Prediction of volatile anaesthetic solubility in blood and priming fluids for extracorporeal circulation

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Volatile anaesthetics are often used during cardiopulmonary bypass (CPB). To understand the kinetics of inhaled anaesthetics during CPB, anaesthetists should understand changes in blood solubility caused by fluid use. We set out to predict the solubility of three volatile anaesthetics, desflurane, iso-flurane and halothane, during CPB by determining: (i) their solubility in fresh whole blood and eight CPB priming fluids at 37°C; (ii) the effect of temperature on the solubility of these anaesthetics in lactated Ringer’s, gelofusin, banked blood and plasma; (iii) their solubility in different mixtures of these four priming fluids at different temperatures; and (iv) their estimated and actual solubility in blood during hypothermic CPB. We calculated solubility using a concept of volume fraction partition coefficient and compared estimated and measured solubilities. For the three anaesthetics tested, solubilities are in the order: fresh whole blood > plasma > banked blood > normal saline = lactated Ringer’s = gelofusin = Haemaccel = hydroxyethyl starch = mannitol. The solubilities of the anaesthetics in all priming fluids increased logarithmically at lower temperatures (P<0.05). The volume-fraction estimates of the partition coefficients were within approximately ±20% of the measured values for all values of solubility. The corresponding estimates of solubility for CPB blood samples were between −36% and +24% of the measured values. During normothermic CPB, blood solubility of volatile anaesthetics would be unchanged when using plasma, slightly reduced when using banked blood and markedly reduced when using crystalloids and colloids.

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During cardiopulmonary bypass (CPB), volatile anaesthetics can be added to the oxygenator to provide anaesthesia,1, 2 regulate systemic vascular resistance3, 4 and reduce hormonal responses to CPB.5, 6 The rate of wash-in and wash-out of volatile anaesthetics via oxygenators depends on their solubility in blood.7 Two important factors affect the solubility of volatile anaesthetics: hypothermia increases solubility,7–12 but crystalloid haemodilution decreases it.11, 12 Although the effect of hypothermia on solubilities in saline and plasma has been studied,12 and although the effects of hypothermia and crystalloid haemodilution on blood solubility of halothane, enflurane and isoflurane have been observed in CPB,13–16 the solubility of volatile anaesthetics in colloid and other priming fluids has not been investigated. Data for the recently introduced agent, desflurane, are limited. To provide such information for predicting blood/gas partition coefficients (λB/G) during CPB, we studied the following: (i) solubility of desflurane, isoflurane and halothane in eight CPB priming fluids; (ii) effect of temperature on the solubility of these anaesthetics in four CPB priming fluids; (iii) estimated and measured solubilities of the three anaesthetics in different combinations of the four priming fluids at different temperatures; and (iv) predicted and measured λB/G of the three agents during hypothermic CPB.

Methods

Liquid/gas partition coefficients (λ) were measured using a two-stage headspace equilibration method (see below) for fresh whole blood, plasma, banked blood, CPB priming fluids, mixtures of different primes, and diluted blood in
CPB. Coefficients were obtained for desflurane, isoflurane and halothane simultaneously by using a mixture of the three anaesthetic vapours in air.

**Solubility of volatile anaesthetics in eight CPB priming fluids**

The solubilities of desflurane, isoflurane and halothane in banked blood (Blood Bank, Beijing, China), plasma (Blood Bank, Beijing), normal saline (China Dazhong, Tianjing, China), lactated Ringer’s solution (China Dazhong, Tianjing, China), gelofusin (Braun, Switzerland), Haemaccel (Behring, Germany), hydroxyethyl starch (Changshu Pharmaceutical, Jiangshu, China) and mannitol (ZhenDa TianQing, Jiangshu, China) were measured by gas chromatography (see below). We used 10 samples of each solution at 37°C. To compare fresh whole blood with banked blood, the solubility in fresh whole blood (Fuwai Hospital) was measured. Collection of fresh whole blood was approved by the Committee of Scientific Research in Fuwai Hospital and informed consent was obtained from each of the 10 male healthy volunteers aged 23 (22–25) yr (mean (range)).

**Effect of temperature on solubility of anaesthetics in CPB priming fluids**

The solubility of desflurane, isoflurane and halothane in lactated Ringer’s solution (a crystalloid solution), gelofusin (a colloid solution), plasma and banked blood was measured at 37, 33, 29, 25, 21 and 17°C. We used six samples of each fluid at each temperature.

**Solubility of anaesthetics in mixtures of different priming fluids**

Lactated Ringer’s solution, gelofusin, plasma and banked blood were mixed in different proportions, as determined by a computer, to give 494 mixtures, each with a volume of approximately 270 ml. We randomly chose 10 of these mixtures which had different proportions of the four constituents (Table 1). The solubilities of desflurane, isoflurane and halothane in these 10 mixtures were measured at 37, 33, 29, 25, 21 and 17°C, using six samples from each mixture at each temperature. Estimated λ of the mixtures was calculated from the equation: estimated \( \lambda = \sum (\lambda_x \times F_x) \) where \( \lambda_x \) is the solubility of the agent in each constituent and \( F_x \) is the fractional concentration of each constituent in the total mixture. We called this the volume fraction partition coefficient.

**Solubility of anaesthetics in blood during CPB**

After approval from the Committee of Scientific Research in Fuwai Hospital, we obtained informed consent from 20 adult patients undergoing valve replacement surgery requiring CPB. Patients were anaesthetized using total intravenous anaesthesia. Lactated Ringer’s solution or gelofusin was administrated before CPB. The CPB circuit were primed with lactated Ringer’s solution and gelofusin. Blood samples were taken 15 min after the beginning of CPB to allow a steady state to be reached after haemodilution by priming fluid. Urine output, type and volume of infused fluids, and CPB priming fluid were noted. Blood loss was estimated before the blood sample was collected. The solubility of desflurane, isoflurane and halothane in each blood sample (measured \( \lambda_{\text{B/G}} \) of diluted blood in CPB) were measured at 37, 33, 29, 25, 21 and 17°C. The solubilities of the three anaesthetics in each blood sample were estimated using the concept of volume fraction partition coefficient. The method is given in the Appendix.

**Gas chromatography and determination of solubility**

Anaesthetic concentration was measured with a GOW-MAC 580 gas chromatograph equipped with a 6 m stainless steel column (0.32 cm internal diameter) packed with Chromosorb-P60/80 mesh maintained at 75°C. We used a 10 ml min\(^{-1}\) nitrogen carrier flow, and a flame ionization detector supplied with hydrogen at 35 ml min\(^{-1}\) and air at 300 ml min\(^{-1}\). The output was passed to a TAI-SSC922 integrator and peak areas were calculated. Under these conditions, the peaks of desflurane, isoflurane and halothane were completely separated. Primary and secondary (compressed gas tank) standards were used for calibration. Primary standards were made by injecting an aliquot of each anaesthetic into a glass flask of known volume with a syringe. Because of the high saturated vapour pressure of desflurane, we took steps to ensure that no desflurane was lost. Liquid desflurane and the syringe were kept at 4°C in a refrigerator. Liquid desflurane was drawn into the cool syringe at 4°C in the refrigerator and was injected into the flask or tank immediately. The primary standards (glass flask) were used to calibrate the secondary standards; the secondary standards (tank) were injected at intervals to calibrate the gas chromatograph during each study. All \( R^2 \) of the linear regression between concentration of anaesthetics

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**Table 1** Composition of mixtures of priming fluids

<table>
<thead>
<tr>
<th>Amount (ml) in mixture</th>
<th>Lactated Ringer’s</th>
<th>Gelofusin</th>
<th>Plasma</th>
<th>Banked blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixture 1</td>
<td>54</td>
<td>108</td>
<td>0</td>
<td>108</td>
</tr>
<tr>
<td>Mixture 2</td>
<td>54</td>
<td>54</td>
<td>108</td>
<td>54</td>
</tr>
<tr>
<td>Mixture 3</td>
<td>102</td>
<td>68</td>
<td>68</td>
<td>34</td>
</tr>
<tr>
<td>Mixture 4</td>
<td>68</td>
<td>34</td>
<td>34</td>
<td>136</td>
</tr>
<tr>
<td>Mixture 5</td>
<td>216</td>
<td>0</td>
<td>0</td>
<td>54</td>
</tr>
<tr>
<td>Mixture 6</td>
<td>39</td>
<td>193</td>
<td>39</td>
<td>0</td>
</tr>
<tr>
<td>Mixture 7</td>
<td>45</td>
<td>45</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Mixture 8</td>
<td>45</td>
<td>135</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>Mixture 9</td>
<td>193</td>
<td>0</td>
<td>77</td>
<td>0</td>
</tr>
<tr>
<td>Mixture 10</td>
<td>39</td>
<td>154</td>
<td>77</td>
<td>0</td>
</tr>
</tbody>
</table>

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Solubilities of volatile anaesthetics in CPB primes and blood
and peak area of gas chromatography output were higher than 0.9995 throughout the study. The regression equation was used to convert peak area to agent concentration. Peak areas were proportional to concentrations over the entire range of the concentrations tested.

A gas mixture of desflurane, isoflurane and halothane for equilibration in solubility determination was prepared as follows. A stainless steel cylinder (8.1 litres) was evacuated to a pressure of about 0.5 atm less than ambient. Liquid desflurane, isoflurane and halothane were aspirated into the cylinder and the cylinder was filled with compressed air. We calculated the volume of the three liquid anaesthetics transferred into the cylinder and the compressed air pressure required in the cylinder to ensure that the total pressure of each anaesthetic in the cylinder was <90% of its saturated vapour pressure. The cylinder was rolled for 30 min to mix the contents in the cylinder thoroughly. The anaesthetic concentrations in the cylinder were then calibrated using the equilibration in solubility determination was prepared as follows.

We transferred into the cylinder and the compressed air pressure required in the cylinder to ensure that the total pressure of each anaesthetic in the cylinder was <90% of its saturated vapour pressure. The cylinder was rolled for 30 min to mix the contents in the cylinder thoroughly. The anaesthetic concentrations in the cylinder were then calibrated using the primary standard; it contained 1.65% desflurane, 1.78% isoflurane and 1.85% halothane.

A 20 ml gas-tight glass syringe, calibrated precisely and capped with a three-way stopcock, was sealed by coating the plunger with a thin layer of silicone grease. The gas-tightness of these grease-sealed syringes was tested before the study: the concentrations of anaesthetic vapours in the syringes decreased by no more than 2% over 8 h. This also showed that the grease did not absorb anaesthetic. Approximately 7 ml of liquid sample was drawn into a syringe and the above-mentioned anaesthetic gas mixture was added to give 18 ml; the three-way stopcock was then closed. The syringe was shaken vigorously and immersed in a waterbath at the chosen test temperature. Every 15 min for 2 h, the syringe was shaken vigorously for 5–10 s. After the third shaking, the plunger of the syringe was withdrawn to the 20 ml position with the stopcock closed, causing a small negative pressure in the syringe. The stopcock was then opened briefly to allow air into the syringe and to restore ambient pressure in the syringe. After this 2 h period (the first equilibration period), the concentration of anaesthetic \((C_1)\) in the gas phase of the syringe was analysed by gas chromatography. All the gas and some of the liquid in the syringe were expelled and exactly 4 ml of liquid sample \((V_L)\) was retained in the syringe for the second equilibration. Vapour-free air was drawn in to move the plunger to the 18 ml position. The syringe was shaken vigorously and immersed in the waterbath with the same temperature as in the first equilibration period. The second equilibration had the same sequence of shaking, volume adjustment and timing as in the first equilibration. At the end of the second equilibration period, the concentrations of anaesthetics in gas phase \((C_2)\) were analysed by gas chromatography.

The total amount of anaesthetic \((\text{ml, in liquid plus gas phase})\) after the second equilibration is equal to that in the liquid phase which was retained in syringe after the first equilibration. This relationship can be expressed as:

\[
C_2 \times V_G + C_{L2} \times V_L = C_{L1} \times V_L
\]

(1)

where \(V_G\) and \(V_L\) are the gas volume and sample volume retained in the syringe for the second equilibration, respectively; \(C_{L1}\) and \(C_{L2}\) are the anaesthetic concentrations in liquid samples at the end of the first and second equilibration, respectively. \(\lambda\) is defined as the ratio of anaesthetic concentration (vol%) in liquid phase to that in gas phase (vol%), e.g. \(C_{L1} = \lambda \times C_1\) and \(C_{L2} = \lambda \times C_2\). Substituting these into equation (1) yields:

\[
C_2 \times V_G + \lambda \times C_{L2} \times V_L = \lambda \times C_1 \times V_L
\]

This equation can be rearranged to give:

\[
\lambda = \left( \frac{V_G}{V_L} \right) \times \left( \frac{C_2}{(C_{L1} - C_2)} \right)
\]

Equation (3) was used to calculate \(\lambda\).

Statistical analysis

Means and standard deviations were obtained for \(\lambda\) in priming fluids and blood. Solubilities in each priming fluid at 37°C for each anaesthetic were compared with those in fresh whole blood using Student’s \(t\)-test. The ratio of mean solubility in each priming fluid and mean solubility in fresh whole blood \((R_{PB})\) was calculated. Repeated-measures analysis of variance was used to determine the difference among the three anaesthetics for each priming fluid. We related \(\log_{10}\lambda\) in each fluid to temperature, and calculated residual standard deviation (RSD) and 95% confidence limits (CL) of the slope and intercept of the regression lines. To assess the concept of volume fraction partition coefficient and the methods of predicting solubility given in the Appendix, regression and Bland and Altman’s ‘limits of agreement’ analysis\(^{17}\) were performed between estimated \(\lambda\) in mixtures of priming fluids and in CPB blood and the corresponding measured \(\lambda\). \(P<0.05\) was accepted as statistically significant.

Results

Table 2 shows the solubility of desflurane, isoflurane and halothane measured at 37°C. Their solubilities in fresh whole blood were not significantly different from those in plasma \((P>0.05)\), but were significantly greater than those in the other priming fluids and in ACD solution \((P<0.05)\).

For all three anaesthetics, the liquid/gas partition coefficients increased as temperature decreased (Table 3; four CPB priming fluids in step (ii), diluted blood during CPB in step (iv)). To estimate \(\lambda\) in CPB blood in step (iv), we used \(\lambda\) values from our previous study.\(^{18}\) The solubilities were in the order: desflurane < isoflurane < halothane in all conditions. As temperature decreased, \(\log_{10}\lambda\) increased linearly \((P<0.05)\). The temperature coefficient of \(\lambda\) (percentage change in \(\lambda\) per degree centigrade) was calculated.\(^{19}\)

Details of the fitted linear equations for the dependence of \(\log_{10}\lambda\) on temperature are given in Table 4. The RSDs are in terms of \(\log_{10}\lambda\) and lie between 0.021 and 0.075 for different
media at different temperatures; these correspond to RSDs of between 2.1% and 7.5% of the arithmetic values of $\lambda$.

As anticipated, $\log_\lambda$ of desflurane, isoflurane and halothane in the prime mixtures increased linearly as temperature decreased. We found a direct linear relationship between logarithm of estimated $\lambda$ ($\log_\lambda$) and measured $\lambda$ ($\log_\lambda_m$) in mixed primes for desflurane, isoflurane and halothane ($P<0.05$, Figure 1A). Figure 1B shows the ‘limits of agreement’ analysis between estimated and measured $\log_\lambda$. The mean difference between estimated and measured $\log_\lambda$ was $-0.115$ and the SD was 0.164, which indicated that the estimated $\lambda$ in the CPB blood were within $+24\%$ and $-36\%$ of the measured values of $\lambda$.

**Discussion**

Although volatile anaesthetics are regularly used for open heart surgery, the solubility of anaesthetics in different CPB priming fluids has not been described before. Partition coefficients in all primes at $37°C$ were smallest with desflurane and greatest with halothane, a similar pattern as in fresh whole blood. This suggests that the differences in pharmacokinetics between the
three anaesthetics will not be affected during CPB. However, when a priming fluid is mixed with blood, the solubility in blood will decrease to an extent which depends on \( R_{PB} \) (Table 2); the smaller the ratio, the greater the reduction in \( \lambda \) for the mixture. For all the three anaesthetics tested, \( R_{PB} \) of the eight priming fluids are in the following order: plasma (1.00) > banked blood > normal saline = lactated Ringer’s solution = gelofusin = Haemaccel = hydroxyethyl starch = ACD solution > mannitol (Table 2). This order implies that, during normothermic infusion and normothermic CPB, \( \lambda \) in diluted blood would be unchanged by using plasma, slightly decreased by using banked blood and greatly decreased by using crystalloidal and colloidal solutions.

As anticipated, reduction in temperature caused an increase in solubility of the anaesthetics in all priming fluids, mixtures and CPB blood. This is consistent with the change for fresh whole blood.\(^7\)\(^{-12}\)\(^{18}\) Table 3 gives the temperature coefficients of \( \lambda \). Taking all agents and all solutions tested in this study into account, no correlation was found between temperature coefficient and its \( \lambda \) at \( 37^\circ\)C. However, for a given solution (with a minor exception for lactated Ringer’s), the magnitudes of the temperature coefficients were in the same order as the partition coefficients at \( 37^\circ\)C: desflurane < isoﬂurane < halothane.

We assumed that the solubility of an anaesthetic in a mixture of different solutions is equal to the sum of the solubility in each component multiplied by its volume fraction, a concept we call ‘volume fraction partition coefficient’. This concept was substantiated in this study. For example, 1 unit of banked blood (250 ml in volume) used in this study consists of 200 ml (80% in volume fraction) whole blood and 50 ml (20% in volume fraction) ACD solution, and solubility of desflurane in banked blood at \( 37^\circ\)C would be 0.52×0.80% + (0.29×20%). The estimated solubilities of desflurane, isoﬂurane and halothane in banked blood were 0.47, 1.18 and 2.10, respectively, almost identical to the measured solubilities of banked blood (0.45, 1.17 and 2.14, respectively; Table 2). We also estimated solubility in 10 mixtures containing different proportions of priming fluids and found a close relationship between measured and estimated solubility (Figure 1A). The limits of agreement (equal to mean±2 SD of the differences, which will include about 95% of the data points) are 0.201 and –0.221 (Figure 1B). We have shown that the solubility of volatile anaesthetics in CPB primes can be predicted and that the concept of volume fraction partition coefficient is useful for the prediction.

For the agents studied, wash-in and wash-out will be quickest for desflurane and slowest for halothane at any given temperature, and slower at low temperature for all agents (Table 3), but quicker on dilution of blood with priming fluids (other than plasma) (Table 2). During CPB, hypothermia will increase anaesthetic blood solubility and haemodilution will reduce it. The effects of these two factors on blood solubility of halothane, enflurane and isoﬂurane have been observed in CPB.\(^{13}\)\(^{-16}\) It is not practical to monitor the solubility of the anaesthetics during CPB. If body temperature and the nature and degree of haemodilution by priming fluid are monitored, solubility can be estimated to within about –36% to 24% of the measured value (Figure 2). Therefore, changes in the rates of wash-in and wash-out of the anaesthetics are likely.

### Table 4 Regression equations for predicting solubility from temperature (°C) for four priming fluids, fresh whole blood (from previous study\(^1\)) and CPB blood. \( \log_2\lambda=\text{slope}\times T/\text{Intercept}. \) RSD= residual standard deviation; \( R^2= \) coefficient of determination for regression line; CL= confidence limit

<table>
<thead>
<tr>
<th>Priming Fluid</th>
<th>Lactated Ringer’s</th>
<th>Gelofusin</th>
<th>Banked blood</th>
<th>Plasma</th>
<th>Fresh whole blood</th>
<th>Diluted blood in CPB</th>
<th>Isoﬂurane</th>
<th>Lactated Ringer’s</th>
<th>Gelofusin</th>
<th>Banked blood</th>
<th>Plasma</th>
<th>Fresh whole blood</th>
<th>Diluted blood in CPB</th>
<th>Halothane</th>
<th>Lactated Ringer’s</th>
<th>Gelofusin</th>
<th>Banked blood</th>
<th>Plasma</th>
<th>Fresh whole blood</th>
<th>Diluted blood in CPB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope (95% CL)</td>
<td>(-0.042) (-0.050, -0.033)</td>
<td>(-0.031) (-0.037, -0.025)</td>
<td>(-0.028) (-0.032, -0.024)</td>
<td>(-0.027) (-0.036, -0.019)</td>
<td>(-0.028) (-0.038, -0.018)</td>
<td>(-0.042) (-0.048, -0.036)</td>
<td>(-0.048) (-0.057, -0.039)</td>
<td>(-0.040) (-0.045, -0.035)</td>
<td>(-0.043) (-0.047, -0.039)</td>
<td>(-0.047) (-0.053, -0.041)</td>
<td>(-0.040) (-0.044, -0.035)</td>
<td>(-0.043) (-0.051, -0.034)</td>
<td>(-0.045) (-0.056, -0.034)</td>
<td>(-0.043) (-0.047, -0.039)</td>
<td>(-0.049) (-0.060, -0.038)</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Intercept (95% CL)</td>
<td>(-0.221) (-0.026, 0.467)</td>
<td>(-0.034) (-0.198, 0.130)</td>
<td>0.266 (0.151, 0.382)</td>
<td>0.309 (0.070, 0.549)</td>
<td>0.437 (0.160, 0.714)</td>
<td>0.536 (0.366, 0.706)</td>
<td>1.102 (0.853, 1.352)</td>
<td>0.883 (0.753, 1.013)</td>
<td>1.518 (1.419, 1.618)</td>
<td>1.604 (1.257, 1.951)</td>
<td>1.700 (1.600, 1.800)</td>
<td>1.636 (1.412, 1.859)</td>
<td>1.413 (1.252, 1.574)</td>
<td>1.237 (1.109, 1.366)</td>
<td>2.132 (1.894, 2.370)</td>
<td>2.254 (1.938, 2.569)</td>
<td>2.547 (2.439, 2.655)</td>
<td>2.297 (1.987, 2.607)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSD</td>
<td>0.0532</td>
<td>0.0354</td>
<td>0.0250</td>
<td>0.0519</td>
<td>0.0660</td>
<td>0.0368</td>
<td>0.0540</td>
<td>0.0282</td>
<td>0.0215</td>
<td>0.0752</td>
<td>0.0217</td>
<td>0.0484</td>
<td>0.0348</td>
<td>0.0278</td>
<td>0.0516</td>
<td>0.0684</td>
<td>0.0234</td>
<td>0.0871</td>
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</table>
Our methods have some limitations. We cannot accurately estimate blood loss and circulating blood volume and did not take metabolism of colloid into account. This may have caused the systematically greater value of limits of agreement in predicting \( \lambda \) in CPB blood than that in prime mixtures (±36% to +24% in CPB blood compared with about ±20% in prime mixtures). However, our method will help anaesthetists judge changes in blood solubility of volatile anaesthetics during CPB, and the effect of this change on their pharmacokinetics.

**Acknowledgements**

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Appendix: estimating the solubility of volatile anaesthetics in CPB blood

(1) Estimate following circulating volumes (ml): (i) fresh whole blood volume ($V_{fwb}$), i.e. patient’s body weight (kg) multiplied by 70 (ml kg$^{-1}$); (ii) net added crystalloid volume ($V_{cry}$), i.e. (infused crystalloid plus primed crystalloid minus urine output)/3; (iii) added colloid volume ($V_{col}$), i.e. infused colloid plus primed colloid; (iv) added banked blood volume ($V_{bb}$), i.e. infused banked blood plus primed banked blood; (v) added plasma volume ($V_{p}$), i.e. infused plasma plus primed plasma; and (vi) total circulating blood volume ($V_{total}$) = $V_{fwb} + V_{cry} + V_{col} + V_{bb} + V_{p}$.

(2) Calculate solubilities for fresh whole blood ($\lambda_{fwb}$), crystalloid ($\lambda_{cry}$), colloid ($\lambda_{col}$), banked blood ($\lambda_{bb}$) and plasma ($\lambda_{p}$) at the temperature at which $\lambda$ is estimated by using the equations in Table 4.

(3) Estimated $\lambda$ of CPB blood = ($V_{fwb} \times \lambda_{fwb}$) + ($V_{cry} \times \lambda_{cry}$) + ($V_{col} \times \lambda_{col}$) + ($V_{bb} \times \lambda_{bb}$) + ($V_{p} \times \lambda_{p}$) / $V_{total}$

References


12 Han YH, Helrich M. Effect of temperature on solubility of halothane in human blood and brain tissue homogenate. Anest Analg 1966; 45: 775–80


