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Osmolarity Determines the Solubility of Anesthetics in Aqueous Solutions at 37° C

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The authors determined whether they could predict accurately the solubility of anesthetics in aqueous solutions at 37° C, knowing the osmolarity and the *p*H of the solution and the solute composition.

The partition coefficients of the four volatile anesthetics, isoflurane, enflurane, halothane, and methoxyflurane, were determined concurrently at 37° C between air and aqueous solutions containing sodium chloride, dextrose, mannitol, or heparin. The osmolarities of these solutions ranged from 0 to 7,000 mOsm/l. The partition coefficients decreased linearly with increasing osmolarity when plotted on a semilogarithmic scale. The effect of osmolarity on the partition coefficient of the alkane anesthetic, halothane, was 20% less ($P < 0.001$) than the effect of osmolarity on the partition coefficients of the three methyl-ethyl ether anesthetics, isoflurane, enflurane, and methoxyflurane. The solubility of anesthetics in aqueous solutions did not depend on either the molecular structure of the solute or the *p*H of the solution. The solubility of volatile anesthetics in aqueous solutions at 37° C is inversely and predictably dependent on the osmolarity of the solutions. (Key words: Anesthetics, volatile: enflurane, halothane, isoflurane, methoxyflurane. Solubility: partition coefficients.)

MARKHAM AND KOBE reported that the solubility of carbon dioxide and nitrous oxide in aqueous solutions decreased linearly as the solute concentration (osmolarity) increased.[1] In addition, other investigators found that the solubility of halothane in normal saline decreased 7% from that in distilled water.[2,3] These studies indicated that the solubility of volatile anesthetics in aqueous solutions decreased when the concentration of solute increased (salting-out effect).[4,5]

However, the relevance of these findings to the solubility of volatile anesthetics in aqueous solutions, in general, is unclear for four reasons: 1) the relationship between osmolarity and the solubility of anesthetics in aqueous solutions has not been determined; 2) the effect of osmolarity on the solubility of anesthetics other than halothane has not been determined; 3) the effect of solutes of different molecular structure on the solubility of anesthetics has not been determined; and 4) the effect of *p*H on the solubility of anesthetics in aqueous solutions has not been determined. That is, at present we cannot predict the solubility of anesthetics in aqueous solutions from a single solubility such as that in distilled water. Specific solubilities must be determined for each aqueous or biologic solution requiring study.[3,6]

Therefore, we determined the effects of changes in the osmolarity and *p*H of aqueous solutions and the effect of solutes of different molecular structure on the solubility of volatile anesthetics in solution. Our results suggest that the solubility of anesthetics in aqueous solutions can be predicted reasonably if the solubility is known for two solutions of different osmolarities.

Methods

Thirty-milliliter glass syringes, each fitted with a nylon stopcock, were sealed air tight by coating the barrel (above the 20-ml mark) with a thin layer of silicone grease. Approximately 4 ml of test solution was added to each syringe, and the syringes were warmed in a waterbath at 37° C for 15 min. After warming, 17 ml of an anesthetic gas mixture was aspirated into each syringe. The syringes were shaken vigorously for 30 s and reimmersed in a waterbath. After 15 min, the gas pressure within each syringe was equilibrated with ambient pressure by briefly opening the stopcock. The syringes again were shaken vigorously for 30 s and reimmersed in the waterbath for 2 h to equilibrate the anesthetic partial pressures between gas and liquid phases. Each syringe was shaken vigorously for 30 s at 30-min intervals during the 2-h equilibration period. Equilibration periods of 1.5, 2, 3, and 4 h yielded similar partition coefficients.

After the 2-h equilibration period, the concentrations of anesthetics in the gas and liquid phases were measured. The concentrations of anesthetics in the gas phase of each syringe were measured by injection of the equilibrated gas directly into a gas chromatograph, whereas the concentrations of anesthetics in the liquid phase of each syringe were measured by extraction of the anesthetics from an aliquot of equilibrated liquid into a large air-filled flask as follows: four 600-ml flasks, whose internal volumes had been measured precisely by water displacement, each were fitted with an aluminum-foil–covered rubber stopper. Each stopper was pierced with a #16-gauge needle fitted with a metal stopcock. The flasks were warmed to 37° C and then evacuated to a negative pressure of 2/3 of an atmosphere. Exactly 2.31-ml of equilibrated liquid

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phase was aspirated into each evacuated flask. To ensure complete extraction of anesthetic from the liquid, the flasks were immersed in the waterbath for 1 h, during which time they were shaken vigorously for 30 s at 15-min intervals. The vacuum within each flask was dissipated by intermittently opening the stopcock. After the hour, a 10-ml aliquot of air was injected into each flask, mixed thoroughly, and then slowly withdrawn. The gas sample from each flask then was analyzed in a gas chromatograph. Extraction periods of 1, 2, and 3 h yielded identical results.

We prepared a gaseous mixture of isoflurane, enflurane, halothane, and methoxyflurane by aspirating these four liquids into a size H cylinder that had been evacuated to a negative pressure of 2/3 of an atmosphere. We determined that volume of the four liquid anesthetics required to ensure complete vaporization of all the liquid within the cylinder. To give the desired concentrations of anesthetics, we then transfilled the cylinder with compressed air and mixed the contents of the cylinder thoroughly.

We measured the solubility of anesthetics in both commercially available aqueous solutions and solutions that we prepared using anhydrous sodium chloride and dextrose as shown in table 1.

The concentrations of four anesthetics were measured concurrently in both the gas and liquid phases in a two-channel gas chromatograph.[7] Nitrogen was used as the carrier gas at a flow rate of 40 ml/min. Each channel contained an 1/8 in × 360 cm column packed with 10% SF-96 on Chromosorb WHP 60/80 mesh and a flame ionization detector with an air/hydrogen gas mixture (flow rates of 283 and 40 ml/min, respectively). The temperature in channel one was set to 100° C for separation of methoxyflurane from the other three anesthetics, whereas the temperature in channel two was set to room temperature for separation of isoflurane, enflurane, and halothane. We prepared calibration curves for a wide range of anesthetic concentrations to validate the linearity of the gas chromatograph for a wide range of sensitivities. We demonstrated that the peak heights from the gas chromatograph were related rectilinearly to the concentrations of anesthetics over the entire range of concentrations studied.

The partition coefficient (λ) between liquid and gas phases for any volatile anesthetic is defined by:

$$\lambda = \frac{C_{liq}}{C_{gas}} \quad (1)$$

where C_{liq} is the equilibrium concentration of anesthetic in the liquid phase expressed in milliliters of anesthetic gas per milliliter of liquid, and C_{gas} is the equilibrium concentration of anesthetic in the gas phase expressed in milliliters of anesthetic gas per milliliter of gas.

TABLE 1. Test Solutions' Characteristics*

Solution	Preparation	Osmolarity (mOsm/l)	pH
Distilled H_2O	Laboratory prepared	0	7.0
Normal saline	Viaflex®	308	5.0
Sodium chloride (2 M)	Laboratory prepared	4,000	7.0
Sodium chloride (3.5 M)	Laboratory prepared	7,000	7.0
D10W	Viaflex®	505	4.0
D50W	Laboratory prepared	2,530	7.0
Mannitol	Viaflex®	1,098	6.0
Heparin (1,000 U/ml)	Upjohn®	308	5–7.5

* The aqueous solutions, their source of preparation, osmolarity, and pH are listed.

Expanding equation (1) yields:

$$\lambda = \frac{(GC^*_{liq}S_{liq})*(Vol_{fl}/Vol_{liq})}{(GC^*_{gas}S_{gas} - GC^*_{liq}S_{liq})} \quad (2)$$

where GC_x refers to the height of the gas chromatograph peak for each anesthetic in phase "x" (gas or liquid) of each syringe, S_x refers to the gas chromatograph sensitivity settings for each anesthetic in phase "x" (gas or liquid), Vol_{fl} refers to the calibrated net gas volume in the flask expressed in milliliters, and Vol_{liq} refers to the calibrated volume of equilibrated liquid phase aspirated into the flask expressed in milliliters.

Because the concentrations of anesthetics in the gas phase of the syringe, $GC^*_{gas}S_{gas}$, were much higher than those in the gas phase of the flask, $GC^*_{liq}S_{liq}$, equation 2 was simplified to:

$$\lambda = \frac{(GC^*_{liq}S_{liq})*(Vol_{fl}/Vol_{liq})}{GC^*_{gas}S_{gas}} \quad (3)$$

We used equation 3 to calculate the partition coefficients for the four anesthetics.

We applied a least-squares linear regression analysis to the data for each anesthetic plotted on a semilogarithmic scale. Using analysis of covariance and the Student–Newman–Keuls multiple range test, we compared the slopes of the regression lines. A significance level of $P < 0.05$ was accepted.

Results

The partition coefficients of the four anesthetics, isoflurane, enflurane, halothane, and methoxyflurane, decreased linearly, on a semilogarithmic scale, when osmolarity increased (fig. 1). The magnitude of the salting-out effect ranged from as little as 3% for methoxyflurane in saline to as much as 24% for enflurane and isoflurane in mannitol when compared with the respective partition coefficients in distilled water (table 2).

The predicted partition coefficients in distilled water (the least-squares linear regression intercept [table 3])

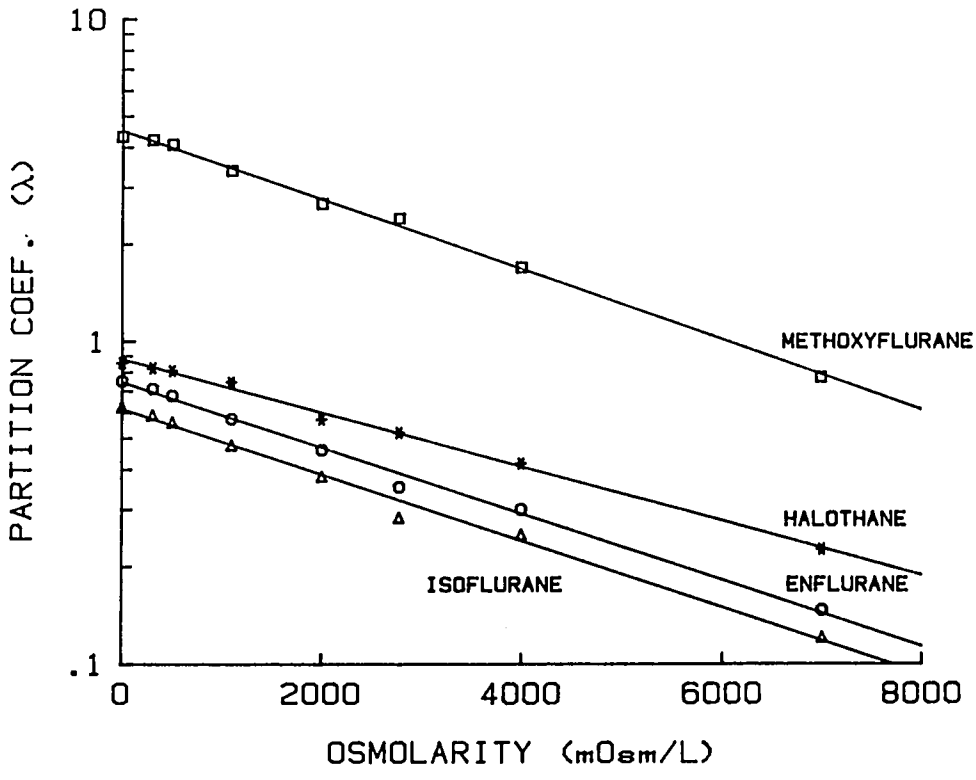

FIG. 1. The linear relationships between the liquid/gas partition coefficients of isoflurane, enflurane, halothane, and methoxyflurane in aqueous solutions and the osmolarity of these solutions are shown on a semilogarithmic scale. The slope of the regression line for halothane is significantly less ($P < 0.001$) than the slopes for isoflurane, enflurane, and methoxyflurane.

were in close agreement with both the experimental (table 2) and literature values.[3,8,9] The slope of the least-squares linear regression line for halothane was significantly less ($P < 0.001$) than the slopes of the regression lines for isoflurane, enflurane, and methoxyflurane (table 3).

We found that the gas chromatograph peak for halothane overlapped an (apparently) volatile preservative, contained in vials of Invenex® bovine heparin and (to a lesser extent) in Upjohn® bovine lung heparin. The size of the peak from the preservative varied from vial to vial, thus making it difficult to standardize the baseline for halothane. This finding may be important if halothane were analyzed at low concentrations in blood anticoagulated with these preparations of heparin. To obviate this problem in subsequent studies, we anticoagulated blood specimens with sodium citrate or EDTA.

Discussion

We found that an increase in the osmolarity of aqueous solutions decreases the solubility of volatile anesthetics in those solutions at 37° C. Furthermore, we found that the salting-out effect applies in a consistent fashion to the four volatile anesthetics, isoflurane, enflurane, halothane, and methoxyflurane, in aqueous solutions over a wide range of osmolarity values. Thus, we now may predict both the partition coefficients and the solubility of these four anesthetics in aqueous solutions of known osmolarity at 37° C.

The effect of osmolarity on the liquid/gas partition coefficients has practical applications for the clinician. Although changes in serum osmolarity within the physiologic range (290–305 mOsm/l) have only a small effect

TABLE 2. Linear Regression Parameters for the Liquid/Gas Partition Coefficients of Anesthetics in Aqueous Solutions*

	Isoflurane	Enflurane	Halothane	Methoxyflurane
Slope	-1.025×10^{-4}	-1.022×10^{-4}	-0.838×10^{-4}†	-1.084×10^{-4}
Intercept	0.617	0.746	0.880	4.51
Correlation coefficient	−0.996	−0.997	−0.998	−0.999

* The slope, intercept, and correlation coefficient for the least-squares linear regression analysis for each anesthetic are presented. The intercepts are the predicted liquid/gas partition coefficients in distilled water.

† Significantly different from the other three slopes, $P < 0.001$.

TABLE 3. Liquid/Gas Partition Coefficients of Anesthetics in Four Aqueous Solutions*

Solution	Osmolarity (mOsm/l)	Isoflurane	Enflurane	Halothane	Methoxyflurane
Distilled H_2O	0	0.626 ± 0.05 (7)	0.754 ± 0.06 (7)	0.859 ± 0.02 (7)	4.33 ± 0.5 (6)
Normal saline	308	0.590 ± 0.01 (6)	0.713 ± 0.01 (6)	0.825 ± 0.02 (6)	4.22 ± 0.30 (6)
Isotonic heparin (1,000 U/ml)	308	0.593 ± 0.01 (5)	0.715 ± 0.01 (5)	—†	4.08 ± 0.22 (5)
Mannitol	1,098	0.476 ± 0.023 (8)	0.575 ± 0.024 (8)	0.747 ± 0.03 (7)	3.38 ± 0.14 (8)

Data are means ± SD.
Sample numbers are noted in parentheses.

* Determined at 37°C.
† Not determined (see text).

on the liquid/gas partition coefficients, changes in the serum osmolarity and the concentration of serum constituents at the extremes of the physiologic range may decrease significantly the liquid/gas partition coefficients. For example, the blood/gas partition coefficient of isoflurane decreases significantly after an infusion of mannitol.[10] This may be attributed to both a transient increase in the osmolarity of blood (the osmolarity of 20% mannitol is 1098 mOsm/l) and a more prolonged decrease in the concentration of serum constituents caused by the influx of water along the osmotic gradient. In addition, the regressions between the liquid/gas partition coefficients and osmolarity can be used to predict the solubility of anesthetics in mixtures of aqueous solutions. For example, the solubility of volatile anesthetics in crystalloid pump prime can be estimated from the osmolarity of the mixture of solutions. The anesthetic partial pressure will equilibrate more rapidly in crystalloid pump prime containing mannitol than in isotonic pump prime, and it will equilibrate far more rapidly in both of these pump-prime solutions than it will in sanguinous pump prime.

We found no correlation between solutes of different molecular structure and the solubility of anesthetics in aqueous solutions. For these solutes that are composed of relatively small molecules with no inherent anesthetic binding sites, the solubility of anesthetics in aqueous solutions was independent of the molecular structure of the solute and dependent only on the osmolarity. Although aqueous solutions containing proteins were not studied, previous work indicates that the solubility of anesthetics in solutions containing very low concentrations of proteins (such as cerebrospinal fluid) is the same as the solubility in aqueous solutions of the same osmolarity.[3] Thus, the results of this study indicate that the solubility of anesthetics in aqueous solutions is unrelated to the molecular structure of nonprotein solutes. Based on previous work, this may be extended to include aqueous solutions containing very low concentrations of protein solutes.

We found no correlation between the *p*H of aqueous solutions and the solubility of anesthetics in solution. The concentration of hydrogen ions in solution (0.0007 mEq/l* at *p*H 7.0) is too small to contribute to the osmolarity of the solution and, therefore, has no measurable effect on the solubility of anesthetics. Neither the solutes studied nor the anesthetics used were affected by changes in *p*H. This finding agrees with previous work that showed no correlation between the solubility of halothane in an aqueous solution containing mono- and polypeptides and the *p*H of the solution.[11]

Although the solubility of all four volatile anesthetics decreased when osmolarity increased, the solubility of the alkane, halothane, decreased about 20% less than the three methyl–ethyl ethers isoflurane, enflurane, and methoxyflurane. Isoflurane, enflurane, and methoxyflurane belong to a homologous series of methyl–ethyl ether compounds and therefore would be expected to behave similarly. Halothane, on the other hand, is an alkyl derivative and not a member of the same structural series as the other three anesthetics. The size of the dipole moment of these compounds and the size of the molecules (*i.e.*, the number of bulky substitutions) together probably account for the relative solubility of these compounds and the differential effect of osmolarity on the partition coefficients.

In preliminary studies, we determined whether the solubility of these four anesthetics in aqueous solutions decreased as a result of competition between the four anesthetics present (as in the methods described above). We found that when present concurrently, the solubilities of both halothane and methoxyflurane in aqueous solutions were identical to the solubilities obtained when the anesthetics were present individually. Furthermore, when the anesthetics were present concurrently, the partition coefficients were in close agreement with those in the literature.[3,8,9]

* mEq/l is the same as mOsm/l for monovalent ions.

We tested the assumption that the concentration of anesthetic in the gas phase of the syringe was much greater than that in the gas phase of the flask (equation 3). Because this assumption is weakest when the concentration of anesthetic in the gas phase of the flask is greatest compared with that of the syringe (that is, when the most soluble anesthetic is dissolved in the solution with the greatest solvent characteristics), we determined the error in the partition coefficient for methoxyflurane dissolved in distilled water. The error was less than 1.4% of the uncorrected partition coefficient. This proves that even when the assumption is weakest, the error in the partition coefficient is insignificant. Therefore, we used equation 3 to calculate the partition coefficients for all four anesthetics in aqueous solutions.

In summary, we found that the solubility of volatile anesthetics in aqueous solutions at 37° C is inversely and predictably dependent upon the osmolarity of the solution.

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