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THE SOLUBILITY OF VOLATILE ANESTHETICS IN WATER AT 25.0 °C USING ^{19}F NMR SPECTROSCOPY

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Abstract

Anesthetic concentration is very important for quantitative analysis of anesthesia theory. Traditionally for analysis, the concentration value was derived from the water/gas partition coefficient. However, the values from many investigators show discrepancy between them. This study determined 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. This method has great advantages in that the dissolved molecule in the solution can be separately quantified from neat anesthetics. And saturated solution of anesthetic agent can be prepared *in situ* in a NMR tube to avoid pressure and temperature change in the solution.

Key words:

- Anesthesia theory
- Enflurane
- Halothane
- Isoflurane
- Methoxyflurane
- Solubility
- ^{19}F NMR

Introduction

The need for accurate data on the solubilities of inhaled anesthetics for quantitative analysis of the molecular mechanisms of general anesthesia has been emphasized [1-3]. Many studies have been performed on the water/gas partition coefficient of general anesthetics. The water solubility of anesthetics has been conventionally 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 analysis of quantitative experiments. However, most investigators [1-3,5] in the field of volatile anesthetics have conventionally quoted one of these solubility values from the literature.

In analyzing the cause of this discrepancy, several problems become apparent. Regarding the problem of preparation of the sample solution, Okuda [6] and Lassberg *et al.* [7] used an injection syringe for the solution container, and leakage of anesthetic gas from the syringe seemed to be significant. Stoeting *et al.* [8] used rubber or glycerin to improve the container seal, however, dissolution of the anesthetic agent in these materials can become a problem. Okuda [6], Stoeting *et al.* [8], Smith *et al.* [9], and Ikeda [10] used a microsyringe for sample transfer. Anesthetic solutions in water are generally very unstable with changes in temperature or pressure, but Smith *et al.* [9] used a water solution of anesthetics for standard solution. They assumed that added anesthetics would completely dissolve in the water , but this cannot be verified, and the prepared standard solution is very unstable with temperature or pressure changes, so it is not appropriate to use as the standard solution. As a problem with measurement, 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 signal from dissolved-state molecules, and if a trace amount of pure anesthetics contaminated the solution, these methods quantified it as it were dissolved. These problems may have greatly influenced the accuracy and probability.

This study determined the water solubility of volatile anesthetics using ^{19}F NMR spectroscopy. With this method, saturated solutions of anesthetic agents can be prepared *in situ* in a NMR tube, and pressure and temperature changes in the sample solution caused by preparative manipulation can be reduced. The ^{19}F NMR spectroscopy method has high sensitivity and can select the signal originating from a fluorine atom only. In addition, the signal from dissolved anesthetics and that from pure anesthetic liquid can be distinguished from each other on spectrum. (solvent effects: If the solvent differs, the signal from a molecule shows a different position on the NMR spectrum.) In the most unfavorable condition of contamination of a pure anesthetic agent in solution, only the molecules in solution can be quantified by this method. We accurately determined the water solubilities of volatile anesthetics using ^{19}F NMR spectroscopy.

Experimental

Reagent: Volatile anesthetics, 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 1 % *o*-dichlorobenzene standard sample (Varian, U.S.A.).

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 µsec (45 degree pulse), and the repetition time was 16.5 sec. (The T_1 of halothane in water was approximately 6.6 sec 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 OD coaxial tube made by Sigemi Standard & Joint Co. Ltd. (Tokyo, Japan) was used. The sample solution was prepared in the outer tube, and the reference solution of toluene-D8 was added to the inner tube (Fig. 2b).

Preparation of reference sample: Toluene was selected as the solvent of the reference solution. The signals in toluene appears near that in water or in ethanol. Methoxyflurane (9.17 mg) was added to toluene-D8 0.93870 g (55.5 mmol/l).

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 due to solvent effects, 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 mM, 9.0 mM, 6.0 mM 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. 2b). 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. 2a. High purity nitrogen gas was bubbled through the liquid anesthetics and water at a rate of 100 ml/min for 20 minutes. The gas was accurately saturated with the anesthetics and water at 25.0 °C as it passed through the outer tube, and a saturated solution of the anesthetics was prepared in the NMR tube (Fig. 2a A). The gas outlet was conducted to the outside through a much thicker duct open to the atmosphere. The saturation of the anesthetic solution was checked by its concentration with time.

Measurement: The NMR tube with saturated solution (Fig. 2a A) was taken out, and the inner tube containing the reference solution (Fig. 2a B) was placed into it (Fig. 2b). The samples were loaded in the NMR probe with the gas flow VT unit maintained at 25.0 °C. Spinning rate was 15 Hz. Three minutes were allowed for thermal equilibrium between the sample and the probe prior to start of the acquisition. Air flow temperature was maintained within ±0.1 °C. The sample temperature cannot be measured directly during the NMR measurement, however, 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 signal from the water solution and the corresponding signal from the toluene solution, and also being as narrow as possible to reduce integral drift from baseline discordance between the lower field baseline of the signal and the higher (Fig. 1). The two corresponding spectral lines, in water and in toluene, but originating from the same fluorine atom, have almost the same line shape, so their integration in a region gives a much better ratio of the integral value. Integral parameters, B_0 and B_1, were selected to coincide with the lower and higher field baseline levels of the signal on graphic display. Sometimes, discordance between the baseline levels gives poor separation of the integration curve. In this case, the original FID data was again Fourier-transformed and its phases adjusted to keep the spectral baseline horizontal. The integral curve obtained was graphed on a chart. The percentage versus the reference was calculated from the chart. 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 versus the reference was determined by the method of least squares (Fig. 1). For methoxyflurane, the relation was determined as follows,

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

The solubility of methoxyflurane was calculated from equation(1).

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 a coupling constant to a proton, $J_{H,F}$(5.6 Hz). Table 1 shows the concentrations of the saturated solution of methoxyflurane 9.1±0.4 mM (n=5), of halothane 18.0±0.5 mM (n=8), of enflurane 11.9±0.3 mM (n=4), and of 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 anesthetics in water. The greatest advantage of this method is that it can distinguish the dissolved anesthetic signal in water from that of pure anesthetics in the spectrum, and completely avoids the inevitable error associated with counting dispersed pure anesthetics in water. This adaptation was not previously applied except by Koehler *et al.* [12][*] , and enables the reduction of systematic error.

Our value for the solubility of methoxyflurane at 25.0 °C agreed well with that reported by Okuda [6] within experimental error. Our 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, our value was slightly lower than the accepted value in the literature of 18.5 mM (Smith *et al.* [9]). All of our solubility values were lower than those previously reported in the literature. We will discuss this in the following sections.

Preparation of Sample: Smith *et al.* [9] and Ikeda [10] bubbled distilled water with anesthetic gas to prepare the sample. With this method, confirmation of an equilibrium between the gas and the water indicates a completely saturated solution. We modified this method and prepared a saturated anesthetic solution under constant temperature during measurement. The apparatus (Fig. 2a) was designed to prepare the saturated solution directly in the NMR tube, and the materials in contact with the anesthetics 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 *et al.* [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 anesthetics and distilled water were stirred in a container and sealed with water. Leakage and oozing through the water

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[*] Koehler *et al.* [16] 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 unreliable.

was inevitable in these containers. The leakage from the system prior to equilibrium was regarded as it were dissolved in the water phase. Larson *et al.* [14], Regan *et al.* [15], and Eger *et al.* [16] sealed the container with a normal rubber stopper. Stoelting *et al.* [8] used a glass stopper with glycerin to seal a 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. The amount dissolved in the stopper or glycerin is regarded as if it is 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 hours anesthetic 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 *et al.* [15], and Eger *et al.* [16] measured the quantity of anesthetics by volume. The density of anesthetics characteristically vary significantly with small changes in temperature. The anesthetics vaporized during volume measurement cannot be ignored. The anesthetics should be measured by weight in order to maintain accuracy. In our preparation of the standard solution of methoxyflurane, its weight was constant. For halothane, enflurane, and isoflurane, the reading was not constant because of evaporation of the anesthetic agents. The anesthetics was weighted to the nearest 0.010 g (ca. 2.5 %).

Gas chromatography, infrared spectroscopy, and gas volumetry are reported methods for quantitative analysis of volatile anesthetics, 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 weight of anesthetics to a flask of known volume completely filled with distilled water. A problem associated with this method is that the anesthetics sometimes does not completely dissolve. A pure anesthetics in 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. In general, it takes a long time to prepare a solution of anesthetics in water by equilibration without stirring. A solution prepared in this way lacks stability as a standard solution with changes in pressure and temperature and is of no use as a standard.

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 anesthetics in water is generally low, and the water solution is very unstable with temperature changes and experimental manipulations, so it is not a suitable standard for quantitative analysis. Ethanol was therefore used as the solvent. Signals in ethanol and in water differ by 100-200 Hz from the reference in toluene, but maintain the same line shape (solvent effects). 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 anesthetics differs with each solvent. The maximum difference in interaction between the anesthetics and solvent is 200 Hz from measurement of chemical shift, therefore, the difference in the molecular interactions of anesthetics with solvents comparing with resonance frequency will be of no practical significance. The fluorine atom in the anesthetic molecule has strong bonds and cannot be exchanged chemically. Therefore, we consider that the calibration curve using ethanol as the solvent can be applied to the solution in water in the same way.

Sample Transfer: A microsyringe is commonly used for transfer of samples according to many previous reports. (Okuda [6], Stoelting *et al.* [8], Smith *et al.* [9], Ikeda [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 withdraw could create a bubble of anesthetic 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. With our 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 *et al.* [8] quantified the gas phase sample and extracted the anesthetics 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 anesthetics, the concentration of only the gas phase could be measured. The quantity of anesthetics in the gas phase was subtracted from the initially added quantity. The amount of anesthetics dissolved in the water phase can be deduced by this method so far as no leakage occurred.

Concentrations derived from the water/gas partition coefficients of certain concentrations of anesthetic 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) However, the concentrations are low enough to apply Henry's law in this region.

Conclusions

The present study established a method of quantitative analysis of methoxyflurane, halothane, enflurane, and isoflurane, and accurately determined their solubilities in water at 25.0 °C using ^{19}F NMR spectroscopy. This study provides a standard methodology and values in the field of anesthetic theory.

Acknowledgements

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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:

$$\frac{\alpha \times p_a}{22.4 \times 760} \quad (\text{mol/l})$$

where

α : Bunsen's absorption coefficient at t °C

p_a: vapor pressure (Torr) of the anesthetics at t °C from the literatures [17-19].

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

$$\frac{\beta \times p_a}{0.0821 \times (273+t) \times 760} \quad (\text{mol/l})$$

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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

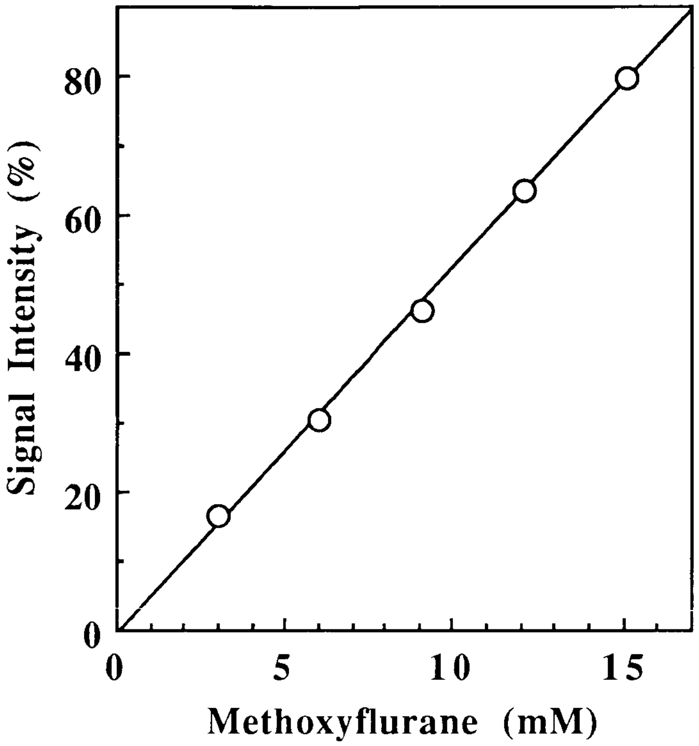
List of Figure Legends

Figure 1. Calibration Curve Plotted as Percentage of Reference for the Set of Methoxyflurane Standards.

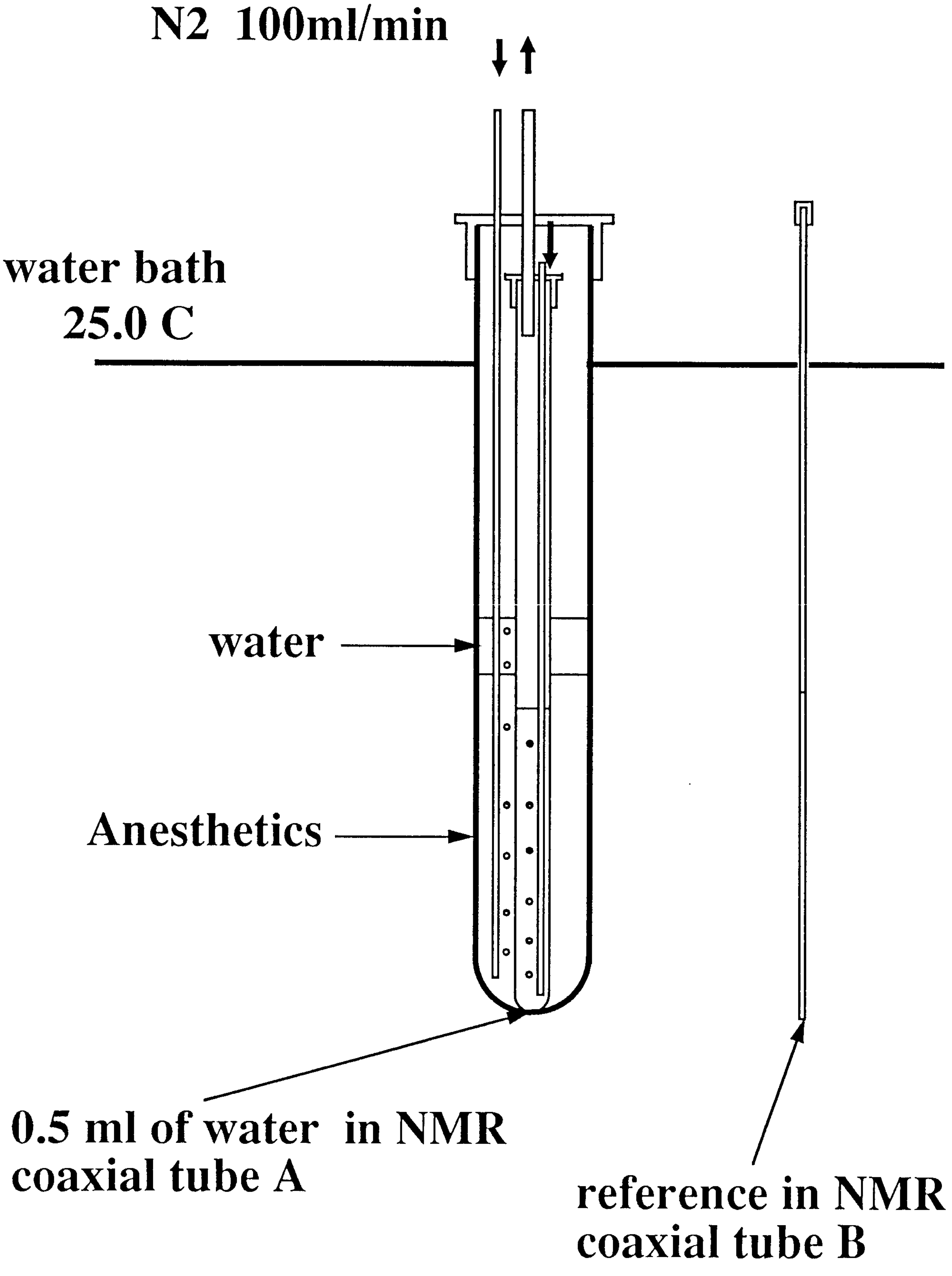
Figure 2a. Apparatus for Preparation of Saturated Solution of Anesthetics in NMR tube(A).

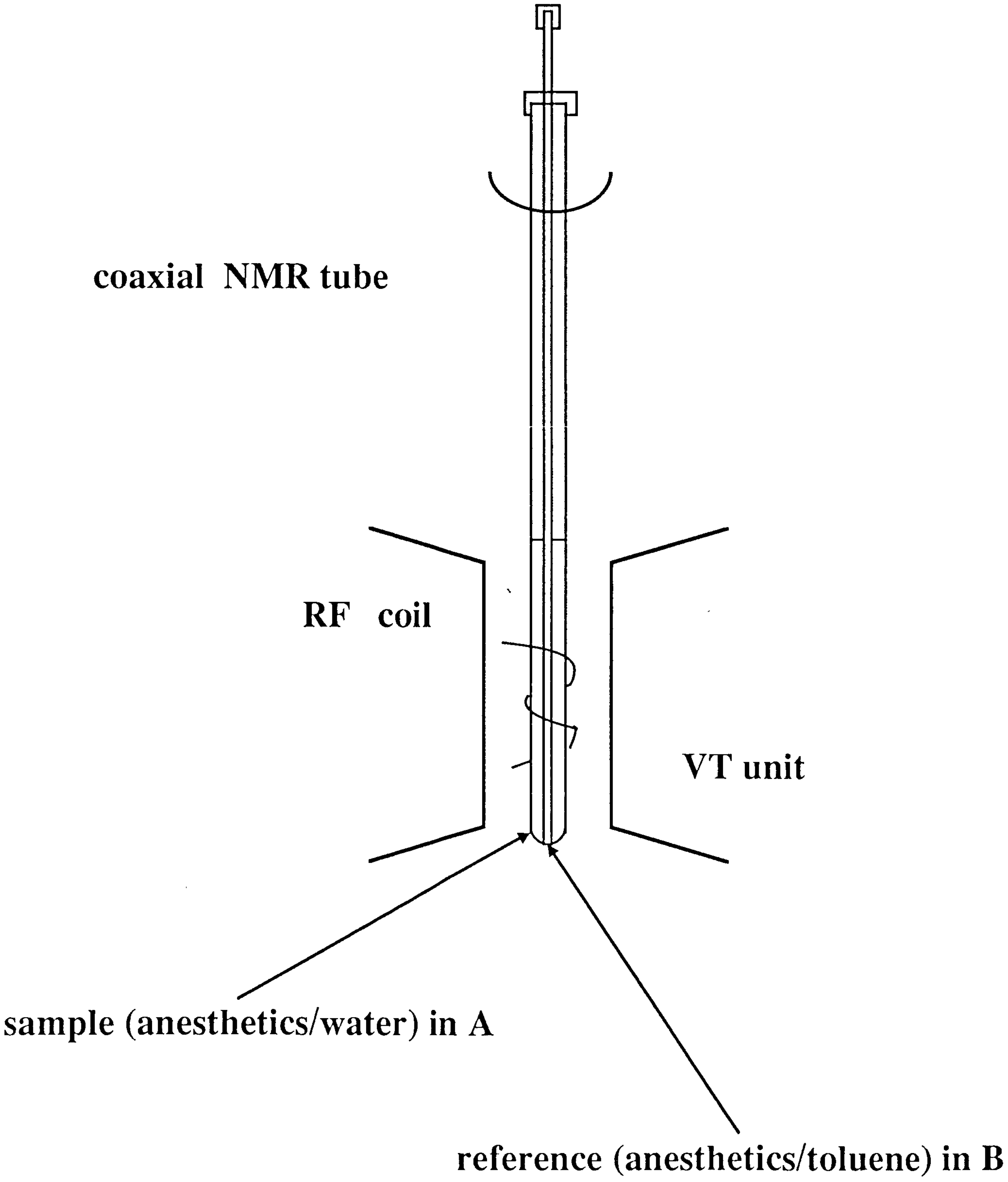
Figure 2b. Measuring procedure of sample solution(A): the reference(B) was inserted to A and they were set together in NMR probe.

Figure 3. ^{19}F NMR Spectrum of Halothane Solution and Reference.



Signal Intensity (%)
80
60
40
20
0
0
5
10
15
Methoxyflurane (mM)


N2 100ml/min
water bath
25.0 C
water
Anesthetics
0.5 ml of water in NMR
coaxial tube A
reference in NMR
coaxial tube B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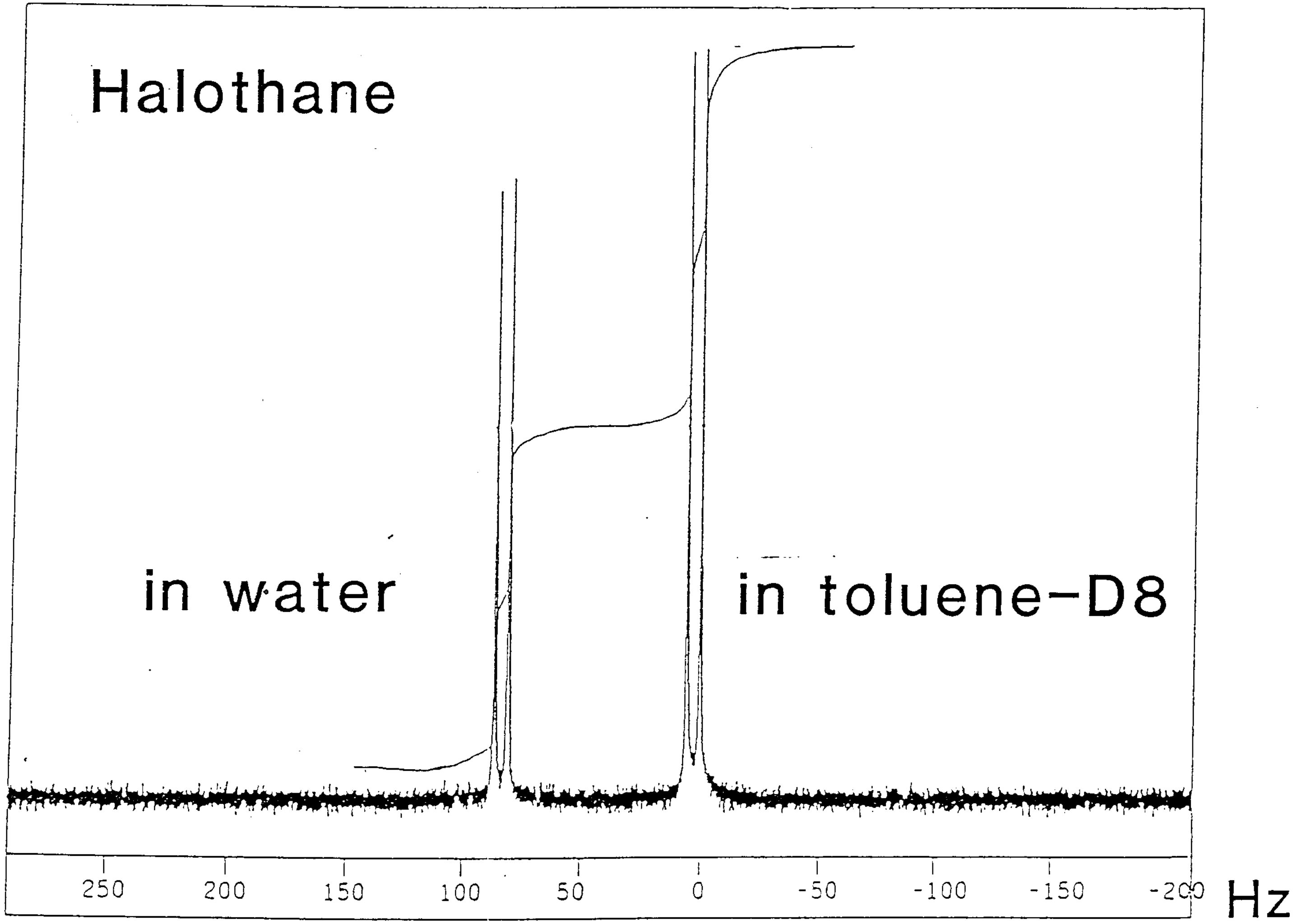
RF coil
VT unit
sample (anesthetics/water) in A
reference (anesthetics/toluene) in B


Halothane
in water
in toluene-D8
250
200
150
100
50
0
-50
-100
-150
-200
Hz