

# Research Methods

## *Flame Ionization Detection of Volatile Organic Anesthetics in Blood, Gases and Tissues*

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THE MAJORITY of chemical and physical methods now available for the quantitative analysis of anesthetics in blood and tissue are tedious and time-consuming.<sup>1-6</sup> Existing chromatographic methods are unnecessarily prolonged by equilibrations, extractions or distillations which require subsequent chromatographic separations.<sup>7-21</sup> In the vast majority of instances, general anesthesia consists of the administration of a single volatile organic anesthetic. In these situations, the concentration of the volatile organic agent in gas, blood or tissue samples may be determined without the use of chromatographic columns. Analyses can be completed within a few seconds by injecting the sample directly into the heated port of a flame ionization detector. The response of the flame ionization detector is specific for organic vapors. Normal blood does not contain measurable quantities of volatile organic materials.<sup>22</sup> The presence of "ketone bodies" or ethyl alcohol in blood does not interfere with the analysis.

### Methods

An F & M Model 1609 flame ionization detector\* and electrometer were used with a Minneapolis-Honeywell one-millivolt recorder and Disc Integrator. The block temperature was maintained at 150° C. The nitrogen, air and hydrogen flows were regulated at rotameter settings of 6, 9, and 6, respectively. These settings were not critical, however, and were increased to shorten the analysis time or

reduced to minimize background noise and improve sensitivity (increase the parts of anesthetic per parts of hydrogen). The best stability was observed when the N<sub>2</sub> and H<sub>2</sub> flows were equal. Once the desired gas flows were obtained, the gases were turned on and off at the main tank valves without altering the two-stage reduction gauges or needle valves. The electrometer, block heater, and injection port heater were left on continuously.

*Injection Port Temperatures.* In the analysis of blood samples and liquid standards, where 1- $\mu$ l. samples were employed, the injection port was maintained between 50 to 60° C. (thermocouple).

For the analysis of weighed blood or tissue specimens (5-25 mg.), the injection port temperature was maintained between 100 and 120° C.

*Syringes.* Hamilton microsyringes† (1, 10, and 50  $\mu$ l.) were employed to inject blood samples, prepare standards, and inject gas samples, respectively. The 1- $\mu$ l. syringes were cleaned with 3 per cent hydrogen peroxide and distilled water whenever an increased resistance to plunger movement was noted. After cleansing, several rinses with the subsequent sample were required to remove residual water.

*Columns.* A 12  $\times$   $\frac{1}{4}$  inch Polyflo tube‡ was packed with 60-80 mesh Chromosorb P.\* Glass wool plugs were inserted in each end. One milliliter of water was injected from a syringe directly onto the column. The moist end of the column was attached to the outlet from the injection port. The column was kept moist by injecting  $\frac{1}{2}$  ml. of water into the

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\* F & M Scientific Corp., Avondale, Pennsylvania.

† Hamilton Company, Inc., Whittier, California. 7001N, 701N, and 705N.

‡ Imperial Eastman, No. 144P  $\times$   $\frac{1}{4}$ , Chicago, Illinois.

proximal end every 2–3 hours. The boundary between the moist and dry Chromosorb P was visible through the polyethylene tube. The moist column served to humidify the carrier gas and stabilize the vapor pressure of water entering the hydrogen flame. Excess water entering the heater block from the column vaporized and blew out the hydrogen flame. A similar Polyflo tube packed from end to end with glass wool or 60–80 mesh glass beads and moistened with water served the same purpose equally well.

Columns packed with a 50–50 mixture (by volume) of phosphorous pentoxide and haloport\* gave the most accurate results. The phosphorous pentoxide ( $P_2O_5$ ) rapidly reabsorbed water vapor and quickly eliminated the pressure surges and fluctuations in carrier gas flow produced by vaporization of water. The life of these columns was dependent upon the number of samples analyzed (amount of water injected) and rarely could be used for more than one day. Ether and trifluoroethyl vinyl ether were hydrolyzed to the corresponding nonvolatile phosphates and alcohols by  $P_2O_5$  at room temperature and could not be analyzed with this column. All columns were employed at room temperature.

When the injection port was kept at room temperature (or below 40° C.), no column packing was required; analysis, however, required one to two minutes longer for completion.

*Gas Sample Loop.* An F & M gas sample loop (0.5 ml.) was installed in the carrier gas line between the nitrogen flow regulator and the injection port. The solubility of anesthetic agents in the sample loop O-rings prevented flow-through sampling. The sample loop was evacuated and filled to atmospheric pressure with standard gas samples or with samples from any point in the anesthetic circuit.

During IPPB (manual or mechanical), the elevated circuit pressure was employed to wash out the sampling line and provide the sample loop with samples. During spontaneous breathing, the vacuum source used to evacuate the loop was employed to wash out the sampling dead space.

With this sample loop, it was possible to analyze circuit gases every 10 seconds during

clinical anesthesia. The sample loop standardizes the injection technique and gas concentrations were directly proportional to peak height. Peak heights were reproducible to within 0.5 per cent.

*Standards.* The preparation of gas standards or gravimetric water or blood standards has been described.<sup>23,24</sup> For routine use, volumetric standards were prepared by adding 5  $\mu$ l. of liquid anesthetic to 20 ml. of distilled water in a capped syringe. § Mercury was added to insure thorough mixing. The concentration (mg. per cent) was calculated from the specific gravity of the agent.

*Blood Samples.* Blood samples were collected in heparinized syringes or capillaries. || The capillary tubes were completely filled except for 4–5 mm. at one end to permit sealing with clay. ¶ Samples were obtained from arteries, veins, and finger or earlobe capillary stabs.

Blood samples or water standards were placed inside the injection port through the silicon rubber septum by means of the 1- $\mu$ l. Hamilton syringes. The small sample size necessitated wiping the sample from the syringe tip by a rotary sweeping motion which deposited the sample on the injection port walls. The extremely rapid elution of the anesthetic from blood made standardization of the injection technique impossible. Reproducible peak heights could not be obtained. The areas under the response curves were determined by means of the Disc Integrator. Since these syringes have a dead space (10 per cent) from which additional anesthetic can distill, the injection was carried out as rapidly as possible to avoid heating the needle. The injection port was cleaned daily with water and pipe cleaners (or after each 50–100 analyses).

*Blood and Tissue Analysis.* The higher injection port temperatures (100–120° C.) required for rapid tissue analyses decreased the accuracy with which 1- $\mu$ l. blood samples could be analyzed. At the lower attenuations

§ Scientific Products, Evanston, Illinois. Crito-caps-K, B-3100.

|| Will Scientific Corp., Buffalo, New York. No. 4965 (1.5 × 75 mm).

¶ Clay-Adams, Inc., New York, New York. Seal-Ease Holder, No. A-2980.

TABLE 1. Ionization Response and Physico-Chemical Properties of Common Anesthetic Agents

Agent	Formula	Molecular Weight	Vapor STP mg./ml.	Specific Gravity	Vapor Pressure 25° C. mm. Hg	Ostwald Partition Coefficient 37° C.†	Anesthetic Blood Concentration mg. %	Flame Ionization Response (Area)				
								μg. × 10 <sup>3</sup>	μg. C × 10 <sup>3</sup>	μMl. × 10 <sup>3</sup>	Standard Deviation*	
											Area	mg. %
Cyclopropane	C <sub>3</sub> H <sub>6</sub>	42.08	1.88	1.42 (air = 1)	90 psi	0.420	8-18	1.66	1.94	7.00	2.2	2.9
Ether	(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> O	74.13	3.31	0.72 <sup>20</sup>	540	15.6	50-100	1.32	1.98	9.80	4.3	0.52
Methoxyflurane	C <sub>2</sub> F <sub>2</sub> Cl <sub>2</sub> HOCH <sub>3</sub>	165.0	7.35	1.42 <sup>20</sup>	33	8.4	10-20	0.37	1.56	6.10	6.6	0.52
Halopropane	C <sub>3</sub> F <sub>7</sub> ClH <sub>3</sub>	150.5	6.72	1.81 <sup>20</sup>	95	5.4	10-20	0.52	2.16	7.02	4.0	0.31
Halothane	C <sub>2</sub> F <sub>3</sub> HBrCl	197.4	8.78	1.86 <sup>15</sup>	300	2.42	10-18	0.27	2.2	5.33	2.8	0.30
Chloroform	CHCl <sub>3</sub>	119.4	5.34	1.47 <sup>20</sup>	200	6.6	10-18	0.22	2.2	2.60	6.2	0.41
Trifluoroethyl vinyl ether (Fluoromar)	C <sub>3</sub> F <sub>3</sub> H <sub>2</sub> OC <sub>2</sub> H <sub>4</sub>	131.4	5.86	1.13 <sup>23</sup>	380	1.45	12-20	0.59	1.61	7.72	4.2	0.36

\* 25 determinations each of blood and water standards.  
† As determined in this laboratory.

(higher sensitivity) required to obtain significant response areas with 1-μl. samples, the pressure produced by vaporization of water at 120° C. produced a significant base line shift. Therefore, when it was necessary to run tissue and blood analyses interchangeably, the samples were weighed prior to analysis.

Tissue specimens, including clotted blood, obtained by biopsy or at autopsy, were blotted to remove excess blood and placed just inside the back end of a 2 ml. polyethylene syringe. The rubber tip of the plunger was covered with a one-inch square of Saran wrap (pre-cut) and placed in contact with the tissue, avoiding air trapping. The plunger was advanced until a small amount of tissue extruded from the front end of the syringe. The syringe was sealed tightly with a metal syringe cap and vigorous pressure applied to the plunger to force any remaining air out of the syringe around the Saran-wrapped plunger. The blood or tissue specimen was injected from the air-free syringe into a tared glass capillary (1.5 × 37.5 mm.) and reweighed on a torsion balance. The capillary was connected to the syringe by means of a Touhy-Borst Adapter.# The weighed specimen (15 to 25 mg.) in the glass capillary was placed in the solid sample injector\* and crushed inside the injection port. Eight to 10 samples could be analyzed before cleaning the port. The port was cleaned by detaching

# Becton-Dickinson Co., East Rutherford, New Jersey. Adapter No. 610-A and 613-A.

the proximal end of the column and the sample injector and flushing 20 ml. of water from a rubber-tipped syringe through the injection port. The syringe was used to blow out excess water and the apparatus reassembled without drying. The entire cleaning procedure required less than one minute.

If it was not possible to analyze the specimen within one or two hours, the tuberculin syringe was attached to flux free core solder (1/8 inch, outside diameter, by 1/16 inch, inside diameter) by means of a Touhy-Borst adapter.# The tissue was extruded through the solder tube by applying vigorous pressure to the plunger until tissue appeared at the distal end. The solder tube was then sealed by crushing both ends with a pair of pliers. Specimens preserved in this manner were kept for two to three weeks without loss of anesthetic. The details of this technique and the means of retrieving the tissue for analysis have been described elsewhere.<sup>25</sup>

*Calculation of Results.* The anesthetic concentration in an unknown sample (X) is given by the following equation:

$$\text{mg. \% } X = \text{mg. \% st'd} \frac{\text{area } X \cdot \text{attenuation } X}{\text{area st'd} \cdot \text{attenuation st'd}} \cdot \frac{\text{Volume st'd}}{\text{Volume } X}$$

The total attenuation in the above equation was obtained by multiplying the instrument range setting by the instrument attenuation setting (*i.e.*, range 1,000 at attenuation 4 X

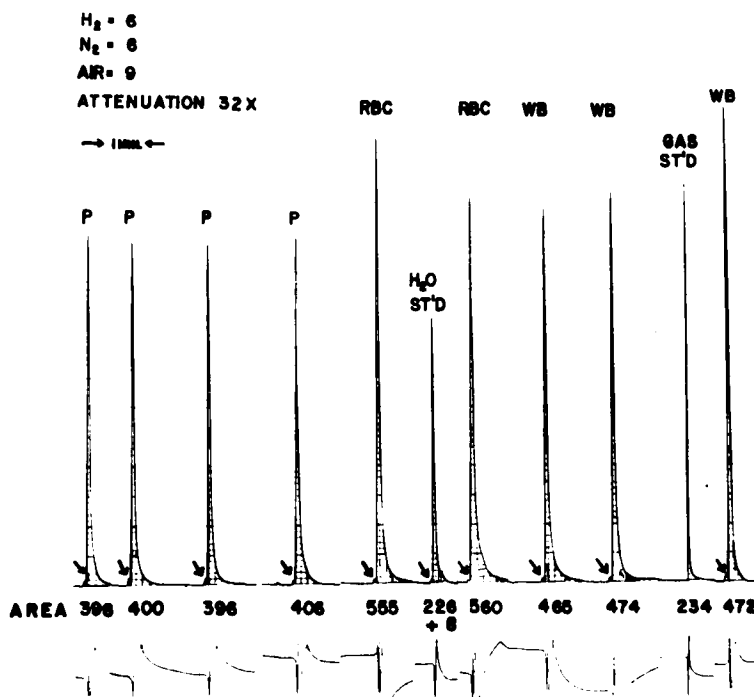


Fig. 1. Distribution of chloroform in whole blood. Conditions as described under methods. P = plasma, RBC = red blood cells, WB = whole blood. The lower tracing is that of the Disc Integrator giving the areas under the response curves, which are shaded for photographic reproduction of the original record. Sample size equal 1  $\mu$ l.  $\rightarrow$  = sample injection.

equals a total attenuation of 4,000). For most purposes the volume or weight of the standard (st'd) or sample was used interchangeably.

### Results

The ionization response (area) for each anesthetic agent was roughly proportional to the micrograms of carbon ( $\mu$ g.C) injected (table 1). The standard deviation of the response area (or mg. per cent) from the mean area obtained from 25 consecutive determinations of blood and water standards for each agent is shown. The response areas of equivalent gas, water, and blood standards were

identical ( $\pm$  one per cent), although the peak heights decreased and the tailing increased in the order listed (fig. 1).

The detector response for each anesthetic agent was linear over a concentration gradient of 200,000 (*i.e.*, 1.0 mg. per cent to 186,000 mg. per cent of halothane). The standard error of analysis increased at the extremes of concentration due to limitations in integrating areas at high concentrations and background electrometer noise at low concentrations (table 2).

The distribution of anesthetic agents in blood was determined by addition of known amounts of anesthetic to blood in a capped

TABLE 2. Standard Error of Analysis as a Function of Blood Concentration—Cyclopropane

Standard mg. %	Determinations	Electrometer		Mean Area	Area/mg. % Maximum Sensitivity	Standard Deviation		
		Range	Attenuation			Area	mg. %	% Error
188*	24	10	256	132.2	1,800	2.2	2.90	1.54
8.3	22	1	32	464	1,789	2.3	0.05	0.50
0.83	20	1	4	370	1,828	7.0	0.016	1.90
0.083	10	1	1	150	1,807	11.0	0.006	7.20

\* 100 per cent cyclopropane.

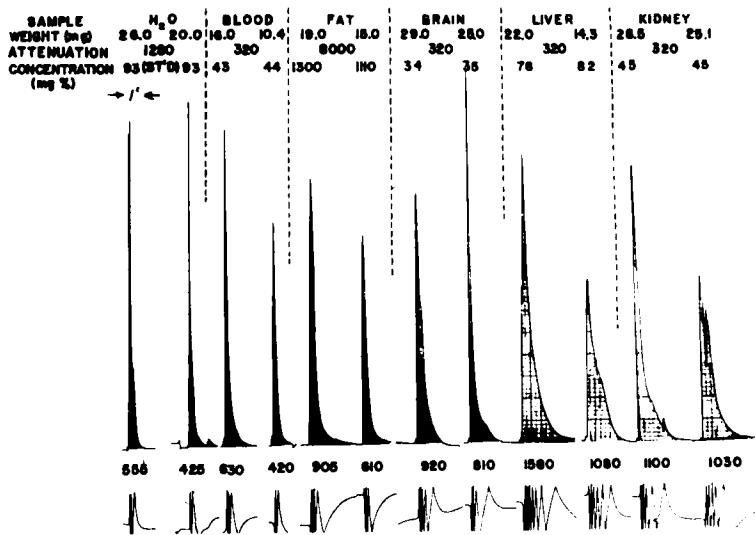


FIG. 2. Analysis of weighed tissues. Conditions for operation are described under Methods.

syringe. After thorough mixing for 15 minutes, the plasma and red cells were separated by centrifugation and the hematocrit recorded. When the plasma and red-cell anesthetic concentrations had been determined, the blood was remixed and the whole blood anesthetic concentration determined. The sum of the plasma and red cell response areas per microliter multiplied by the respective fractional composition (1 minus hematocrit and the hematocrit) equaled response area per microliter of whole blood. The plasma-red cell partition coefficients are shown in table 3 (fig. 1).

The difference in the solubility of the anesthetic in red cells and plasma (table 3) required that the samples be thoroughly mixed prior to whole blood analysis. The red cells separate rapidly in samples collected in heparinized capillaries and cannot be readily remixed unless the capillary is stored in the horizontal position. Mixing, in this situation, was accomplished by rapid rolling of the capillary on a flat surface or by means of a vibrator. If the red cells had settled out in a vertically placed capillary tube, the sample was centrifuged to determine the hematocrit and the red cell and plasma fractions analyzed separately.

Mice were exposed to 1 per cent halothane for three hours and sacrificed by obstructing the trachea. The tissues, obtained by biopsy,

were transferred to weighed capillaries and analyzed as described under Methods. Typical results of duplicate determinations of fat, brain, liver, and kidney specimens are shown in figure 2. The experimental conditions were the same as those described under methods. Each tissue analysis was completed within one to two minutes. Since each determination represented an analysis of a nonhomogenous specimen with varying amounts of connective tissue and blood, duplicate determinations frequently varied by several milligrams per cent. Determinations on homogenized tissue specimens (albeit with loss of anesthetic vapor) usually agreed within  $\pm 1$  per cent of the absolute concentration.

In several hundred preanesthetic determinations, only two out of 30 patients with diabetes showed any significant amounts of "ketone bodies." The long retention time of "ketone bodies" or ethyl alcohol on the water moistened column permitted accurate anesthetic determinations in the presence of these compounds.

When more than one volatile organic anesthetic agent was present, a 2- to 4-foot silicon rubber or "Tide" column placed in series with and proximal to the moist glass wool column permitted the separation and analysis of binary, tertiary, or even quaternary mixtures. The time required for analysis was increased by several minutes.

In addition to the anesthetic agents listed in table 1, vinethene, methylene chloride, ethylene, and divinyl ether have been analyzed in gases and blood.

### Discussion

Flame ionization detectors are specific for volatile organic compounds and do not respond to fixed gases such as O<sub>2</sub>, N<sub>2</sub>, N<sub>2</sub>O, CO<sub>2</sub>, ammonia, or water. Hydrogen flame detectors are 10<sup>3</sup> to 10<sup>4</sup> times more sensitive than thermal conductivity detectors. One part of organ vapor in one billion parts of hydrogen may be detected. In practice, the maximum sensitivity is achieved by employing the lowest nitrogen (carrier gas) and hydrogen flows compatible with stability of the instrument. The explosion hazard of the flame ionization detector is comparable with electronic devices employed in the operating room. The flame is enclosed in a chamber which is constantly purged with 200 to 300 ml. of air per minute.

In the operating room, the anesthesiologist can determine blood anesthetic concentrations every 20 to 30 seconds without assistance. The stability of the instrument does not require standardization more than three or four times daily. The linearity of response permits calibration with a single anesthetic standard.

Under a given set of conditions, the ionization coefficient of each anesthetic vapor relative to cyclopropane may be determined and used as a basis for standardization. If this method of standardization is selected, the relative ionization coefficients should be de-

termined with the particular instrument employed. The use of the constants in table 1 is not recommended, since these values are dependent upon the conditions of operation and instrumental characteristics.

One of the principal advantages of the method described is the ability to determine blood, gas, plasma, or red cell concentrations directly. The determination of partition coefficients is greatly simplified. The plasma-red cell distribution for halothane of 1.15 (table 3) is at variance with the value reported by Lawson *et al.*<sup>6</sup> The plasma-red cell distribution of anesthetic agents in ACD blood was consistently lower than the corresponding values determined in fresh whole blood.

Stewart *et al.*<sup>26</sup> have recently reported on the use of electron capture detectors<sup>27</sup> for the analysis of halogenated anesthetics in gases. This detector is approximately 100 times more sensitive than the flame ionization detector. Instruments of this type will undoubtedly play an ever increasing role in clinical anesthesia.

### Summary

A method is described for the analysis of any single volatile organic agent within 15 to 60 seconds in gas, blood, or tissue samples—interchangeably. Extraction procedures and chromatographic separations are avoided; since, in the vast majority of instances, only a single volatile agent is administered. The conditions for operation of the flame ionization detector are the same for all volatile organic agents, and the determination of any

TABLE 3. Distribution of Anesthetic Agents in Blood (plasma/red cells)

Agent	mg. %	Response Area per Microliter			Whole Blood Distribution (Calculated)*			
		<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>	<i>g</i>
		Plasma	Red Cells	Whole Blood	Plasma <i>a</i> (1 - 0.45)	Red Cells <i>b</i> (0.45)	Whole Blood <i>d</i> + <i>e</i>	Plasma Red Cells = $\frac{a}{b}$
Methoxyflurane	142	410	610	500	225	274	499	0.66
Halothane	186	264	230	250	145	104	249	1.15
Ether	72	990	777	902	544	350	894	1.27
Cyclopropane	18	240	448	339	132	202	334	.53
Chloroform	148	399	558	469	220	251	471	.73
Trifluoroethyl vinyl ether	113	364	298	316	200	134	334	1.22

\* Calculated from hematocrit which was 0.45 red blood cells.

agent may be performed in sequence with other agents. Standardization may be carried out with water solutions of each agent; or all agents may be standardized against pure cyclopropane as a primary standard. Techniques are described for the anaerobic transfer and storage of specimens in core solder without loss of anesthetic vapor for periods of two to three weeks. Complex mixtures of anesthetic vapors may be analyzed by addition of appropriate chromatographic columns; however, the instrumental analysis time may be increased by one to three minutes.

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