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Solubility of NH_3 and apparent pK of NH_4^+ in human plasma, isotonic salt solutions and water at 37°C

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Abstract

The solubility of ammonia, αNH_3 (mM/mmHg), was determined at 37°C and low ammonia partial pressure (0.02–1 mmHg) in pure water ($n = 24$) as 46.7 ± 0.40; aqueous isotonic salt solutions ($n = 7$) as 46.8 ± 0.81; and human plasma ($n = 5$) as 42.0 ± 0.66. The last figure increases to 45.3 ± 0.63 if expressed in molal units (mmol/kg plasma water · mmHg) instead of molarity with respect to the water content of the plasma (mean from four healthy and fasting donors: 0.908 ± 0.005 kg H_2O/kg plasma; mean density at 37°C: 1.020 ± 0.002 kg/l). In pure water, the solubility value is the mean of three different methods: (a) extrapolation of the salting-out effect of ammonia in aqueous NaOH to zero concentration; (b) slope of Henry–Dalton's law and (c) directly measured in pure water and 0.001 M aqueous NaOH. Based on the Henderson–Hasselbalch equation for the system NH_4^+/NH_3 in isotonic salt solutions and human plasma, both constants, apparent pK and solubility, can be derived from total ammonia concentration and pH at equilibrium with defined ammonia gas phase, if additionally the concentration of NH_4^+ or NH_3 is known. This was verified, in the first case, by determining the concentration of NH_4^+ by the experimental conditions, and in the second, by two measurements of total ammonia concentration at two different pH values. Total ammonia concentration was measured by a specific enzymatic standard test and pH with the glass electrode. The mean apparent pK was 8.968 ± 0.013 in isotonic salt solutions ($n = 7$), and in human plasma ($n = 10$) it was 9.014 ± 0.033.

Keywords: Ammonia; Solubility; Apparent pK; Human plasma; Isotonic salt solutions; Water

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1. Introduction

The exact knowledge of the characteristic constants of the buffering system NH_4^+/NH_3 in biological fluids, i.e., the acid dissociation constant of ammonium ($pK\ NH_4^+$) and the solubility of ammonia (αNH_3), is of great importance for calculations in physiological processes in which ammonia is involved. In the organism, ammonia is released in the course of amino acid metabolism, and because of its high toxicity any accumulation above a critical level must be avoided. Most effectively, ammonia is rapidly diminished by conversion into urea in the liver with subsequent urinary excretion, and in the kidneys by NH_4^+ elimination. The fraction of total ammonia present as base NH_3 or as corresponding acid NH_4^+ depends on the actual pH in the body fluid, its p*K* value and solubility in that fluid, and on the partial pressure of ammonia.

The most recommended values of the characteristic constants for the ammonia system in plasma (P) and in pure water (H_2O) at 37°C are those given by Siggaard-Andersen [1]: $pK\ NH_4^+(P) = 9.03$, $\alpha NH_3(P) = 40.5$ mM/mmHg, and $\alpha NH_3(H_2O) = 28$ mM/mmHg. However, these figures are not based on own experimental measurements. The p*K* value of NH_4^+ originally dates back to Bates and Pinching [2], who determined thermodynamic p*K* over a wide temperature range (0 to 50°C) with a value of 8.890 at 37°C by interpolation, and on Bank and Schwartz [3], who studied the influence of urinary salts on p*K* in aqueous solutions. The last two authors presented a quantitative relationship between thermodynamic p*K*, ionic strength and apparent p*K* based upon the Debye–Hückel approximation, and it should be noted that their ammonium p*K* (9.03) was not obtained from plasma directly, but from aqueous electrolyte solutions with ionic strength (*I*) in the physiological range (0.150 M) and without defined ammonia gas phase. Uniquely, the solubility of ammonia (41.5 mM/mmHg) determined by Jacquez et al. [4] was derived from human plasma at 37°C and defined ammonia partial pressure (0.2 and 0.5 mmHg). This value was calculated from measured pH and total ammonia concentration by the Henderson–Hasselbalch equation for the ammonia system using as known the apparent p*K* of NH_4^+ in human plasma which was assumed to be the same as in physiological salt solution and set equal to the value of Bank and Schwartz. It also became obvious that the solubility value of ammonia in pure water at 37°C by Siggaard-Andersen (28 mM/mmHg) is the same as that given by Jacquez et al. and was not measured by both of the authors, but taken from the literature [5]. Other values from literature data are much higher, e.g., 42.8 [6] or 44.6 at 35°C [7], so that the true solubility value is uncertain. With respect to a value of 28 mM/mmHg in pure water, ammonia dissolved in human plasma is salted-in probably by the presence of the plasma proteins. In order to separate this possible salting-in effect in plasma due to the proteins and to the electrolytes, the characteristic constants of the ammonia system were redetermined at ammonia

partial pressure in the range 0.1 to 1 mmHg at 37°C: α in pure water, and both α and apparent pK in human plasma and isotonic salt solutions ($I = 0.155$ M).

2. Materials and methods

2.1. Chemicals

The chemicals in this investigation were used without further purification from different sources. Fluka (Buchs, Switzerland): hydrochloric acid standard solution (1 M); 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (HEPES) ($>99.5\%$); $Na_2B_4O_7 \cdot 10H_2O$ (Borax) (certified purity $>99.5\%$); sodium hydroxide standard solution (1 M). Fresenius (Bad Homburg, Germany): water (Ampuwa, sterile and apyrogenic). Merck (Darmstadt, Germany): D,L-alanine (99%); NaCl (99.5%); NH_4Cl (99.5%). Serva (Heidelberg, Germany): L-histidine (99%). Sigma (St. Louis, MO, USA: glycine ($>99\%$). The gases were from Linde (Mainz, Germany): purified nitrogen (99.999%), oxygen (99.995%) and carbon dioxide (99.9%), and two special gas mixtures, one with 1440 ppm$\pm$2% (v/v), the other with 29.4 ppm$\pm$5% ammonia in nitrogen.

2.2. Human plasma

From freshly drawn heparinized venous blood (Na-Heparin Braun 25 000 IU/5 ml, Braun, Melsungen, Germany: 25 IU/ml of blood; 20 ml from vena cubitalis) of four healthy and fasting adult volunteers (three males, one female: mean age 44$\pm$12), two portions each of 5 ml were distributed on two tonometers (Instrumentation Laboratory IL 237) and conditioned at 37$\pm$0.1°C with constant oxygen partial pressure ($pO_2 = 100$ mmHg), zero carbon dioxide tension and nitrogen to eliminate carbon dioxide. The partial pressure of the different blood gases was set using the pure gases (oxygen, carbon dioxide and nitrogen) and a precision mixing chamber (Corning, 192 Precision Gas Mixer). From this blood, when free of carbon dioxide by control of pH (about 30 min; pH $\cong$ 8.03), the plasma was separated by centrifugation (10 min, 4000 rpm; g-force: $\sim$1400). The plasma from the different donors (1 to 4) was characterized by its mean water content (kg H_2O/kg plasma): (1) 0.905$\pm$0.003; (2) 0.903$\pm$0.003; (3) 0.913$\pm$0.002 and (4) 0.912$\pm$0.002 derived from measurement of the total plasma proteins (g/l): 81; 76; 71 and 73, the density of the plasma at 37°C (kg/l): 1.0201; 1.0224; 1.0190 and 1.0188, and by drying at 90°C to constant weight (8–15 h). In a first series (a), two samples, each of 1 ml of isolated plasma, were adjusted by 1 M aqueous NaOH such that pH of one sample was in the range of the pK of ammonium (pH $\sim$ 9), that of the other about two units above (pH $\sim$ 11). In a second series (b), the treatment of blood

prior to separation of plasma was the same, except the addition of HCl to produce a negative base excess (BE, mM). This decreased the initial pH of the separated plasma and was directly used for equilibration with gaseous ammonia. All plasma samples were equilibrated within a Laué tonometer with the same ammonia partial pressure ($pNH_3 \sim 1$ mmHg).

2.3. Calibration of the pH meter

For measuring pH in blood, plasma and in the solutions at 37°C, a glass electrode system with calomel reference cell and saturated KCl as a salt bridge (Radiometer BMS2 MK2, Blood Mikro System) was used, which was calibrated by two precision buffer solutions (Radiometer S 1500 phosphate buffer (1:1): pH = 6.841, and S 1510 phosphate buffer (1:4): pH = 7.383). However, because of the experimental pH range being significantly above that of normal blood, and because of the known alkaline error of the glass electrode, additional recommended buffer solutions were used to standardize the glass electrode. The following buffer systems with reference pH at 37°C have been used from which an empirical correction formula was derived: Borax/NaOH buffer [8]: 0.010 M/none (pH: 9.089); 0.0125 M/0.0167 M (9.804); 0.0125 M/0.0233 M (10.504); glycine/NaOH buffer [9]: 0.050 M/0.012 M (8.932); 0.050 M/0.0454 M (10.388); Na_2HPO_4/NaOH buffer [8]: 0.025 M/0.0051 M (10.8); 0.025 M/0.0111 M (11.2); 0.025 M/0.0269 M (11.7). All measured pH values (X) in the range pH = 9–11 were corrected by the following formula ($n = 21$): $Y = 3.1439 \cdot e^{0.1166 \cdot X}$ with $R^2 = 0.9945$, and used in all further evaluations. Whereas measured pH at 9.5 by correction changes only slightly to 9.518, a measured pH at 10.5 would increase to a value of 10.695, i.e., by about 0.195 pH units.

2.4. Equilibration at low ammonia partial pressure

Two samples, each of 1 ml were equilibrated in a Laué tonometer [10] by shaking while gaseous ammonia in nitrogen that was prior saturated with pure water vapour at 37°C ($pH_2O = 47.1$ mmHg) was streaming over the liquid phase. Two gas mixtures of ammonia in nitrogen contained in gas cylinders were used, 1440 ppm and 29.4 ppm (v/v), corresponding to ammonia partial pressure of about 1 mmHg and 0.021 mmHg, respectively, and which can slightly vary depending upon ambient barometric pressure (pB, mmHg). Other intermediate ammonia partial pressures (0.096, 0.405 and 0.694 mmHg) were mixed from the higher gas (1440 ppm) by dilution with nitrogen in a peristaltic pump (Ismatec mp-13 GJ-10, Verder) distributed over ten equal tygon tubes with the same inner diameter (2.79 mm). The dilution ratios, in good agreement with the fraction of tubes supplied with the NH_3 gas, were additionally controlled by

diluting a carbon monoxide test gas (2347 ppm CO in nitrogen) with nitrogen under the same conditions, and measuring the relative concentration of CO before and after mixing with nitrogen in a CO-analyzer (Ultramat M, Siemens). The time necessary to reach equilibrium of the dissolved ammonia was in the order of 6–8 h when a gas flow of 1440 ppm ammonia in nitrogen and about 50 ml/min per tonometer was used.

2.5. Measurement of ammonia

For determination of total ammonia in the samples a specific enzymatic method [11] was used which was available as a standardized test kit from Boehringer (Mannheim, Germany). The principle of the method is the reaction of 2-oxoglutarate with ammonia in the presence of glutamate dehydrogenase and reduced nicotinamide-adenine dinucleotide (NADH) to L-glutamate, whereby NADH is oxidized. The extent of NADH that is oxidized to NAD^+ is proportional to the amount of ammonia and was photometrically determined by the decrease of the UV-absorbance in the maximum of NADH at 340 nm wavelength. Assuming that the law of Lambert–Beer is valid, the ammonia concentration (mM) was calculated by the following formula:

$$c\mathrm{NH_3} = \frac{V_{\mathrm{tot}}}{V_{\mathrm{sample}} \cdot a_{340} \cdot d} \cdot \Delta A_{340} \quad (1)$$

V_{sample} means the volume of the sample and was variably chosen due to the expected range of the measurement (5, 10, 20 or 100 μl), V_{tot}, the total volume of the substrate (3.0 ml) + that of the sample + that of the enzyme (20 μl), d, the light path of the cuvette (1 cm), a_{340}, the millimolar absorptivity of NADH at 340 nm with a value of 6.3 $\mathrm{mM^{-1}/cm}$, and ΔA_{340}, the decrease of light absorbance from an initial value to a value measured 20 min after addition of the enzyme. As a blank which was subtracted from the absorbance difference, the same procedure was applied to all solutions, but without addition of NH_4Cl and prior to contact with the ammonia gas phase. In pure water or aqueous NaOH solutions (0.001; 0.010; 0.010/0.145 M NaCl; 0.155; 0.3; 0.5; 0.75 M NaOH), this blank value for different sample volumes was: 10 μl ($n=17$): 0.022 ± 0.007; 20 μl ($n=4$): 0.019 ± 0.003; 100 μl ($n=4$): 0.022 ± 0.004; in isotonic salt solutions or plasma of different initial pH: 5 μl ($n=16$): 0.022 ± 0.004, and in plasma: 10 μl ($n=5$): 0.027 ± 0.005. The reliability and accuracy of the method was controlled by three NH_4Cl standard solutions: 4.64 mM (Boehringer), and 40 and 80 mM prepared from analytically pure NH_4Cl by weight. The mean values found with standard deviation (S.D.), variation coefficient (%), and number of measurements (n) were the following: 4.54 ± 0.062 ($\pm1.37\%$) $n=10$; 40.28 ± 0.71 ($\pm1.76\%$) $n=34$; 80.70 ± 1.52 ($\pm1.88\%$) $n=23$.

2.6. Determination of α in pure water

2.6.1. From salting-out effect by aqueous NaOH

In aqueous solution at pH at least two units above the pK of ammonium (~9.0), almost 99% of the total ammonia will be present as dissolved NH_3, if a small amount of NaOH is added (0.001 M). However, if the addition of NaOH is continued, the salting-out effect on ammonia will become effective, and increasing pH will decrease total ammonia concentration. It was just this effect quantitatively described by the well-known Setchenov relationship [12], that was used to derive the solubility of ammonia in pure water. In its logarithmic form the following equation was applied:

$$\log_{10}\alpha NH_3 = \log_{10}\alpha^{\circ}NH_3 - k_S c\mathrm{NaOH} \quad (2)$$

In Eq. (2), α is the measured solubility of NH_3 in aqueous NaOH of known concentration (cNaOH), α° that to be determined in pure H_2O, and k_S, a constant called Setchenov or salting-out coefficient. A plot of the left side of Eq. (2) versus cNaOH yields a straight line, and from the intercept at zero concentration of NaOH the solubility of NH_3 in pure water can be obtained.

2.6.2. From Henry–Dalton's law

In this approach, Henry–Dalton's law was used to derive the solubility of ammonia in pure water from the slope of the plot ammonia concentration (cNH$_3$) versus ammonia partial pressure (pNH$_3$) in the range 0.02 to 1 mmHg:

$$c\mathrm{NH}_3 = \alpha\mathrm{NH}_3 \cdot p\mathrm{NH}_3 \quad (3)$$

pNH$_3$ was calculated from the gas fraction of ammonia (ppmNH$_3$), the tension of water vapour at 37°C (47.1 mmHg), and ambient barometric pressure (pB, mmHg) by Dalton's law: $p\mathrm{NH}_3 = (p\mathrm{B} - 47.1)\cdot \mathrm{ppmNH}_3 / 10^6$.

2.7. Simultaneous determination of α and apparent pK

For all calculations of α and apparent pK, the familiar Henderson–Hasselbalch equation applied to the ammonia system in aqueous salt solution was used:

$$\mathrm{pH} = \mathrm{p}K + \log_{10}\frac{c\mathrm{NH}_3}{c\mathrm{NH}_4^+} \quad (4)$$

According to Eq. (4), the apparent pK can be calculated, if pH and the concentrations of ammonia (cNH$_3$) and of ammonium ($c\mathrm{NH}_4^+$) are separately known. Furthermore, if the solubility of ammonia shall be obtained from ammonia concentration, Eq. (3), ammonia partial pressure (pNH$_3$) must also be

known. Then, for total ammonia concentration ($c_{tot}NH_3$) in the solution in equilibrium with a definite ammonia gas phase, the following mass balance must hold:

$$c_{tot}NH_3 = cNH_3 + cNH_4^+ \quad (5)$$

2.7.1. Isotonic salt solutions

The criterion that in addition to measurement of pH and total ammonia concentration ($c_{tot}NH_3$) at equilibrium with known ammonia partial pressure (~1 mmHg), the ammonium concentration (cNH_4^+) must also be known to determine α and apparent p*K*, was verified by three types of aqueous electrolyte solutions isotonic to human plasma (0.155 M):

1. A solution of 0.048 M ammonium chloride, NH_4Cl, approximately equal to physically dissolved ammonia under the chosen experimental conditions, and NaCl (0.107 M) to adjust the ionic strength to the value of human plasma. During the equilibrium process with gaseous ammonia, the concentration of NH_4Cl did not change and was equal to the ammonium concentration.
2. A solution of 0.045 M hydrogen chloride, HCl, and NaCl (0.110 M) which was quantitatively converted by ammonia into ammonium chloride. The initial amount of HCl before contact to ammonia gas phase determined the ammonium concentration produced after equilibrium was reached.
3. Solutions consisting of NaCl and the pure weak acid of a buffer the apparent p*K* of which was experimentally determined: HEPES/NaCl (0.025 M/0.130 M and 0.050 M/0.106 M), L-histidine (0.100 M/0.106 M), glycine (0.100 M/0.120 M) and D,L-alanine (0.100 M/0.124 M). In this case, ammonium concentration was equal to the concentration of the corresponding base (A^-) formed from the pure acid (HA) during equilibration with ammonia: $cNH_4^+ = \Delta cA^-$.

$$HA + NH_3 = NH_4^+ + A^-$$

The change in concentration of formed base was calculated by the Henderson–Hasselbalch equation for that particular buffer from measured initial pH, equilibrium pH, and its apparent p*K*. For HEPES, glycine and D,L-alanine, the concentration of base at the initial pH was negligible, only for L-histidine with $pH_{initial} = 7.380$ it was 3.27 mM and had to be respected. For the purpose of stable and reliable measurement of pH, the concentrations in all solutions were such that after reaching the equilibrium with ammonia pH was shifted into the p*K* range of ammonium (~9). With cNH_4^+ known, cNH_3 can be calculated from total ammonia concentration by Eq. (5), and α from Eq. (3) with known pNH_3. Further, from Eq. (4), known cNH_3, cNH_4^+ and pH yield apparent p*K*.

2.7.2. Human plasma

In human plasma, no similar stoichiometric reaction with ammonia at constant ammonia partial pressure was known. Therefore, α and apparent pK of the ammonia system could not be derived from a single measurement of pH and total ammonia concentration by the Henderson–Hasselbalch equation, Eq. (4). At least a second measurement of pH and total ammonia concentration was necessary. With free choice of pH, one measuring pair pH′, $c'_{tot}NH_3$ was in the range pH = 9–9.5 (pK range of ammonium), and the other pair pH″, $c''_{tot}NH_3$ in the range pH = 10.5–11.0, both pH sufficiently separated from each other. Hence, the constants α and pK can be calculated by eliminating cNH_4^+ from Eq. (5), applying twice the Henderson–Hasselbalch equation to each pair and subtracting from each other:

$$pH' - pH'' = \log_{10} \frac{c'NH_3}{c'_{tot}NH_3 - c'NH_3} - \log_{10} \frac{c''NH_3}{c''_{tot}NH_3 - c''NH_3} \quad (7)$$

At the right-hand side, the difference of the logarithms can be replaced by a single logarithm of a quotient. Then, taking the inverse of the logarithm to base 10, and by introducing the solubility of ammonia in human plasma as being independent of pH, i.e., $c'NH_3 = c''NH_3 = \alpha NH_3 \cdot pNH_3$, the following equation for calculating the solubility of ammonia was obtained:

$$\alpha NH_3 = \frac{c'_{tot}NH_3 \cdot 10^{pH'-pH''} - c''_{tot}NH_3}{(10^{pH'-pH''} - 1) \cdot pNH_3} \quad (8)$$

In Eq. (8), all quantities at the right-side were known from experimental measurement, and with the pH values corrected for the alkaline error of the glass electrode, the solubility of ammonia in human plasma could be calculated. Thus, knowing the concentration of dissolved ammonia (cNH_3), the apparent pK of ammonium in human plasma could also be obtained from any pair of measured total ammonia concentration ($c_{tot}NH_3$) and pH by use of the Henderson–Hasselbalch equation, Eq. (4).

3. Results

3.1. Solubility of ammonia in pure water

3.1.1. Derived from the salting-out effect by NaOH

The salting-out effect on ammonia by aqueous NaOH in the concentration range 0.155 to 0.750 M was determined at 37°C and 1.01 mmHg ammonia partial pressure. The solubility of ammonia decreased with increasing con-

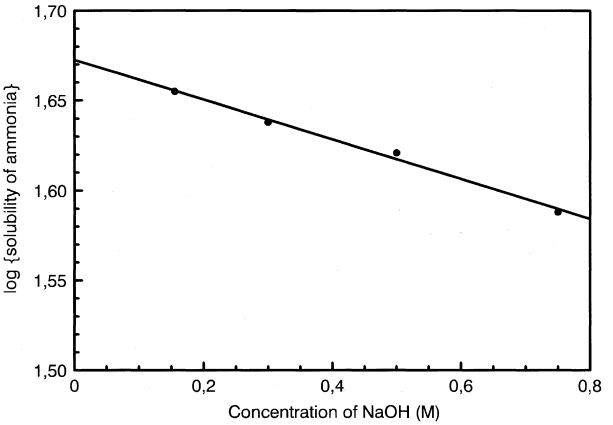

Fig. 1. Solubility of ammonia in pure water derived from the intercept by Setchenov plot ($n=4$): $\log_{10} \alpha NH_3$ versus NaOH concentration.

centration of NaOH, and a plot due to Eq. (2), also called Setchenov plot, yielded a straight line (Fig. 1). The slope, k_S, and the intercept, log $\alpha°NH_3$, calculated by linear regression analysis ($n=4$, $r=-0.9956$) were found to be: 0.109 ± 0.007 M^{-1} and 1.6721 ± 0.0035, respectively. From the intercept at zero concentration of NaOH, a solubility value of 47.00 ± 0.38 mM/mmHg was derived for NH_3 in pure water at 37°C.

3.1.2. From Henry–Dalton's law

To test the validity of Henry–Dalton's law, two series of measurements were run at ammonia partial pressure in the range 0.021 to 0.99 mmHg at 37°C: one series consisting of dilute NaOH (0.010 M), the other of dilute NaOH plus NaCl (0.010 M + 0.145 M). In both series, Henry–Dalton's law proved to be valid, as can be demonstrated by a plot of total ammonia concentration versus ammonia partial pressure in 0.010 M NaOH (Fig. 2), which gives a straight line. The slope of that line through the origin is the solubility of NH_3 at 37°C in this solution. In the two series, obviously, there was no significant difference to be observed, even in the presence of NaCl at 0.145 M. Treatment of the data by linear analysis ($n=8$) yielded a slope of 46.32 ± 0.14 in the first series without NaCl ($r=0.9999$), and, respectively, 46.55 ± 0.31 in the second series with NaCl ($r=0.9996$). Therefore, the solubility of ammonia was derived from both series ($n=16$) and corrected for the small amount of 0.010 M NaOH by use of the Setchenov equation, Eq. (2), and the known salting-out coefficient (0.109) for ammonia by aqueous NaOH to the value of pure water, which is slightly increased to 46.56 ± 0.26 mM/mmHg.

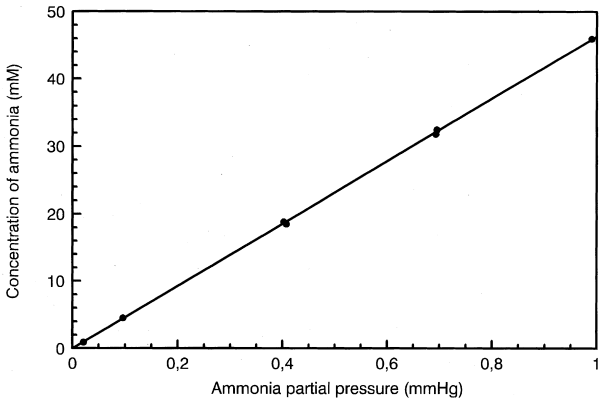

Fig. 2. Solubility of ammonia in pure water derived from slope by Henry–Dalton's law ($n=8$): concentration of ammonia versus partial pressure of ammonia.

3.1.3. Directly measured in pure H_2O

The solubility of ammonia in pure water was also directly determined by measuring total ammonia concentration under equilibrium conditions at 37°C and 1.01 mmHg ammonia partial pressure in pure water (two samples, $n=10$): 48.31 ± 0.57 mM, and in 0.001 M aqueous NaOH (two samples, $n=4$): 48.31 ± 0.73 mM. Obviously, there was no difference between the two series of measurements, and can be comprised by one representative mean (four samples, $n=14$): 48.31 ± 0.59. This total ammonia concentration, however, still included a small amount of unknown ammonium and was roughly corrected by means of the Henderson–Hasselbalch equation for the ammonium system with thermodynamic $pK=8.890$ at 37°C in pure water, and with assumed pH = 10.675 (0.001 M NaOH; $pK_{37}H_2O=13.675$) for all solutions because exact pH was difficult to measure in the unbuffered system. Applied to the total mean, and division by ammonia partial pressure, this leads to a solubility of NH_3 in pure water: 47.06 ± 0.57. In Table 1, all solubility values obtained by the different methods in pure water are summarized: mean, standard deviation, and number of samples (n_i). From these, one total representative mean, and one total standard

Table 1
Solubility of ammonia in pure water at 37°C and low ammonia partial pressure derived from various methods

Solubility in pure water (mM/mmHg)		Method
47.0 ± 0.38 ($\pm0.81\%$)	$n=4$	From intercept of Setchenov plot: $\log_{10}\alpha NH_3$ versus cNaOH
46.6 ± 0.26 ($\pm0.56\%$)	$n=16$	From slope (0/0) of Henry–Dalton's law
47.1 ± 0.57 ($\pm1.21\%$)	$n=4$	Directly measured in pure H_2O and 0.001 M NaOH
46.7 ± 0.40 ($\pm0.86\%$)	$n=24$	Total mean

deviation was derived taking the number of samples as weights by known statistical formula: 46.72±0.40

3.2. Solubility and apparent pK of the system NH_4^+/NH_3

3.2.1. In isotonic salt solutions

All results and experimental conditions of the aqueous electrolyte solutions isotonic to human plasma (0.155 M) and used to determine α and apparent pK are summarized in Table 2. The initial conditions before contact to ammonia gas phase at $t=0$ are defined in the first three columns, where pK_a is the apparent pK of the buffers used to calculate the ammonium concentration, $c\mathrm{NH}_4^+$, which is equal to that of the formed base by use of the Henderson–Hasselbalch equation for that buffer. All apparent pK values of the buffers were experimentally determined by measuring the pH of equimolar solutions (M) of the acid (HA) and its corresponding salt (by addition of half the acid concentration as NaOH) adjusted to an ionic strength of 0.155 M by NaCl: HEPES (0.025/0.0125/0.1425; 0.050/0.025/0.130); L-histidine (0.100/0.050/0.105); glycine (0.100/0.050/0.105) and D,L-alanine (0.100/0.050/0.105). The agreement is good to moderate with values derived from the literature at 37°C and at the same ionic strength: HEPES [13,14] (7.339); L-histidine [15] (8.780); glycine [16] (9.382) and D,L-alanine [16] (9.502). The equilibrium conditions are contained in column 4 to column 9, and the results are in the last two columns. A mean of 46.8±0.81 mM/mmHg was derived for the solubility of ammonia from the various systems which is practically identical to that of pure water, and the mean apparent pK of ammonium was 8.968±0.013.

Table 2
Solubility and apparent pK of the ammonia system in aqueous isotonic salt solutions at 37°C and 1 mmHg ammonia partial pressure: results and experimental conditions

Initial conditions ($t=0$)			Equilibrium conditions						Results	
HA/NaCl		pK_a	pH_{eq}	$c_{tot}NH_3$ (mM)	I_{tot} (mM)	$c\mathrm{NH}_4^+$ (mM)	$c\mathrm{NH}_3$ (mM)	$p\mathrm{NH}_3$ (mmHg)	Solubility (mM/mmHg)	pK
(mM/mM)										
NH_4Cl[a]	47.7/107	–	8.983	95.48±0.36	154.7	47.68	47.80	1.01	47.33	8.982
HCl	45/110	–	8.990	90.90±0.06	155.0	45.00	45.90	0.99	46.36	8.981
HEPES	25/130	7.345	9.244	73.02±0.15	154.7	24.69	48.33	1.01	47.85	8.952
	50/106	7.345	8.960	96.27±0.07	154.9	48.82	47.45	1.01	46.98	8.972
L-histidine	100/105.8	8.851	8.923	96.66±0.06	156.8	50.87	45.79	1.01	45.34	8.969
glycine	100.3/120	9.333	9.085	82.97±0	156.9	36.21	46.76	0.99	47.23	8.974
D,L-alanine	100/123.8	9.456	9.121	78.80±0.06	156.7	31.65	47.15	1.01	46.68	8.948
					Mean±S.D.		Solubility		46.82±0.81	
					$n=7$		Apparent pK		8.968±0.013	

[a] Mean of $n=6$ independent NH_4Cl solutions.

3.2.2. In human plasma

In human plasma, two series of measurements were done which differ in the treatment of blood and plasma prior to the equilibration procedure with ammonia gas phase (see initial conditions in the first two columns of Table 3). In a first series (a) for simultaneous determination of α and apparent pK, isolated plasma was used and adjusted by 1 M NaOH to two different pH values, the lower by 8–10 mM in the range of ammonium pK, the higher by ca. 60 mM. Additionally, for plasma at pH $\cong$ pK, solid NH_4Cl in the order of dissolved NH_3 (ca. 40–49 mM) was added for accelerating the equilibrium process with gaseous ammonia. However, this effect was not significant, and all plasma samples, also including those without NH_4Cl, reached equilibrium after 2 to 5 h. From each combination ($n=5$) consisting of two different measuring pairs of pH and total ammonia concentration of the same plasma (the first three pairs: donor

Table 3
Plasma from human blood equilibrated with ammonia (0.144% in nitrogen) at 37°C and barometric pressure

Initial conditions ($t=0$)		Equilibrium conditions			Results	
$NaOH/NH_4Cl$ (mM/mM)	$pH_{initial}$	pH_{eq}	$c_{tot}NH_3$ (mM)	pNH_3 (mmHg)	Solubility (mM/mmHg)	Apparent pK
(a) Isolated plasma adjusted by 1 M NaOH						
60	10.820	10.507	43.62±0.05 (3)	1.01	41.90	8.995
10/48.6	–	9.443	57.42±0.88 (2)	1.01	(45.39)	
55	10.829	10.568	42.64±1.41 (3)	1.01	41.12	8.996
8/47.7	9.017	8.951	87.61±1.01 (3)	1.01	(44.54)	
65	10.923	10.648	43.02±0.17 (2)	1.00	41.74	8.960
10/39.8	9.214	9.023	78.60±0.41 (2)	1.00	(44.86)	
58	10.947	10.454	44.90±0.11 (2)	1.01	42.89	9.016
8/46.6	9.006	8.994	88.92±0.89 (3)	1.01	(46.16)	
58	11.271	10.822	43.53±0.28 (2)	1.01	42.34	9.073
8	8.875	9.652	54.05±0.61 (2)	1.01	(45.57)	
			Mean±S.D.	$n=5$	42.00±0.66 (45.30±0.63)	9.008±0.042
(b) Separated plasma from blood (B) or isolated plasma (P) prior adjusted at $pO_2=100$ mmHg, $pCO_2=0$, and negative BE (mM)						
− 5 (B)	7.700	9.418	60.64±0.81 (2)	1.01		9.025
−30 (B)	7.043	9.283	65.49±1.15 (2)	1.01		9.000
−30 (B)	6.922	9.293	67.65±0.54 (2)	1.01		9.050
−30 (B)	6.910	9.300	66.40±0.54 (2)	1.01		9.034
−30 (P)	6.888	9.255	66.40±0.41 (2)	1.01		8.989
			Mean±S.D.	$n=5$		9.020±0.025

Column 1: (a) concentration of added NaOH and NH_4Cl to plasma (b) negative BE (mM) by addition of HCl; column 4: number of measurements in parenthesis: these reflect the variation of total ammonia concentration at equilibrium measured from hour to hour; column 6 (in parenthesis): mmol/kg plasma water·mmHg.

1 to 3; the last two pairs: donor 4), the solubility of ammonia was calculated by Eq. (8), and by Eq. (4) with ammonia concentration thus known, also apparent p*K* from pH and total ammonia concentration preferably measured in the range of p*K*. All the results of both solubility and apparent p*K* from series (a) are shown in the last two columns of Table 3 with mean and S.D.: 42.00±0.66, and 9.008±0.042, respectively. Also given in parenthesis is the solubility in molal units (mmol/kg plasma water·mmHg) calculated from the density of the plasma (kg/l) at 37°C and the water content of the plasma (kg H_2O/kg plasma) with a mean: 45.30±0.63. In a second series (b), the blood was treated prior to separation of the plasma and equilibration with gaseous ammonia in the same manner, but with a negative base excess (mM, BE<0) to expel CO_2 from blood and to decrease the initial pH in the plasma with no other addition of substance. At equilibrium with gaseous ammonia at 1.01 mmHg ammonia partial pressure, total ammonia concentration in plasma was measured at only one pH in the range of p*K* of ammonium (9.2–9.4). In measuring total ammonia concentration at a high level (pH≅p*K*), 5 μl of plasma were used and a high NH_4Cl–Standard (80 mM) as a control, and at a lower level (pH≅10.5–11) 10 μl of plasma and a lower NH_4Cl–Standard (40 mM). For calculation of apparent p*K* from series (b) by Eq. (4), the solubility of ammonia in plasma derived from series (a) can be used. However, because all plasma samples in series (b) are from the same donor, the corresponding solubility value in series (a) was taken (the mean of the last two values: donor 4, 42.62±0.39, leading to a mean apparent p*K* of 9.020±0.025 from $n=5$ measurements. From both series of measurements ($n=10$) which give essentially the same result, a total mean of apparent p*K* was calculated: 9.014±0.033.

4. Discussion

In pure water, the solubility of ammonia at 37°C and 1 mmHg was 46.7±0.40 mM/mmHg, and is the average from three different methods: (a) from Setchenov plot by extrapolation to zero concentration of NaOH; (b) by the slope from Henry–Dalton's law; and (c) directly measured as total ammonia concentration in 0.001 M NaOH. For comparison with literature data, Table 4, attention must be paid to the choice of references with respect to similar experimental conditions of temperature and ammonia partial pressure that should be the nearest to 37°C and 1 mmHg. For the solubility of ammonia in pure water, most of the references do not perfectly meet these conditions. The highest (60.8), derived from Hales and Drewes [17] who measured separately the distribution of ammonia in the gas phase (10^{-7} to 10^{-9} M) and in aqueous solution (total ammonia 10^{-4} to 10^{-5} M) in a temperature range 5 to 25°C, was

Table 4
Solubility of ammonia and apparent pK of ammonium at 37°C and 1 mmHg ammonia partial pressure: comparison with literature data

αNH_3 (mM/mmHg)	Conditions	Reference
In pure water		
42.7	Interpolated between 36 and 38°C	Perman EP. 1903 [6]
43.2	Interpolated between 30 and 40°C	Sherwood TK. 1925 [19]
46.4	Calculated by empirical eq. at 37°C	Hakuta T, Edwards TJ, Prausnitz JM. 1977 [20]
60.8	Calculated at 37°C by empirical eq.	Hales JM, Drewes DR. 1979 [17]
43.3	Calculated at 37°C by empirical eq.	Dasgupta PK, Dong S. 1986 [21]
47.1±0.35	Measured at 37°C, 1 mmHg	This work
In human plasma		
41.4±0.72	Measured at 37°C; 0.2/0.5 mmHg	Jacquez JA, Popell JW, Jeltsch R. 1959 [4]
42.0±0.66	Measured at 37°C; 1 mm Hg	This work
Apparent pK		
In isotonic salt solution		
9.025	From thermodynamic pK at 37°C; Calculated by DH at 0.155 M	Bates RG, Pinching GD. 1950 [2]
8.923	Measured at $I=0.15$ M at 35 and 45°C; interpolated to 37°C	Everett DH, Landsman DA. 1954 [18]
9.023	Data from Bates and Pinching calc. at $I=0.155$ M by DH	Jacquez JA, Popell JW, Jeltsch R. 1959 [4]
9.032	Measured at physiological conditions: 37°C; $I=0.150$ M	Bank N, Schwartz WB. 1960 [3]
8.968±0.013	Measured at physiological conditions: 37°C; $I=0.155$ M; 1 mmHg	This work
In human plasma		
9.014±0.033	Measured at 37°C; 1 mmHg	This work

DH: Debye–Hückel equation.

extrapolated to 37°C and 1 mmHg ammonia partial pressure. It should be noted that this is the only measurement which is comparable to the true physiological range in human blood (10 to 60 μM), however, if it is applied, a large sample volume (0.2 l) will be necessary in the solubility cell. All the others are in the range 42 to 46 mM/mmHg, the temperature range being inter- or extrapolated to 37°C with ammonia partial pressure not too far from 1 mmHg, and are close to the value found in this work. The value of 28 mM/mmHg referred by Siggaard-Andersen and not contained in Table 4, was probably calculated from a solubility value at high ammonia partial pressure (~760 mmHg) and should not be used furthermore. The solubility of ammonia in isotonic salt solutions, 46.8 mM/mmHg, was essentially the same in comparison to the mean of 46.7

mM/mmHg in pure water, but strongly decreased (−10.1%) to 42.0 mM/mmHg in human plasma. The last is in good agreement with the value 41.5 of Jacquez et al. [4], directly measured in human plasma by a gasometric method. However, this is based on the assumption that apparent p*K* in human plasma is the same as in isotonic salt solution of ionic strength 0.155 M without proteins. If ammonia solubility in plasma is corrected for plasma water (0.75 ml/g plasma protein), its value increases by a factor of 1.06 (mean plasma proteins: 75±4.3 g/l) to 44.5 mM/mmHg, and its deviation from pure water reduces to only just −4.7%. This approximated value referred to the free space of solution in plasma is in good agreement with the experimental solubility value in molal units: 45.3 mmol/kg plasma water·mmHg. In isotonic salt solutions consisting of aqueous ammonium and sodium salts of HCl, HEPES and amino acids at concentration 0.155 M, the salt effect was negligible in contrast to human plasma with a significant salting-out effect, but no salting-in of ammonia could be observed.

On the other hand, the mean apparent p*K* at 37°C in aqueous isotonic salt solutions (0.155 M) was 8.968±0.013 obtained from five different systems combined with ammonia gas phase at 1 mmHg: NH_4Cl, HCl, HEPES, L-histidine, glycine and D,L-alanine, and this was compared with literature data, Table 4. Depending on the experimental conditions, the different authors can be divided into three categories: The first, Jacquez et al. [4] calculated apparent p*K* at ionic strength $I=0.150$ M from the known thermodynamic p*K* at 37°C by Bates and Pinching [2] with the Debye–Hückel approximation. The second, Everett and Landsman [18] measured apparent p*K* at ionic strength $I=0.15$ M, but at a different temperature, 35°C and 45°C, and it had to be interpolated to 37°C. Only the third, Bank and Schwartz [3] measured apparent p*K* under physiological conditions at 37°C and at 0.150 M ionic strength with the glass electrode showing that the Debye–Hückel equation was consistent with their results. In isotonic salt solutions, the apparent p*K* ranges from 8.923 by Everett and Landsman to 9.032 by Bank and Schwartz with the value 8.968 in between found in this work. In contrast to these measurements, all other groups did not use a defined ammonia gas phase in their experiments. With reference to the value of Bank and Schwartz taken as the most reliable and frequently used, the apparent p*K* measured with defined ammonia gas phase was decreased from 9.032 to 8.968. This means that the apparent dissociation constant of ammonium was significantly increased from $0.929\cdot10^{-9}$ M to $1.076\cdot10^{-9}$ M by 15.8%. In human plasma, the apparent p*K* measured at 37°C and 1 mmHg ammonia partial pressure was 9.014±0.033, and increased relative to that in isotonic salt solutions without proteins. Compared to the apparent p*K* of Bank and Schwartz it is decreased, but to a lesser extent that corresponds to an increase of the apparent dissociation constant by 4.1%, an uncertainty which is acceptable, if the old value was used. For direct comparison in human plasma, no literature data were available.

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