (Lowe, 1964; Cole, Salamonsen and Fish, 1975) and solvent extraction methods (Wolfson, Ciccarelli and Siker, 1966; Wortley et al., 1968; Douglas, Hill and Wood, 1970; Allott, Steward and Mapleson, 1971). Solvent extraction provides one of the most accurate and reproducible methods for determining drug concentrations and is the method of choice in the present work.

METHODS AND MATERIALS

Halothane and trichloroethene (trichloroethylene) were obtained from I.C.I. Pharmaceuticals Ltd; toluene and carbon disulphide were "Gold Label" reagents from Aldrich Chemicals Ltd; carbon tetrachloride and chloroform were Analar reagents from British Drug Houses Ltd. Krebs solution of the

following composition was used throughout the work: NaCl, 118 mmol/litre; $NaHCO_3$, 25 mmol/litre; KCl, 4.7 mmol/litre; KH_2PO_4, 1.2 mmol/litre; $MgSO_4$ $7H_2O$, 1.2 mmol/litre; $CaCl_2 2H_2O$, 2.5 mmol/litre; glucose, 11 mmol/litre. The solution was aerated before use with 5% carbon dioxide in oxygen and had a pH of 7.45 (± 0.05) units.

Measurement of Ostwald solubility coefficients

A constant flow (0.3 litre/min) of known concentrations of anaesthetic vapour in 5% carbon dioxide in oxygen was bubbled through fresh Krebs solution (20 ml), allowing 45 min for equilibration. Anaesthetic concentrations in the gas mixture were in the range 10–50% v/v for cyclopropane; 1–3% v/v for halothane; 0.5–2% v/v for trichloroethene. A Riken 18A refractometer gave accurate estimations of gaseous concentrations of halothane and trichloroethene; gaseous cyclopropane concentrations were estimated using a Servomex Oxygen Analyser (Type OA 272), allowing for the carbon dioxide which was also present in the gas phase. Ostwald solubility coefficients were measured for cyclopropane, halothane and trichloroethene in the temperature range 295–310 K. Four samples of Krebs solution at each concentration were analysed in duplicate by gas chromatography. Halothane was estimated chromatographically using the method described by Allott, Steward and Mapleson (1971).

Chromatographic analysis of trichloroethene

Carbon disulphide was a suitable solvent for tri-chloroethene extraction from aqueous solutions having a high extractive efficiency and being easily

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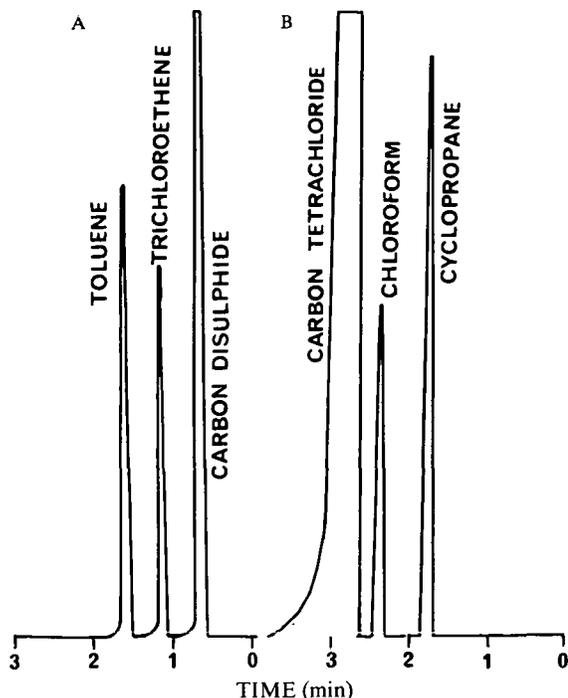
FIG. 1. Chromatograms of A: trichloroethene; B: cyclo-propane.

separated chromatographically from trichloroethene. More common solvents either contained impurities which interfered in the analysis or were not fully resolved from trichloroethene in the concentration range of interest. Precautions were taken to avoid exposure to carbon disulphide fumes; solutions containing carbon disulphide were stored and handled in a fume cupboard and distilled water was added to each solution, forming a protective surface layer which reduced evaporation to a minimum. An internal standard, toluene, was added to the extracting solvent and trichloroethene concentrations were determined from the ratio of trichloroethene to toluene peak heights.

Carbon disulphide 0.5 ml was placed in a 5-ml glass tube containing distilled water 1 ml; Krebs solution 1 ml containing the unknown concentration of trichloroethene was added; after roller mixing for 30 min 0.7 μlitre of the lower layer was injected into the chromatograph. Figure 1A shows a typical chromatogram. Calibration for trichloroethene was carried out daily by injecting a series of standard solutions in the concentration range 0.4–3.0 mmol/litre prepared by dilution of a stock 8.0-mmol/litre solution

of trichloroethene in carbon disulphide. Figure 2 shows the linear response of the chromatograph to these solutions.

Chromatographic analysis of cyclopropane

Cyclopropane was extracted from Krebs solution into carbon tetrachloride containing chloroform as the internal standard in a manner similar to that described by Allott, Steward and Mapleson (1971) for the extraction of halothane from biological media. Figure 1B shows a typical chromatogram. The chromatograph was calibrated daily using standard solutions in the concentration range 0.6–8.0 mmol/litre prepared by dilution of a stock 30-mmol/litre solution of cyclopropane in carbon tetrachloride. All cyclopropane solutions were protected by a layer of distilled water. The response of the detector to these solutions is shown in figure 2.

Stock cyclopropane solutions were prepared using the gas-handling system shown schematically in figure 3. The system was evacuated to a residual pressure of ~ 15 Pa; cyclopropane ~ 60 kPa was allowed into the system, purified by vacuum distillation at 77 K and a known pressure was expanded into the vessel, V. A flow of nitrogen swept any remaining cyclopropane out of trap, T, and to vent through a sulphuric acid trap which removed cyclopropane from the gas flow (Linde and Price, 1958). The vessel was removed from the gas-line, weighed, opened to a carbon tetrachloride reservoir and filled. The concentration of this stock solution was obtained directly from weighings and by calculation knowing the pressure of cyclopropane inside the vessel.
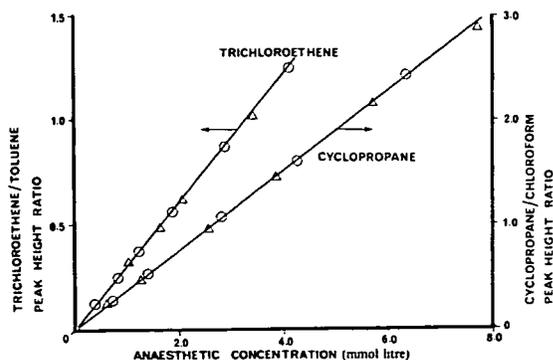

FIG. 2. Linear FID response to increasing concentrations of cyclopropane and trichloroethene; O: standards prepared from fresh stock solution; $\triangle$: standards prepared from stock solution 2 months old.
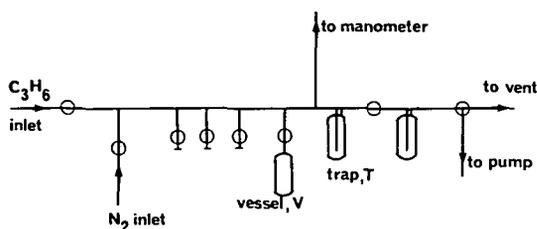
FIG. 3. Schematic illustration of the gas-handling system.

Chromatographic details

Cyclopropane, halothane and trichloroethene analyses were carried out with a Pye 104 Chromatograph, equipped with a flame ionization detector and potentiometric recorder, using a glass column (3 m × 4 mm) packed with 5% OV-101 on 100/120 mesh Chromosorb W and operated at column temperature, 353 K; detector temperature 473 K; injection port temperature, 423 K; carrier gas (nitrogen) flow, 50 ml/min; hydrogen flow, 50 ml/min; and air flow, 500 ml/min.

Evaluation of methodology

A standard solution was allowed to age and was sampled daily to determine the peak height ratio, mean ± SEM ($n = 6$). In the same trial, a fresh standard solution was prepared daily and its peak height ratio, mean ± SEM ($n = 6$), was determined also. The sets of ratios obtained from the old and new solutions on any given day were then compared using an unpaired t test and the trial was continued until there was a significant difference in the peak height ratios at the 0.05 level of significance. In a typical trial a 3-day-old, 1.42-mmol/litre cyclopropane standard solution with a protective water layer, peak height ratio 0.541 ± 0.004, was significantly less than a similar fresh solution, peak height ratio 0.595 ± 0.007. Similar trials were performed with extracted samples.

RESULTS AND DISCUSSION

Fresh trichloroethene standards prepared from the same stock solution over a period of 3 months showed no deterioration, but any set of standards which was allowed to age showed significant deterioration after 4 weeks. In practice new standards were prepared fortnightly and stock solutions were renewed every 2 months. Extracted samples were stable for at least 1 week and in practice were analysed within 24 h of extraction.

Figure 4 shows the decay characteristics of a 2.64-mmol/litre cyclopropane standard solution. With no water layer present the rate of decay was very rapid and even with a protective water layer significant
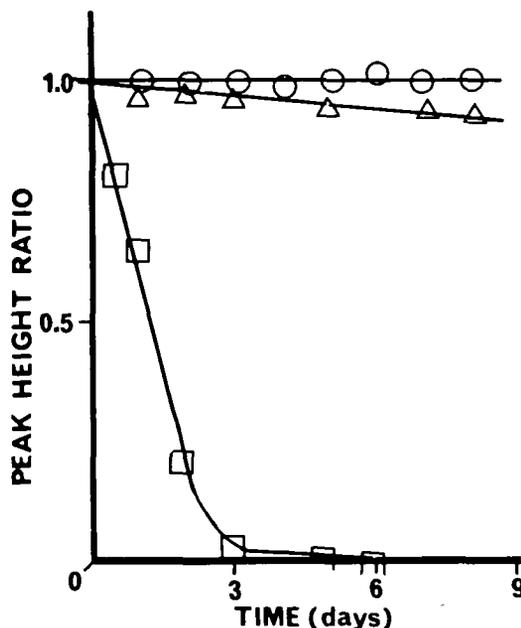

FIG. 4. Decay characteristics of a standard cyclopropane solution (2.64 mmol/litre) fresh solution (O) compared with ageing solution with (△) and without (□) a protective water layer.

differences in peak height ratios were found after 3 days. Therefore fresh standard solutions were prepared daily. The rate of decay of the stock cyclopropane solution was very much slower, enabling the same stock solution to be used over a period of 2 months without a significant deterioration. Extracted samples deteriorated also after a few days and were analysed within 24 h of extraction.

Aqueous solutions of known anaesthetic concentration were prepared and analysed in order to determine the efficiency of the extraction procedure. The mean values (± SEM) of extractive efficiency were 94.5 ± 0.3% for trichloroethene and 98.0 ± 0.4% for cyclopropane. When six samples of Krebs solution, each equilibrated with 2.2% v/v trichloroethene at 310 K, were extracted the mean trichloroethene concentration (± SEM) was 2.249 ± 0.014 mmol/litre, representing 95% confidence limits of 3.0%.

The procedures described here have been in routine use in our laboratories for a period of 12 months and

TABLE I. *Effect of temperature on Ostwald solubility coefficients* ($\lambda \pm$ SEM) *of cyclopropane, halothane and trichloroethene in Krebs solution*

Cyclopropane		Halothane		Trichloroethene	
Temp. (K)	$\lambda \pm$ SEM	Temp. (K)	$\lambda \pm$ SEM	Temp. (K)	$\lambda \pm$ SEM
297.6	0.237 ± 0.009	298.1	1.30 ± 0.04	300.5	2.23 ± 0.03
301.6	0.212 ± 0.014	301.6	1.15 ± 0.01	302.5	2.08 ± 0.05
304.8	0.197 ± 0.013	304.6	0.97 ± 0.01	305.2	1.96 ± 0.04
310.0	0.181 ± 0.009	310.5	0.78 ± 0.02	310.0	1.54 ± 0.05

have provided reliable and reproducible results during that time. The trichloroethene analysis, although less rapid than the method described by Cole, Salamonsen and Fish (1975), does not rely on aqueous trichloroethene standard solutions which in our experience require lengthy preparation and are subject to loss of anaesthetic vapour to the atmosphere. A feature of the cyclopropane analysis described here is that gaseous cyclopropane, which is explosive in air, is handled only when preparing stock solutions and is contained in a vacuum system from which air is excluded. This is in contrast to some previous methods which require the handling of gas standards and samples (Cowles, Borgstedt and Gillies, 1971). The results presented here refer only to analyses of Krebs solution, but preliminary experiments indicate that these methods can be successfully used in the determination of blood and tissue concentrations of these anaesthetics.

Table I gives the Ostwald solubility coefficients of cyclopropane, halothane and trichloroethene at various temperatures, the number of determinations being six in each case. The performance of the apparatus used in the determination of solubility coefficients was tested by measuring the solubility of these anaesthetics in water at 310 K: values ($\pm$ SEM) of 0.212 ± 0.024, 0.80 ± 0.01 and 1.64 ± 0.02 were found for cyclopropane, halothane and trichloroethene respectively, the number of determinations being 5 in each case. These results compare favourably with the preferred values of 0.21, 0.80 and 1.7 respectively quoted by Steward and others (1973). Those authors also reported that at 310 K the solubility coefficients of most anaesthetic gases in 0.9% saline were approximately 94% that in pure water. Our results show a similar relationship between solubility coefficients in Krebs solution and in pure water; the former ($\pm$ SEM) being 87 ± 0.6, 93.0 ± 0.4 and $96.0 \pm 0.4\%$ of the latter for cyclopropane, halothane and trichloroethene, respectively.

Allott and others (1973) reviewed the literature on the temperature-dependence of solubility of inhaled anaesthetics in various biological media and calculated temperature coefficients for several anaesthetics. We have analysed our data for anaesthetic solubility in Krebs solution by the method suggested by Hildebrand, Prausnitz and Scott (1970) and find temperature coefficients (per cent/K at 310 K) of -2.27, -4.18 and -3.81 for cyclopropane, halothane and trichloroethene respectively, compared with the mean values of -2.11, -4.01 and -3.94 respectively reported by Allott and others (1973). Differences between the mean values, which include data from several different aqueous media, and the Krebs only values are to be expected, but the results are in general agreement, showing that the effect of temperature on anaesthetic solubility in Krebs solution parallels that in other aqueous media.

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DETERMINATION PAR LA CHROMATOGRAPHIE EN PHASE GAZEUSE DES COEFFICIENTS DE SOLUBILITE D'OSTWALD POUR LE CYCLO-PROPANE, L'HALOTHANE ET LE TRICHLORO-ETHENE (TRICHLOROETHYLENE)

RESUME

On décrit et évalue dans cet article des méthodes d'analyse par chromatographie en phase gazeuse, et par extraction de solvants, du cyclopropane et du trichloroéthène (trichloro-éthylène). Le cyclopropane a été extrait à l'aide de tétra-chlorure de carbone et le trichloroéthène à l'aide de bisulfure de carbone, en se servant respectivement de chloroforme et de toluène comme contrôle interne. Les coefficients de solubilité d'Ostwald ont été mesurés pour le cyclopropane, l'halothane et le trichloroéthène dans une solution de Krebs: à 310 K les valeurs respectives, ± erreur type des moyennes, des coefficients d'Ostwald sont 0,181 ± 0,009, 0,78 ± 0,02 et 1,54 ± 0,02; sur une plage de températures de 295–310 K, les coefficients de température respectifs de solubilité sont: −2,27, −4,18 et −3,81 en unités de pourcentage de K à 310 K.

GASCHROMATISCHE BESTIMMUNG DER OSTWALD-LÖSLICHKEITSKOEFFIZIENTEN FÜR CYCLOPROPAN, HALOTHAN UND TRICHLOROETHEN (TRICHLORATHYLEN)

ZUSAMMENFASSUNG

Gaschromatische Methoden unter Verwendung von Lösungsextraktionen für die Analyse von Cyclopropan und Trichloroethen (Trichloräthylen) werden beschrieben und bewertet; Cyclopropan wurde in Tetrachlorkohlenstoff und und Trichloroethen in Schwefelkohlenstoff extrahiert, wobei jeweils Chloroform und Toluen als interne Norm verwendet wurden. Die Ostwald-Löslichkeitskoeffizienten für Cyclo-propan, Halothan und Trichloroethen wurden in einer Krebs-Lösung gemessen: bei 310 K sind die jeweiligen Werte ± SEM der Ostwald-Koeffizienten 0,181 ± 0,009, 0,78 ± 0,02 und 1,54 ± 0,02; über den Temperaturbereich 295–310 K sind die jeweiligen Temperaturkoeffizienten der Löslichkeit −2,27, −4,18 und −3,81 in Einheiten von Prozent/K bei 310 K.

DETERMINACION MEDIANTE CROMATOGRAFIA EN FASE GASEOSA DE COEFICIENTE OSTWALD DE SOLUBILIDAD PARA EL CICLOPROPANO, HALOTANO Y TRICLOROETENO (TRICLOROETILENO)

SUMARIO

Se describen y evalúan métodos cromatográficos en fase gaseosa empleando extracción con disolvente para el análisis de ciclopropano y tricloroeteno (tricloroetileno): el ciclopropano fue extraído con tetracloruro de carbono y el tricloeteno con disulfuro de carbono, empleando cloro-formo y tolueno respectivamente como patrón interno. Se midieron los coeficientes Ostwald de solubilidad para el ciclopropano, halotano y tricloroeteno en solución de Krebs: a 310 K los valores respectivos ± ETM de los coeficientes Ostwald son 0,181 ± 0,009, 0,78 ± 0,02 y 1,54 ± 0,02; en los límites de temperatura de 295–310 K los respectivos coeficientes de temperatura de solubilidad son −2,27, −4,18 y −3,81 en unidades de por ciento/K a 310 K.