A new method for measurement of anaesthetic partial pressure in blood

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Summary
We have developed a simple, reliable method for rapid analysis of the partial pressure of volatile anaesthetic agents, based on a two-stage, headspace analysis. It is designed to solve the problems associated with reduced solubility of modern anaesthetics. After equilibration and analysis of a 2-ml sample of blood at 37°C, 1 ml is transferred to another vial for a second equilibration. This ensures that there is no vapour in the headspace before the second equilibration. Measurements were performed on human blood samples equilibrated with 1% sevoflurane, 2.5% isoflurane or 3% desflurane in a tonometer. The mean error in the sample measurements was ±2.3% of the tonometer reading and the 95% confidence interval for an individual measurement was ±8.5%. Blood samples may be stored overnight without any significant change in the results. (Br. J. Anaesth. 1997; 78: 449–452).

Key words

The rarity of clinical studies which measure partial pressures of anaesthetics in blood is a testimony to the troublesome nature of the assay. Blood content can be measured relatively simply by comparison, but partial pressure can be calculated only if the blood-gas partition coefficient is known. The classical headspace analysis technique for measurement of partial pressure involves equilibrating the anaesthetic between the blood sample and air (the headspace) within the containing vessel.1 2 The partial pressure in the headspace can then be measured easily, but is no longer equal to the initial partial pressure in the blood sample. However, if the headspace is repeatedly flushed with fresh gas and repeated equilibrations performed, then a curve of the exponential washout of the anaesthetic from the blood can be plotted. Projecting this curve one step backwards from the first equilibration should give the initial partial pressure of the anaesthetic vapour. The blood-gas solubility of the sample can be determined from the rate of washout if the volumes of blood and headspace are known. Blood can be equilibrated within the glass syringe used to obtain the sample1 2 (which has the advantage of maintaining ambient pressure in the headspace) or it can be transferred to a vial sealed with a PTFE-faced septum3 (which has a cost advantage). If the ratio of air to blood is very small, such as when a bubble is introduced into a syringe of blood and allowed to equilibrate, the reduction in partial pressure of anaesthetic within the blood is negligible and a single step measurement is adequate.4 We have found this technique troublesome in practice and in principle it is less appropriate for less soluble anaesthetics.

When we measured partial pressures using this classical technique our results grossly underestimated the partial pressure of desflurane in blood equilibrated in a tonometer, even though we were able to measure isoflurane partial pressures reasonably accurately. We thought that the error may be caused by trapping of desflurane in bubbles which prevented efficient flushing of the headspace between equilibrations. This would have a greater effect with desflurane than isoflurane because there is more desflurane in the gas within a bubble than in an equivalent volume of blood—the reverse is the case for isoflurane. We describe a simple method for accurate, reproducible and rapid analysis of the partial pressure of a volatile anaesthetic in small volumes of blood which does not depend on an assumed blood-gas partition coefficient.

Methods

EQUIPMENT
All gas samples were analysed using a Pye Series 204 gas chromatograph. The carrier gas was nitrogen flowing at 30 ml min−1. A 0.9-m glass column (2 mm id) packed with 3% OV-17 on GasChromQ was maintained at 125°C, producing a retention time of 0.5 min. The volatile agent was detected by a flame ionization detector operating at 175°C. All samples were injected into a 20-µl sampling loop (Valco) on the chromatograph inlet using a 100-µl
gas-tight syringe fitted with a valve (SGE, Melbourne).

Blood samples were prepared by equilibration in an IL-237 tonometer. A 2.5-ml gas-tight syringe (SGE, Melbourne) was used to transfer blood from the tonometer and between vials.

A cylinder of isoflurane in air was prepared and used as a standard to confirm stability of the gas chromatograph throughout the measurements. An approximate measurement of its concentration was made using a Datex Ultima (Datex Instrumentarium, Helsinki), calibrated according to the manufacturer’s recommendations.

PREPARATION OF BLOOD SAMPLES

Blood was obtained (with the approval of the chairman of the local Research Ethics Committee) from healthy volunteers and patients undergoing major surgery. A 6-ml blood sample was placed in the tonometer and allowed to equilibrate with a gas mixture flowing at 200 ml min⁻¹ for a period of 15 min. Mixtures of 1% sevoflurane, 2.5% isoflurane or 3% desflurane in air, and a mixture of 3% desflurane, 5% carbon dioxide and 21% oxygen (balance nitrogen) were used. A gas sample was obtained from the outflow of the equilibration chamber into the 100-μl syringe and injected into the gas chromatograph. This was taken to be the “true” partial pressure of anaesthetic in the blood. Two 2-ml aliquots of blood were then transferred accurately into nominally 5-ml vials (mean 4.77 (SEM 0.01) ml, measured by water displacement) and closed by a screw cap sealed with a Teflon-faced septum. The analysis was generally undertaken immediately but in order to investigate the effect of delay in measurement, one sample of some pairs was stored in a refrigerator and analysed 24 h later.

ANALYSIS TECHNIQUE

The vials were agitated in a water bath at 37°C for 1 min. Headspace gas was then sampled using a 100-μl syringe by puncturing the vial septum and making several movements of the plunger. Two samples were obtained and injected into the gas chromatograph: the greater reading (almost invariably the first, but differences were generally less than 2%) was used in the calculations. Exactly 1 ml of blood was transferred into another 5-ml vial and sealed with a new cap and septum for a second period of equilibration at 37°C and subsequent analysis of the headspace gas (see fig. 1). The syringe was flushed several times with room air between samples to avoid carry over, and frequent air samples were injected into the gas chromatograph to confirm that syringe contamination was negligible.

CALCULATIONS

When the sample is equilibrated in the water bath the pressure within the headspace increases because of warming, humidification and exchange of gases with the blood sample. When the headspace sample is injected into the gas chromatograph it is at room temperature and pressure: the concentration of desflurane is unchanged but the partial pressure is reduced, so the chromatograph reading must be increased to compensate for this. The increase in pressure caused by temperature should be given by a factor of:

\[(273 + 37)/(273 + T_{lab})\]

where \(T_{lab}\) = laboratory temperature in Celsius and typically gives a 5% increase. Humidification increases pressure by 6 kPa, but when the sample is aspirated into the chromatography syringe it cools and the vapour pressure of water reduces to approximately 3 kPa, and our calculations include this factor to correct for the “dilution” of the anaesthetic by water vapour in the headspace sample.

Carbon dioxide in blood also equilibrates with the headspace increasing the total headspace pressure. Some correction for this should be made, but the relationship between carbon dioxide content and tension is complex. We have crudely assumed that the content is 0.1 ml (ml of blood)⁻¹ kPa⁻¹. Then a 2-ml blood sample equilibrated with \(x\) kPa carbon dioxide contains 0.2x ml of carbon dioxide. After equilibration with 3 ml headspace the total vial carbon dioxide content is \(0.2y/101.3\) kPa, where \(y = \text{carbon dioxide tension after equilibration in kPa}\). Assuming that no carbon dioxide is lost from the vial we deduce that \(y = 0.2x/0.23 = 0.87x\), so the pressure within the headspace increases by 0.87 of the fractional partial pressure of carbon dioxide in blood before the first equilibration. A similar effect occurs during the second equilibration, although the volumes of blood and headspace have changed, and we allow for an increase in headspace pressure of 0.63x at the time the second sample is obtained. Finally, the volatile anaesthetic itself increases the

Figure 1 Methodology flowchart.
headspace pressure (although this is only likely to be important for desflurane). Conversion of gas chromatograph counts to partial pressure of the syringe sample \((P_{\text{syringe}})\) is achieved by sampling intermittently from a cylinder containing a known concentration of the vapour concerned, \(P_{\text{syringe}}\) is converted to partial pressure within the vial \((P_{\text{vial}})\) by

\[
P_{\text{vial}} = \frac{P_{\text{syringe}} \times 310}{(273 + T_w) \times (1 + F_{\text{water}} + F_{\text{CO}_2} + F_{\text{anaesthetic}})}
\]

where \(F_{\text{water}}\) is taken to be 0.03, \(F_{\text{CO}_2}\) fractional carbon dioxide tension in the vial, and \(F_{\text{anaesthetic}}\) is taken to be \(F_{\text{syringe}}\) expressed as a fraction of ambient pressure.

Simple back projection no longer works because the ratio of blood to air is not constant. Let \(P_0\) be the partial pressure of desflurane in the sample when it was first drawn; let \(P_1\) and \(P_2\) be the partial pressures in the vial after the first and second equilibrations, respectively; let \(V_o, V_1\) and \(V_2\) be the volumes of the vial, the first blood sample (2 ml) and the sample transferred (1 ml), respectively; let \(\lambda\) be the blood gas coefficient at 37°C. The gas chromatograph readings are corrected for pressure changes in the vial caused by humidification and warming of air. Then equating the amount of desflurane in the vial before and after each equilibration:

\[P_0 V_o \lambda = P_1 V_1 \lambda + P_1 (V_o - V_1)\]

and

\[P_0 V_o \lambda = P_2 V_2 \lambda + P_2 (V_o - V_2)\]

The second equation is solved for \(\lambda\) and this value is used to determine \(P_0\) in the first. The calculations were performed using Microsoft Excel v4.

**Results**

Data are presented as percentage of the tonometer concentration and are shown in figure 2. Analysis of variance revealed no difference between the results for the different anaesthetics. Overall the mean error of the 93 samples was 2.3%, with the 95% confidence interval of the mean \((-3.2\%\, \pm\, 1.4\%)\) failing to include zero, that is there was a systematic error. The mean difference (confidence interval of the mean) between 11 paired samples analysed 24 h apart was 2.0% \((-0.8\%\, \pm\, 4.8\%)\) of the tonometer reading.

**Discussion**

When we attempted to measure the partial pressure of desflurane using the method described by Landon and colleagues for isoflurane, we consistently obtained values less than the tonometer. We blamed incomplete flushing of the headspace between equilibrations caused by trapping of desflurane vapour in froth and bubbles, noting that such errors are more significant the less soluble the agent. We therefore decided to transfer an aliquot of blood to a new vial to overcome this problem.

When the samples are equilibrated in vials it is necessary to allow for pressure changes in the headspace. Our corrections for this are exclusively based on simple, theoretical physical principles, yet they are not commonly used in this context. When a physiological concentration of carbon dioxide was present in the tonometer gas our results would have underestimated by approximately 5% without the appropriate correction factor. We have been able to ignore the exchange of oxygen between blood and sample because the carrier gas for the tonometer was air and the headspace also initially contains air. There may however be an effect if blood is not fully saturated with oxygen because uptake of oxygen by blood reduces absolute headspace pressure.

When multiple headspace equilibrations are used, successive measurements lie on an exponential washout curve and all points contribute to its determination. It is simple to calculate the best fit to such a function if the data are assumed to be homoscedastic, that is measurements at each equilibration have equal variances. We experimented with multiple sample transfers and washouts, but it seemed unlikely that our data would be homoscedastic given that blood volume was reduced at each step, and it was not clear how to weight the contribution of each equilibration in the new washout function. We therefore calculate the sample blood-gas coefficient from the results of the second equilibration and use that result with the first measurement to calculate the initial partial pressure of desflurane in the sample.

Although there is a systematic error in our application of this technique, it is small compared with the confidence interval for a measurement and it is even possible that equilibrium within the tonometer was incomplete. The important result for an individual sample is the interval about it within which the true value is expected to lie. The confidence interval of 8.5% of the reading for an individual sample because the carrier gas for the tonometer was air and the headspace also initially contains air. There may however be an effect if blood is not fully saturated with oxygen because uptake of oxygen by blood reduces absolute headspace pressure.

![Figure 2](image-url) Figure 2 Results from the four equilibration gas mixtures. The solid bars indicate the 95% confidence interval of the mean, the error bars indicate the 95% prediction interval for an individual measurement.
performed four headspace equilibrations. When they tested this method using blood tonometered with 0.24% isoflurane they found a mean error of less than 1% of the true value and a variability similar to ours in absolute terms, but because we used 1% isoflurane in the tonometer, our variability was less as a proportion of the true value. Dwyer and co-workers\textsuperscript{5} performed two headspace equilibrations of 5-ml samples within syringes and found a systematic error of $-4\pm7\%$ (cf. our $-2.3\pm8.5\%$) when measuring blood equilibrated with isoflurane in a tonometer. Several groups have used a single headspace equilibration to determine the partial pressure of gas within the sample. The headspace measurement is related to initial partial pressure by a nomogram which either relies on an assumed blood-gas coefficient or requires tonometry of a separate blood sample with a known gas mixture. This presents problems if the blood-gas coefficient is variable (e.g. during cardiopulmonary bypass). Frei and co-workers\textsuperscript{6} described a technique of single headspace equilibration of 0.5 ml of blood in a 5-ml vial. When measuring isoflurane they obtained a coefficient of variation of 2.8% (c.f. our 4.3%). Their method allows one analyst to process 40 samples per hour using a given nomogram. Flynn and co-workers\textsuperscript{7} injected each of five 5-ml vials with 0.6 ml of blood from the same sample, taking the mean value as the result for that sample. They claimed a coefficient of variation of less than 4% for isoflurane, which was less than the error in the slope of their nomogram (1.93±0.11). Katoh and colleagues\textsuperscript{8} measured sevoflurane partial pressures in arterial blood from a single headspace equilibration. They measured the blood-gas solubility in each case by obtaining a blood sample before anaesthesia and equilibrating it with a known amount of sevoflurane vapour, but did not validate their technique.

In summary, the two-stage method described in this article allows rapid measurement of the partial pressure of desflurane and blood-gas solubility in a single 2-ml sample of blood with accuracy and repeatability similar to published results on the measurement of isoflurane.

References