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The solubility of volatile anaesthetics in water at 25.0°C using ^{19}F NMR spectroscopy

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Abstract: Anaesthetic concentration is very important for the quantitative treatment of anaesthesia theory. Traditionally concentration values have been derived from the water/gas partition coefficient. However, the values from many investigators show discrepancies. This study reports the accurate solubility of methoxyflurane (9.1 mM), halothane (18.0 mM), enflurane (11.9 mM) and isoflurane (13.5 mM) in water at 25.0°C using ^{19}F NMR spectroscopy. The method has advantages in that the dissolved molecule in solution can be separately quantified from undissolved anaesthetic. Saturated solutions of the anaesthetic agents were prepared *in situ* in a NMR tube to avoid pressure and temperature changes in the solution.

Keywords: *Anaesthesia theory; enflurane; halothane; isoflurane; methoxyflurane; solubility; ^{19}F NMR.*

Introduction

The need for accurate data on the solubilities of inhaled anaesthetics for quantitative analysis of the molecular mechanisms of general anaesthesia has been emphasized [1–4]. Many studies have been performed on the water/gas partition coefficients of general anaesthetics. The water solubility of anaesthetics has been conveniently calculated from the water/gas partition coefficient. Table 1 shows the solubility values of methoxyflurane, halothane, enflurane and isoflurane derived from these reported water/gas coefficients (the Appendix contains the process of this conversion). The solubility values are not consistent between different reports, and it is not reasonable to use any of these values for the analysis of quantitative experiments. However, most investigators [1–3, 5] in the field of volatile anaesthetics have conventionally quoted one of these solubility values from the literature.

In analysing the cause of this discrepancy, several problems become apparent. Regarding the problem of preparation of the sample solution, Okuda [6] and Laasberg and Hedley-Whyte [7] used an injection syringe for the solution container, and leakage of anaesthetic gas from the syringe was significant. Stoelting and Longshore [8] used rubber or glycerin to improve the container seal; however, dissolution of the anaesthetic agent in these materials can become a problem. Okuda [6], Stoelting and Longshore [8], Smith *et al.* [9] and Ikeda [10] used a microsyringe for the sample transfer. Anaesthetic solutions in water are generally very unstable with changes in temperature or pressure, yet Smith *et al.* [9] used aqueous solutions of anaesthetics as standards. They assumed that added anaesthetics would completely dissolve in the water, but since this cannot be verified it is inappropriate to use such solutions as standards. The gas chromatography method [11] is not good for accurate quantitative analysis, because the sample signal is influenced by huge interrupting signals from water or air. All previously reported methods do not measure signals from dissolved-state molecules, and if a trace amount of undissolved anaesthetic contaminates the solution, these methods quantified it as if it were dissolved.

This study determines the water solubility of volatile anaesthetics using ^{19}F NMR spectroscopy. With this method, saturated solutions of anaesthetic agents can be prepared *in situ* in a

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Table 1
Solubilities of methoxyflurane, halothane, enflurane and isoflurane in water at 25.0°C, measured and converted from water/gas partition coefficient

	Solubility (mM)	Concentration (%)	Analysis	Reference
Methoxyflurane				
	9.1 ± 0.4 (n = 5)	saturated (3.9)	F NMR	this study
	9.7	0.39–0.79	GC	[6]
Halothane				
	18.0 ± 0.5 (n = 8)	saturated (39.4)	F NMR	this study
	18.0	1	GC	[10]
	20.6	1.6	GC	[7]
	21.1	0.87	GC	[9]
	23.5	0.65–1.3	GC	[6]
	23.7	1.2	IR	[15]
	47.0	saturated (39.4)	F NMR	[12]
Enflurane				
	11.9 ± 0.3 (n = 4)	saturated (26.4)	F NMR	this study
Isoflurane				
	13.5 ± 1.2 (n = 6)	saturated (38.4)	F NMR	this study
	18.5	1.8	GC	[9]

GC, gas chromatography; IR, infrared spectroscopy; F NMR, ^{19}F NMR spectroscopy.

NMR tube, and pressure and temperature changes in the sample solution caused by preparative manipulation can be reduced. The method has high sensitivity and selects the signal originating from a fluorine atom only. In addition, the signals from dissolved anaesthetic and that from pure anaesthetic liquid can be distinguished in the spectrum (solvent effects: if the solvent is changed, the signal from a molecule may show a different position in the NMR spectrum). In the most unfavourable condition of contamination of a pure anaesthetic agent in solution, only the solute molecules are quantified by this method. Water solubilities of several volatile anaesthetics have thereby been determined using ^{19}F NMR spectroscopy.

Experimental

Analytes and reagents

Volatile anaesthetics, methoxyflurane (2,2-dichloro-1,1-difluoroethylmethyl ether), halothane (2-bromo-2-chloro-1,1,1-trifluoroethane), enflurane (2-chloro-1,1,2-trifluoroethyldifluoromethyl ether) and isoflurane (1-chloro-2,2,2-trifluoroethyldifluoromethyl ether) were distilled twice under reduced pressure. Water was distilled three times. Ethanol (95%, spectrograde; Nacalai Tesque, Kyoto, Japan) was used without further purification. Toluene-D8 (99%, CEA, France) was used as the external field lock solvent.

Spectrometer

The ^{19}F NMR spectra were measured at a resonance frequency 254.05 MHz using a JEOL GX 270 NMR spectrometer. A 5-mm FH dual probe was used. Homogeneity of the magnetic field was tuned to 0.5 Hz, and resolution was verified by a 1% *o*-dichlorobenzene standard (Varian, USA).

Parameters of measurement

The observation frequency was always set midway between the sample and reference signals. The frequency range was 1000 Hz and the digital filter was 500 Hz. All observed signals were within ±250 Hz of the observation frequency. Under these conditions, preservation of electric linearity of the spectrometer was maintained. The pulse width was 10.5 μs (45 degree pulse), and the repetition time was 16.5 s. (The T_1 of halothane in water was approximately 6.6 s at 25.0°C.) This ensured that signal saturation did not occur. The digital resolution was 0.06 Hz. To preserve linearity during Fourier transformation, a window function was not used. In order to ensure a good signal to noise ratio, and hence accurate integration data, the number of accumulations was typically between 64 and 512.

Sample tube

A 5-mm o.d. coaxial tube made by Sigemi Standard & Joint Co. Ltd. (Tokyo, Japan) was used. The sample solution was prepared in the

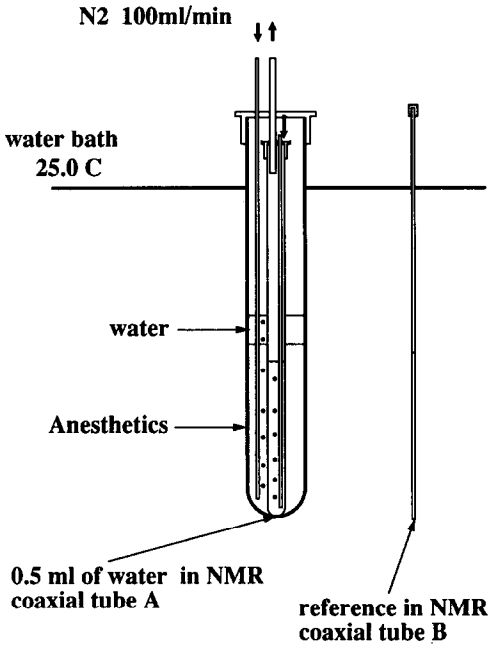

Figure 1(a)
Apparatus for the preparation of saturated solutions of anaesthetics in a NMR tube (A).

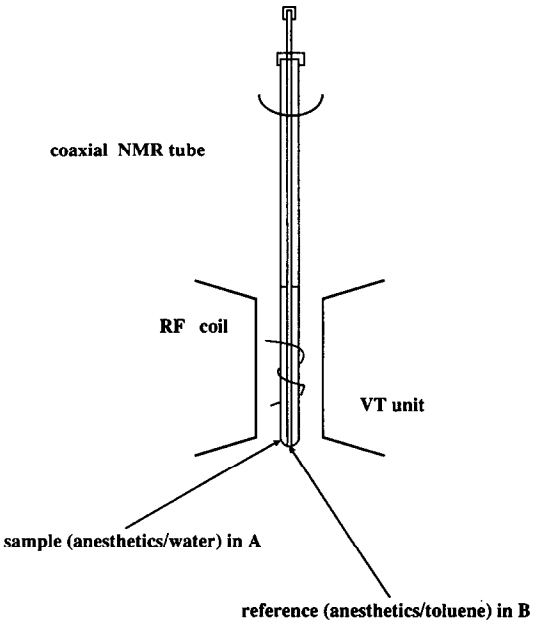

Figure 1(b)
Measuring procedure of sample solution (A): the reference (B) was inserted coaxially into A and the pair were set together in the NMR probe.

outer tube, and the reference solution of toluene-D8 was added to the inner tube (Fig. 1b).

Preparation of reference sample

Toluene was selected as the solvent of the reference solution. The signals in toluene appear near that in water or in ethanol. Methoxyflurane (9.17 mg) was added to toluene-D8 0.93870 g (55.5 mmol l^{-1}).

Preparation of standard sample

Solutions of known concentration of methoxyflurane were prepared in order to obtain a calibration curve for methoxyflurane. Methoxyflurane (248 mg) was added to a 100-ml volumetric flask, and then made to 100 ml with ethanol (95%). This gave a standard solution (15 mM). Ethanol was used since (1) the ^{19}F NMR signals from the sample in ethanol and the reference in toluene-D8 do not overlap with each other and (2) the two signals are relatively close so that a narrow integration region can be selected in order to reduce error. The standard solution was diluted with ethanol to give 12.0, 9.0, 6.0 and 3.0 mM solutions, and a calibration curve was plotted for the set of standards. Each standard was added to the NMR outer tube, and the inner tube containing the reference solution was placed inside it (Fig. 1b). The spectrum was measured and integrated as described later.

Preparation of sample solution

Distilled water (0.5 ml) was added to the NMR outer tube. The tube was placed into the apparatus as shown in Fig. 1(a). High purity nitrogen gas was bubbled through the liquid anaesthetic and water at a rate of 100 ml min^{-1} for 20 min. The gas was saturated with the anaesthetic and water at 25.0°C as it passed through the outer tube, and a saturated solution of the anaesthetic was prepared in the NMR tube (Fig. 1a,A) The gas outlet was conducted to the outside through a much thicker duct open to the atmosphere. The saturation of the anaesthetic solution was checked by plotting its concentration with time.

Measurement

The NMR tube with saturated solution (Fig. 1a,A) was taken out, and the inner tube containing the reference solution (Fig. 1a,B) was placed into it (Fig. 1b). The samples were loaded in the NMR probe with the gas flow VT unit maintained at 25.0°C. The spinning rate was 15 Hz. Three minutes were allowed for thermal equilibrium between the sample and the probe prior to start of the acquisition, and air flow temperature was maintained within

±0.1°C. The sample temperature cannot be measured directly during the NMR measurement, and the error will be a maximum of ±0.5°C. Sample temperature in the probe during off-measurement was calibrated using a D 641 system (Takara Thermistor Co.).

Integration

Spectra obtained were displayed and integrated on graphic monitor using a GX 270 system program. The integral region was selected so as to include both the signal from the water solution and the corresponding signal from the toluene solution, this being as narrow as possible to reduce integral drift from baseline discordance between the lower and higher field baselines of the signals (Fig. 3). The two corresponding spectral lines, in water and in toluene, have almost the same line shape, so their integration gives an accurate ratio. Integral parameters, B_0 and B_1, were selected to coincide with the lower and higher field baseline levels of the signal on a graphic display. Sometimes, discordance between the baseline levels gave poor separation of the integration curve. In this case, the original FID data were again Fourier-transformed and the phases adjusted to keep the spectral baseline horizontal. The integral curve obtained was plotted on a chart from which the percentage of the reference was calculated. Reproducibility of integration mostly depended on signal separation between the sample and the reference. Reproducibility, including procedures of preparation, measurement, spectral phases, and integration, was ±4% for methoxyflurane, ±3% for halothane, ±3% for enflurane and ±9% for isoflurane.

For halothane, enflurane and isoflurane, standard solutions and the reference solution were prepared in the same way and the solubilities were measured.

The relationship between the concentrations of the standard solutions and their integrated intensities (S) represented by the percentage of the reference was determined by the method of least squares (Fig. 2). For methoxyflurane, the relation was as follows, and the solubility of methoxyflurane was calculated from this equation:

$$S(\%) = 5.281 \times C(\text{mM}) - 0.635. \quad (1)$$

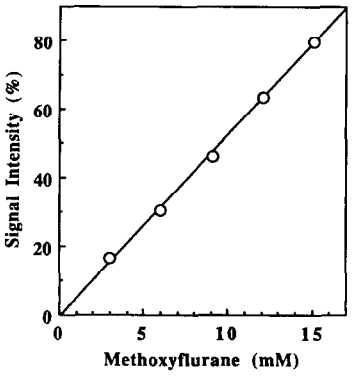

Figure 2
Calibration curve plotted as percentage of reference for the set of methoxyflurane standards.

Results

Figure 3 shows a typical ^{19}F NMR spectrum of the halothane solution, the reference and the integration curve. Each resonance appears as a doublet as a result of coupling to a proton, $J_{H,F}$(5.6 Hz). Table 1 shows the concentrations of saturated solutions of methoxyflurane 9.1 ± 0.4 mM (n = 5), halothane 18.0 ± 0.5 mM (n = 8), enflurane 11.9 ± 0.3 mM (n = 4) and isoflurane 13.5 ± 1.2 mM (n = 6) at 25.0°C, respectively.

Discussion

The ^{19}F NMR spectroscopy method used in this study has a high sensitivity for measuring the solubility of anaesthetics in water. The greatest advantage of the method is that it can distinguish the dissolved anaesthetic signal in water from that of undissolved anaesthetic in the spectrum, and it avoids the error associated with counting dispersed anaesthetics in water. This procedure has not previously been applied except by Koehler *et al.* [12],* and enables the reduction of systematic error.

The value for the solubility of methoxyflurane at 25.0°C agreed well with that reported by Okuda [6] within experimental error. The value for halothane at 25.0°C agreed well with that reported by Ikeda [10], but was smaller than the values obtained by other investigators. For enflurane, there are no reported values at 25.0°C for comparison. For isoflurane, the value was slightly lower than the literature one of 18.5 mM (Smith *et al.* [9]). All

*Koehler *et al.* [12] reported a solubility value of 47 mM for halothane in Mes buffer solution using ^{19}F NMR. However, this value was markedly different from that obtained in water, and is therefore inappropriate to aqueous solubility.

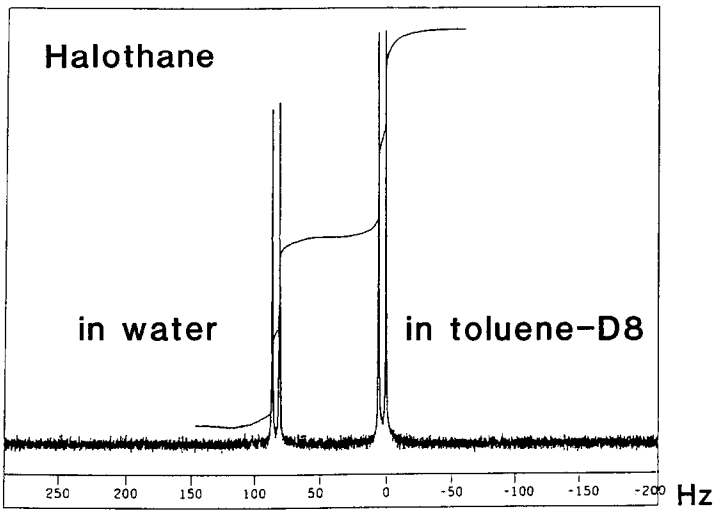

Figure 3
^{19}F NMR spectrum of halothane solution and reference.

of the present solubility values were lower than those previously reported, as discussed below.

Preparation of sample

Smith *et al.* [9] and Ikeda [10] bubbled distilled water with anaesthetic gas to prepare the sample. With this method, confirmation of an equilibrium between the gas and the water indicates a completely saturated solution. This method was modified in the present method to give a saturated anaesthetic solution at constant temperature during measurement. The apparatus (Fig. 1a) was designed to prepare the saturated solution directly in the NMR tube, and the materials in contact with the anaesthetics were limited to Teflon, glass and stainless steel. The gas in the apparatus was opened to the atmosphere, in order to maintain atmospheric pressure inside the NMR tube.

As containers for preparation, Okuda [6] and Laasberg and Hedley-Whyte [7] used an injection syringe. Air tightness of the syringe was maintained by the water between the ground surfaces of the piston and the cylinder. Saidman *et al.* [13] used a method in which anaesthetics and distilled water were stirred in a container and sealed with water. Leakage and oozing through the water was inevitable in these containers. Larson *et al.* [14], Regan and Eger [15], and Eger and Shargel [16] sealed the container with a normal rubber stopper. Stoelting and Longshore [8] used a glass stopper with glycerin to seal the flask (the partition coefficient of glycerin is much higher than that of water). The use of rubber or other unselected materials may cause systematic error since the amount dissolved in the stopper or glycerin is regarded as if it were dissolved in the water phase, so the water/gas partition coefficient may increase.

In this study, saturation of solution was confirmed by the absence of a change in concentration after up to 6 h anaesthetic exposure. Demonstrating saturation is difficult, but Ikeda [10] and Larson *et al.* [14] also checked it in this way.

Standard solution

Larson *et al.* [14], Regan and Eger [15], and Eger and Shargel [16] measured the quantity of anaesthetics by volume. The density of anaesthetics vary significantly with small changes in temperature, while vaporization of anaesthetic during volume measurement cannot be ignored. Anaesthetics should be measured by weight in order to attain accuracy. In the present preparation of the standard solution of methoxyflurane, its weight remained constant. For halothane, enflurane and isoflurane, the weight was not constant because of evaporation of the anaesthetic agents. The anaesthetics were weighed to the nearest 0.010 g (*ca* 2.5%).

Gas chromatography, infrared spectroscopy and gas volumetry are reported methods for the quantitative analysis of volatile anaesthetics; however, little detailed information is available with respect to the calibration of each method. Smith *et al.* [9] prepared a standard

solution for gas chromatography by adding a weighted amount of anaesthetic to a flask of known volume completely filled with distilled water — the problem here is that the anaesthetic sometimes does not completely dissolve. Undissolved anaesthetic in a solution may easily adhere to the wall of the glass flask and become very difficult to agitate when water is added to reach the top of the flask. It generally takes a long time to prepare a solution of an anaesthetic in water by equilibration without stirring. A solution prepared in this way lacks stability as a standard solution with changes in pressure and temperature.

In the present study, the calibration curve was plotted from the ethanol solution of the standards. To assay the sample dissolved in water, the solution in water is usually used for plotting the calibration curve. However, the solubility of anaesthetics in water is generally low, and the water solution is very sensitive to temperature changes and experimental manipulations, so it is not a suitable standard for quantitative analysis. Ethanol was therefore used as the solvent. NMR signals in ethanol and in water differ by 100–200 Hz from the reference in toluene, but maintain the same line shape. This enables complete separation of the signal of the sample and the reference, in order to quantify their integration. Some investigators would question the use of the same calibration curve in ethanol and the sample in water, because the interaction of the anaesthetics differs with each solvent. The maximum difference in interaction between the anaesthetics and solvent is 200 Hz from measurement of chemical shift, therefore, the difference in the molecular interactions of anaesthetics with solvents compared with resonance frequencies is of no practical significance. The fluorine atom in the anaesthetic molecule is strongly bound to carbon and cannot be exchanged chemically.

Sample transfer

A microsyringe is commonly used for transfer of samples according to many previous reports [6, 8–10]. A reduction in sample pressure during withdrawal of the water phase cannot be avoided and its temperature may vary. The fall in pressure of the sample system during its withdrawal could create a bubble of anaesthetic gas in the solution. The gas phase sample is compressed during injection into the measuring apparatus and changes in volume, so determination of the volume of the gas phase sample injected becomes inaccurate. In the present method, the saturated solution was prepared in a NMR tube, contributing to the experimental precision of this method.

Premises in calculation of the water/gas partition coefficient in previous studies

Okuda [6] and Ikeda [10] measured samples from the gas phase and from the water phase to calculate the ratio of concentrations, and then determined the water/gas partition coefficient. Stoelting and Longshore [8] quantified the gas phase sample and extracted the anaesthetic from the water phase, in order to calculate its concentration in the water phase. Error may result from this extraction procedure. Using the gas chromatography method and the infrared spectroscopy method, by preparing a water solution of a known amount of anaesthetic, the concentration of only the gas phase could be measured. The quantity of anaesthetic in the gas phase was subtracted from the initially added quantity. The amount of anaesthetic dissolved in the water phase can be deduced by this method provided no leakage occurs.

Concentrations derived from the water/gas partition coefficients of certain concentrations of anaesthetic gases cannot be directly compared with the solubility measured from the saturated solution. Concentration dependence of the water/gas coefficient might explain the somewhat higher values reported in the literature (the higher the concentration, the lower the water/gas coefficient).

Conclusions

The present study has established a method of quantitative analysis of methoxyflurane, halothane, enflurane and isoflurane, and enabled accurate determination of their solubilities in water at 25.0°C using ^{19}F NMR spectroscopy. The study provides a standard methodology and solubility values applicable to the field of anaesthetic theory.

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References

[1] N.P. Franks and W.R. Lieb, *Nature* **274**, 339–342 (1978).

[2] N.P. Franks and W.R. Lieb, *Nature* **310**, 599–601 (1984).

[3] D.A. Haydon and B.W. Urgban, *J. Physiol.* **373**, 311–327 (1986).

[4] E.I. Eger II, *Anesthesiology* **65**, 122–123 (1986).

[5] A. Steward, P.R. Allott, A.L. Cowles and W.W. Mapleson, *Br. J. Anaesth.* **45**, 282–293 (1973).

[6] Y. Okuda, *Arch. Jap. Chir.* **37**, 700–716 (1968).

[7] L.H. Laasberg and J. Hedley-Whyte, *Anesthesiology* **32**, 351–356 (1970).

[8] R.K. Stoelting and R.E. Longshore, *Anesthesiology* **36**, 503–505 (1972).

[9] R.A. Smith, E.G. Porter and K.W. Miller, *Biochim. Biophys. Acta* **645**, 327–338 (1981).

[10] S. Ikeda, *Anesthesiology* **37**, 87–91 (1972).

[11] C.O. Rutledge, E. Seifen, M. Alper and W. Flacke, *Anesthesiology* **24**, 862–867 (1963).

[12] L.S. Koehler, E.T. Fossel and K.A. Koehler, *Biochemistry* **16**, 3700–3707 (1977).

[13] L.J. Saidman, E.I. Eger II, E.S. Munson and J.W. Severinghaus, *Anesthesiology* **27**, 180–184 (1966).

[14] C.P. Larson Jr, E.I. Eger II and J.W. Severinghaus, *Anesthesiology* **23**, 349–355 (1962).

[15] M.J. Regan and E.I. Eger II, *Anesthesiology* **28**, 689–700 (1967).

[16] E.I. Eger II and R. Shargel, *Anesthesiology* **24**, 625–627 (1963).

[17] R. Thomason, G. Light and D. Holaday, *Anesth. Analg.* **41**, 225–229 (1962).

[18] R.C. Rogers and G.E. Hill, *Br. J. Anaesth.* **50**, 415–423 (1978).

[19] G.A. Bottomley and G.H.F. Seiflow, *J. Appl. Chem.* **13**, 399–402 (1963).

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Appendix

Conversion of water/gas partition coefficient to solubility value

The water/gas partition coefficient at t°C reported in the literature was converted to the solubility value using the following equation:

$$\frac{\alpha \times p_a}{22.4 \times 760} \text{ (mol l}^{-1}\text{)},$$

where α is Bunsen's absorption coefficient at t°C; p_a is the vapour pressure (torr) of the anaesthetics at t°C from the literature [17–19].

The partition coefficient is represented by the Ostwald solubility coefficient (β), and the conversion is carried out by use of the following equation:

$$\frac{\beta \times p_a}{0.0821 \times (273 + t) \times 760} \text{ (mol l}^{-1}\text{)}.$$