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Determination of Liquid/Air Partition Coefficients for Dilute Solutions of Ethanol in Water, Whole Blood, and Plasma

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Abstract

Liquid/air partition coefficients were determined for dilute solutions of ethanol in water, whole blood, and plasma at various equilibrium temperatures from 20° to 40°C. Ethanol was determined in air and liquid samples by gas chromatography. The partition coefficients decreased exponentially as the temperature of equilibrium increased. The slopes of the regression lines were not significantly different and the mean temperature coefficient of solubility was 6.5%/1°C. At 37°C, the partition coefficients for water/air, whole blood/air, and plasma/air were 2133, 1756, and 2022, respectively. The blood/air relationships were well correlated with the water content of the samples ($r = 0.67$, $p < 0.001$). With sodium fluoride as the blood anticoagulant at 2.0, 5.0, and 10.0 mg/mL, the concentration of ethanol in the equilibrated air phase rose by 3.2%, 5.4%, and 8.9%, respectively compared with heparinized blood.

Introduction

To predict the uptake or excretion of a volatile substance through the lungs, investigators need to know its solubility in blood at 37°C. The solubility of a gas or vapour in blood is usually expressed as a partition coefficient, which can be determined by experiments *in vitro* on the distribution of the substance between blood and air at constant temperature. The ratio of the concentrations reached in the two phases at equilibrium denotes the partition coefficient (1).

Partition coefficients for many anesthetic gases and volatile organic solvents are available in the literature (2-4); but for agents having a high solubility in water, such as ethanol, conflicting values have been published. In a recent publication, a figure of 1300 was given for the blood/air partition coefficient of ethanol at 37°C (5); whereas, among workers in biological alcohol research, a figure of 1692 is generally accepted (6). Ethanol partition between water and air is of basic importance when using breath-alcohol simulators (7).

In the course of work on the use of breath analysis for estimating the concentration of ethanol in blood, accurate values for blood/air partition coefficients were needed. Experiments were made with solutions of ethanol in water, whole blood, and plasma wherein samples of the equilibrated air and liquid phases were analysed by gas chromatography (GC). Blood/air partition coefficients of ethanol were measured at various temperatures between 20°-40°C with blood samples from healthy men and women. The effect of biochemical constituents, hematological profiles, water contents, and the concentration of NaF as anticoagulant were studied.

Materials and Methods

Ethanol Standards

Standard solutions of ethanol were prepared in distilled water by dilution of absolute alcohol (99.6% v/v). A 10% w/v stock solution was prepared and working standards at concentrations of 0.50, 1.00, 2.00, 2.50 and 3.00 mg/mL were made when needed. The accuracy of the ethanol standards was checked by chemical analysis (8) and recoveries ranged from 98-102%. The 10% w/v solution of ethanol was added to blood and plasma samples used for the equilibrium studies.

Blood Sampling and Mixing with Ethanol

Samples of venous blood were obtained from healthy men and women when they attended a hospital clinic as blood donors. When the clinic staff had collected the volume needed, an additional 50 mL was taken from a forearm vein into heparinized vacutainers (10 mL) containing heparin to give a concentration of 0.2 mg/mL. In this way, samples from 20 men, mean age 35 yr (range 19-45) and 15 women, mean age 28 yr (range 20-35) were obtained. Larger volumes of blood from three other healthy men were divided into 50-mL portions and stored in flasks with heparin (0.2 mg/mL) or sodium fluoride (2.0, 5.0, or 10.0 mg/mL) as the anticoagulants. Plasma was separated from one set of heparinized blood samples by centrifuging. In some experiments, blood samples were taken before and after the test subjects drank a moderate dose of ethanol. All blood samples were stored in a refrigerator at +4°C until used.

Calibrated flasks (10 or 25 mL) were half filled with well-mixed whole blood or plasma and aliquots of a 10% w/v solution of ethanol were added from an Agfa micrometer syringes. The flasks were made up to the mark with more blood and

thoroughly mixed. The volumes of ethanol added were adjusted to give solutions of 0.50 to 1.50 mg/mL. Dilution of the blood and plasma was never greater than about 2% and alcohol was always added on the same day the equilibrium studies were made.

Equilibrium Procedure

Equilibration of ethanol between air and liquid was done using glass flasks fitted with sleeve-type rubber septums. For water/air equilibrium, 500-mL flasks were used and each contained 50 mL of the liquid phase. Blood/air and plasma/air studies were made with 150-mL flasks containing 10 mL of the biological samples. All flasks were immersed in water up to the tops of the septum caps. Equilibrium was maintained at ±0.05°C of a desired temperature from 20°-40°C. After about 30 minutes, a hypodermic needle was thrust through the septums to adjust to atmospheric pressure before taking samples for analysis.

Headspace samples were removed with a warmed (50°C) glass syringe with a 19 gauge needle. The syringe was filled with 5 mL of room air, which was injected through the rubber septum and mixed with the gas phase. The syringe plunger was moved up and down to the 5 mL graduation mark four times to give an equilibrated sample. Then, 5 mL of vapour was removed and the concentration of ethanol was determined by GC. Three determinations were made at each temperature studied. Because the liquid/air partition coefficient of ethanol is so large (6), many samples of air can be taken without depleting the concentration of ethanol in the liquid. This was confirmed by analysing the liquid before and after sampling the vapour.

Determination of Ethanol

Blood, plasma, and water solutions of ethanol were analysed with a Perkin-Elmer (F-11) gas chromatograph. The oven temperature was 75°C. The column was copper measuring 152 cm × 0.476 cm i.d. Carbowax 400 (10% w/w) on Chromosorb W (80/100 mesh) was used as packing material, nitrogen as the carrier gas (70 mL/min) and hydrogen (38 mL/min) and air (260 mL/min) for the flame detector. A 1-μL volume of sample was injected after diluting 1:10 with *n*-propanol (0.08 mg/mL) as internal standard (IS). The resulting ethanol/propanol peak height ratio was measured and used for quantitative analysis.

The standard deviation (SD) of a single determination of blood ethanol was 0.012 mg/mL at a mean concentration of 0.92 mg/mL (9). The detector response was linear for solutions of ethanol in blood, plasma, and water up to at least 4.0 mg/mL and the regression lines passed through the origin. The reproducibility of replicate injections was very high. The coefficient of variation (CV) for a solution of ethanol of 1.0 mg/mL was 0.38%.

The concentrations of ethanol in the vapour phase were determined with a MkII Gas Chromatograph Intoximeter. The oven temperature was 100°C. Porapak Q was used as stationary phase packed into a column made of stainless steel measuring 30.5 cm × 0.30 cm i.d. A mixture of 60% hydrogen and 40% nitrogen served as a combustible carrier gas for the detector. Air was supplied from a small pump. The optimal flow rates of these gases were controlled by valves factory-set for this instrument. Headspace vapour samples were removed from test flasks and injected into a gas sampling valve kept at 100°C inside the temperature controlled oven. The 5-mL sample was sufficient to flush out dead space in the valve and provide the assay volume (0.25 mL). The detector response for ethanol was integrated and displayed as a peak area on a digital voltmeter. The retention time for ethanol was about 2 minutes and the detector response was linear up to 4.0 mg/mL. The precision of the method, expressed as CV of replicate determinations, was 0.83% at a mean concentration of ethanol in the liquid phase of 0.5 mg/mL (10).

Ethanol-Vapour Standards

A headspace technique based on static equilibrium was used to generate known concentrations of ethanol in air. A standard solution of ethanol (100 mL) was equilibrated at 25° ± 0.05°C for 30 minutes. The vapour phase was adjusted to atmospheric pressure and samples were analysed by GC. The air/water ethanol partition ratio ($k_{a/w}$) at 25°C was determined in a new way (11). This involved measuring the change in concentration of ethanol in water (1 mL) after equilibration with a much larger volume of air (2000 mL). The mean value of $k_{a/w} \times 10^1$ was 0.212 (range 0.209-0.217, n = 10) being in good agreement with the results of Harger *et al.* (6) who reported 0.217 ± 0.005 (± SD, n = 13). Indeed, Dubowski (7) evaluated several earlier studies on this subject and found that $k_{a/w} \times 10^1$ on average was 0.2149. The air/water partition coefficient at 25°C, 0.212×10^{-1} ($k_{a/w} = 4717$) was used to calculate the concentration of ethanol in air equilibrated with known strength solutions of ethanol in water at 25°C.

Calculation of Partition Coefficients

From the analysis of air equilibrated with standard solutions of ethanol in water at 25°C, the detector response of the GC could be calibrated. To compare with solutions equilibrated at temperatures higher than 25°C, the expansion of the vapour phase must be adjusted for. Thus, the concentration of ethanol in vapour at t°C equals the concentration (mg/L) at 25°C × (273 + 25)/(273 + t). The concentrations of ethanol determined in air and liquid (water, whole blood, plasma) at equilibrium were used to calculate the partition coefficients ($k_{w/a}$, $k_{b/a}$, $k_{p/a}$).

$$\frac{\text{Concentration of ethanol in the liquid phase}}{\text{Concentration of ethanol in the vapour phase}} = \text{Constant}$$

Biochemical Analysis of Blood Samples

Hematocrit values and other blood constituents were determined by conventional methods of assay used in clinical chemistry. The water content of blood samples was determined by freeze-drying weighed aliquots in an Edwards Model B5 high-vacuum rotary freeze dryer with phosphorus pentoxide as the drying agent. About 0.5 g of well mixed whole blood was freeze dryed for 24 hours at 0.01 torr. After a preliminary weighing, the samples were freeze-dried to constant weight. The water content of blood was calculated from the average change in weight based on duplicate samples and expressed as g/100 g whole blood.

Results

Equilibrium of Ethanol

Figure 1 shows linear relationships between the concentra-

tion of ethanol in water (1 to 4 mg/mL) and the ethanol-vapour concentration in headspace at equilibrium temperatures from 20°-40°C. The slopes of the straight lines correspond to the air/water concentration ratios of ethanol at equilibrium and are thus proportional to partition coefficients. The slopes become steeper as temperatures rise and follow a non-linear trend. Ethanol solubility in water clearly decreases as temperature increases.

Partition Coefficient-Temperature Relationships

The liquid/air partition coefficients are seen to decrease exponentially as equilibrium temperature rises (Figure 2A). The logarithms of partition coefficients plotted against temperature (Figure 2B) follow a rectilinear course. The slopes of the regression lines for water, whole blood and plasma solutions of ethanol were parallel as shown by analysis of covariance ($F = 1.21$, $df = 2$ and 11, $p > 0.05$), but the lines were on different elevations ($F = 100.7$ $df = 2$ and 13, $p < 0.001$). Table I gives the results of a correlation regression analysis with partition coefficients transformed to natural logarithms ($\log_e k$). This device of log transformation gives the rate of change in partition coefficient not only per unit of temperature, but also per unit of partition coefficient. These relative rates of change per 1°C (regression coefficients) are conveniently multiplied by 100 and thereby expressed as percentage rates of change per 1°C. For the temperature change of 20°-40°C, the average temperature coefficient of solubility was 6.47% per 1°C. The value of a partition coefficient within this range of temperature may be derived from the regression equations given in Table I. The quotients $k_{w/a}:k_{p/a}$ and $k_{w/a}:k_{b/a}$ are independent of equilibrium temperature (Figure 2B) but the absolute values may hold only for these particular experimental conditions.

Influence of Water Content of Blood on Partition Coefficients

Table II gives mean values of the partition coefficients of ethanol at equilibrium temperatures of 34°C and 37°C compared with the water content and hematocrit of blood taken from 20 men and 15 women. Partition coefficients for the men were on average 2% lower than those for the women ($p < 0.001$). The water content of blood and its hematocrit were highly correlated $r = 0.78 \pm 0.126$ ($t = 6.18$ $p < 0.001$). As expected, the hematocrit of blood taken from women was less than blood from men ($p < 0.001$) and the water content of blood from men was accordingly higher ($p < 0.001$).

Figure 3 shows a plot of blood/air partition coefficient of ethanol (y) against the water content of blood (x) for samples equilibrated at 34°C. Significant correlations were evident with blood from men ($r = 0.63 \pm 0.188$, $p < 0.01$) and with blood from women ($r = 0.58 \pm 0.225$, $p < 0.05$), as well as for the whole material ($r = 0.67 \pm 0.131$, $p < 0.001$). The regression equation for men and women together was $y = 88.8 + 26.0x$, which implies that over the range of the x-variate, 77-82 g water per 100 g blood, blood/air partition coefficients increase by 26 for each 1 g/100 g increase in water content.

Effect of NaF on Vapour Phase Concentrations of Ethanol

Table III shows that the concentration of ethanol in headspace

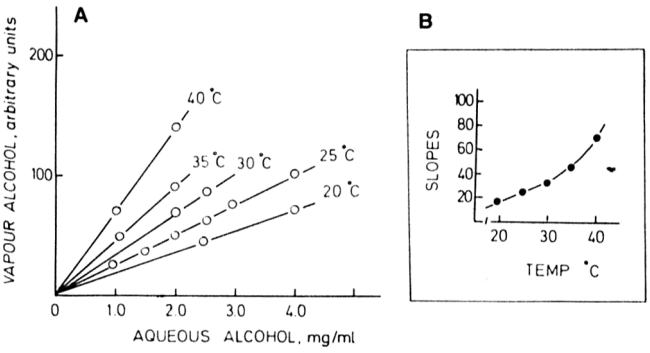

Figure 1. A) Relationships between the concentrations of ethanol in water and in an equilibrated air-space at temperatures from 20°-40°C, B) depicts the slopes plotted against the temperature of equilibrium and shows that the air/water ratios of ethanol are related to temperature by an exponential function.

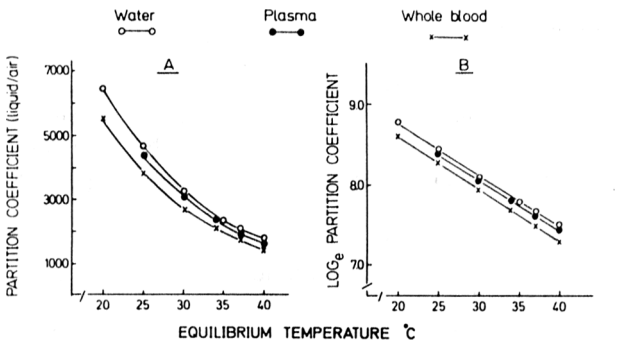

Figure 2. A) Liquid/air partition coefficients of ethanol for water, whole blood and plasma at temperatures of equilibrium from 20°-40°C, and B) the logarithm of partition coefficient and temperature.

Table I. Relationships between Partition Coefficient and Temperature of Equilibrium for Solutions of Ethanol in Water, Whole Blood and Plasma

Partition coefficient	Regression equation	Slope of regression (±SE)	Solubility coefficient (%/1°C)
Water/air	$\log_e y = 10.044 - 0.0643x$	-0 0643±0 0014	-6.43±0.14
Blood/air	$\log_e y = 9.931 - 0.0665x$	-0.0665±0 0014	-6.65±0.14
Plasma/air	$\log_e y = 9.969 - 0.0637x$	-0.0637±0.0013	-6.37±0.13

SE = standard error. Blood samples were from male subjects Correlation coefficients were 0 999 for all three regression lines.

Table II. Comparison of Results Obtained with Blood Samples taken from Healthy Men and Women

Test Variable	Men (n=20)*	Women (n=15)*	Student's t
Blood/air partition coefficient at 34°C	2157±9.6	2195±10.9	3.2**
Blood/air partition coefficient at 37°C	1783±8.1	1830± 7.8	4.1***
Blood/water (g/100 g)	79.3±0.25	81.1± 0.13	4.0***
Hematocrit (%)	44.3±0.72	40.0± 0.77	6.1***

*Values given are mean ±SE
**p<0 01
***p<0.001 by Student's t-test for independent groups.

vapour increases in the presence of NaF (2, 5, and 10 mg/mL). Heparin (0.2 mg/mL) was used as the anticoagulant to give the zero values when testing the effect of various concentration of NaF. This amount of heparin caused no significant rise in the vapour concentration over aqueous solutions of ethanol. Enhanced vapour phase concentrations of ethanol were evident when an ionizable inorganic salt (NaF) was present in solution. Besides the data given in Table III, when 5.0 mg/mL NaF was added to blood samples from three other subjects, the increases in ethanol vapour were 5.5%, 5.1% and 5.3% above heparinized blood. This gives a mean increase of 5.4% (n = 5) associated with 5 mg/mL NaF, an amount commonly used to prevent blood from clotting.

Effect of Other Factors

No clear relationships were seen between partition coefficient of ethanol and other blood constituents. These included hemoglobin, albumin, total protein, urea, electrolytes, and glucose, which were all within normal ranges for healthy subjects. Neither was there any marked effect if samples of blood were taken before or 60 minutes after a subject ate a fat-rich meal. Furthermore, the partition coefficient of ethanol was about the same when ethanol was added to blood *in vitro* as when blood samples were drawn from inebriated subjects.

Discussion

Ethanol and water mix together in all proportions and the equilibrium concentrations reached in body fluids and tissue can be estimated from their relative water contents (12). Liquid/air partition coefficients of ethanol in water, plasma, and whole blood take high values and at 37°C, the blood/air partition coefficient of ethanol is about 1800 on the average. This figure is higher than the values obtained with other gases and volatile organic solvents (1-4). Studies *in vivo* have shown that only about 2-5% of ingested ethanol is excreted through the lungs (12). This follows from the high blood/air partition coefficient of ethanol; the high solubility in blood prevents rapid ventilation.

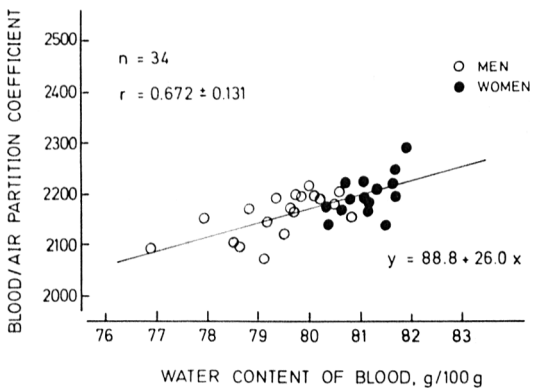

Figure 3. Scatter plot of the relationship between blood/air partition coefficient of ethanol at 34°C and the water content of blood taken from healthy men and women.

Figure 1 suggests that dilute solutions of ethanol in water (1-4 mg/mL) obey Henry's Law (13) connecting the solubility of gases and vapours with their concentrations in the equilibrated vapour phase. The mean temperature coefficient of solubility was 6.5% per 1°C between 20°-40°C for solutions of ethanol in water, plasma, and whole blood. This suggests that ethanol freely equilibrates between the water fractions of biological fluids and the overlying air phases. Ethanol binds insignificantly to the endogenous constituents of plasma and whole blood. This notion gains support from calculations on the water content of plasma (94.5 g/100 mL) and whole blood (85.0 g/100 mL) given by Diem and Lenter (14). If one assumes that ethanol exists only in the water phase of bio-fluids, then $k_{w/p} = 1.06$ and $k_{w/b} = 1.18$. The regression equations in Table I predict that at 37°C, $k_{w/a} = 2133$ $k_{p/a} = 2022$ and $k_{b/a} = 1756$ which gives the quotients $k_{w/p} = 1.05$ and $k_{w/b} = 1.21$. These experimentally derived values agree well with the results estimated from the water content of the liquids.

Normal variations in the biochemical composition and the hematological profile of blood samples, apart from water contents, had no significant effect on the blood/air partition coefficient of ethanol. The differences noted between blood drawn from men and women depended on variations in the water content of the samples. The blood/air partition coefficients were highly correlated with the water contents with a systematic sex-difference (men◂women) of about 2%. If blood contained inorganic salts as anticoagulants, such as NaF, the concentrations of ethanol in headspace vapour rose significantly above bloods preserved with heparin. The solubility of many non-electrolytes decreases in the presence of easily ionizable salts (15). Ethanol becomes 'salted-out' and at a concentration of 5.0 mg/mL NaF, the air-phase concentrations rose by 5.4% on the average. This problem was avoided by the use of heparin as the anticoagulant, which was effective at much lower concentrations (0.2 mg/mL) and did not change the vapour phase concentration of ethanol. The results of Harger *et al.* (6) were obtained with 5.0 mg/mL of NaF added to blood to prevent clotting and must therefore be adjusted by 5.4% to allow for 'salting-out' effects. After this correction, the values of 2137 at 34°C and 1783 at 37°C agree well with the present results of 2143 (34°C) and 1756 (37°C)

Table III. Effect of Sodium Fluoride on 'Salting-out' of Ethanol from Solutions in Water and Blood

NaF (mg/mL)	Ethanol in water*			Increase over zero NaF (range %)	Ethanol in blood*		Increase over zero NaF (range %)
	1	2	3		1	2	
0	99.0	42.8	86.5	0.0- 0.0	51.3	19.4	0.0-0.0
2	101.0	43.5	88.2	1.6- 2.0	52.5	20.4	2.3-5.1
5	104.0	45.8	91.0	5.1- 7.0	53.9	20.6	5.1-6.2
10	110.0	47.4	95.2	10.0-11.1	56.2	21.0	8 2-9.6

*Concentrations of ethanol in water and blood ranged from 0 50-1.50 mg/mL Vapour phase concentrations are expressed in arbitrary units. Vapour/liquid equilibrium was at 34°C.

derived from Table I. Earlier studies on the partition of ethanol between air and water were recently reviewed by Dubowski (7). The results reported here $k_{w/a}$ at 34°C of 2587 agrees well with the average result from most previous workers (16-18).

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