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Joël Lemaire, Michel Heninger, Essyllt Louarn, Ira Katz, Renaud Tissier, et al.. Argon Pharmacokinetics: A Solubility Measurement Technique. *Medical Gas Research*, 2022, 10.4103/2045-9912.351106 . hal-03855485

HAL Id: hal-03855485

<https://hal.science/hal-03855485>

Submitted on 16 Nov 2022

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Argon Pharmacokinetics: A Solubility Measurement Technique

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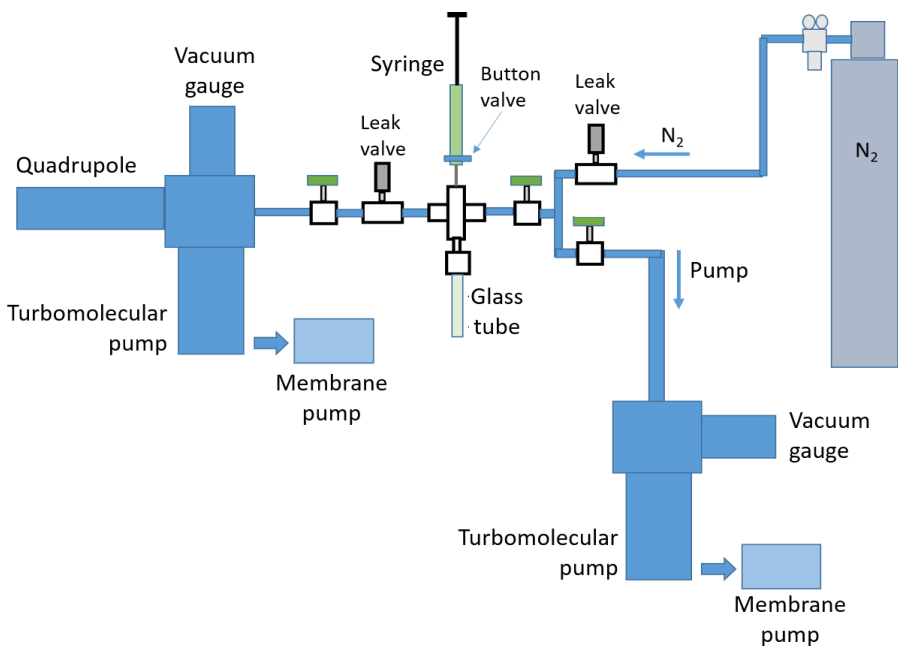
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Running title: Argon Solubility Measurement

Graphical Abstract



Schematic of the system.

Abstract 250 word max

The Noble gas argon has demonstrated biological activity that may prove useful as a medical intervention. Pharmacokinetics (PK), the disposition of the drug molecule in the body through time, is fundamental necessary knowledge to drug discovery, development and even post-marketing. The fundamental measurement to perform in pharmacokinetic studies is blood concentration of the molecule (and its metabolites) of interest. While a physiologically based model of argon PK has appeared in the literature, no experimental data has been published. Thus, argon pharmaceutical development requires measurement of argon solubility in blood. This paper reports on the development of a technique based on mass spectrometry for measuring argon solubility in liquids, including blood, to be further employed in pharmacokinetics testing of argon. Based on a prototype, results are reported from sensitivity experiments using ambient air, water and rabbit blood. The key takeaway is that the system was sensitive to argon during all of the testing. We believe the technique and prototype of the quadrupole mass spectrometer gas analyzer will be capable of inferring argon pharmacokinetics through the analysis of blood samples.

Introduction

The noble gas argon has demonstrated biological activity that may prove useful as a medical intervention in several therapeutic indications.¹ For example, a neonatal rat model study indicated that argon provides neuroprotection against both moderate and severe hypoxia-ischemic brain injury likely via reduction of apoptosis.² Indeed, due to its neuroprotective properties,^{3,4} argon has been considered as an additional treatment to cooling for neonatal encephalopathy. Broad et al. showed the potential of this therapy by administering 45–50% inhaled argon from 2–26 h using a mechanical ventilator in a neonatal piglet model.⁵ Furthermore, cardiovascular safety of this argon therapy was assessed in newborn piglets in a study and found that argon ventilation did not result in a significant change of heart rate, blood pressure, cerebral oxygen saturation, electrocortical brain activity, or blood gas values.⁶

Pharmacokinetics (PK), the disposition of the drug molecule in the body through time, is a scientific discipline that is fundamental necessary knowledge to drug discovery, development and even post-marketing.⁷ PK characteristics of a drug molecule and its formulation have indeed a strong impact on its efficacy on targeted organs and proper dosing. It is mandatory to characterize and optimize this aspect during the drug development process. The fundamental measurement to perform in pharmacokinetic studies is blood concentration of the molecule (and its metabolites) of interest. While we have published a physiologically based model of noble gas PK including argon,⁸ no experimental data has been published. Thus, argon pharmaceutical development requires measurement of argon solubility in blood.

For gas solubility, it is necessary to determine the partition coefficient (PC) between the concentrations in the gas phase in equilibrium with the dissolved concentration in the liquid. Gas solubility measurements are typically made using headspace gas chromatography. In general, this method involves the sampling of the gas phase in the headspace of the liquid in a test vial. Then the component gas concentrations can be determined using gas chromatography.⁹ Progress in xenon concentration measurements has been made, in part due to the need for its detection as a performance enhancing drug.¹¹ Unfortunately the developed methods are not adaptable to argon measurement. Indeed, it has long been understood that the chromatographic separation

of argon and oxygen (normally present in any medical application of argon, and in any animal or human blood sample) is difficult.¹⁰ Due to the high ambient level of argon (close to 1%), injection of the gas sample into the device for gas measurement without air contamination, is also an important complicating factor.

This paper reports on the development of a technique based on mass spectrometry for measuring argon solubility in liquids, including blood, to be further employed in pharmacokinetics testing of argon. To differentiate from headspace gas chromatography, in this method the experimental result is sensitive to the total number of moles leaving a liquid sample, not to argon concentration in the gas phase at equilibrium with the liquid phase. Based on a prototype, results are reported from sensitivity experiments using ambient air, water and rabbit blood.

Methods

System Description

The prototype for measuring argon solubility in liquids, including blood, based on mass spectrometry was developed at the Institut de Chimie Physique, CNRS, Université Paris Saclay (ICP). A photograph of the prototype and a schematic of the system are shown in Figures 1 and 2, respectively. It is based on a quadrupole mass spectrometer gas analyzer (Stanford Research Systems, Sunnyvale, CA) in which the sample injection has been modified. The operation consists of first flushing the complete system with the background gas (nitrogen) to remove ambient argon from the system. The background gas will also act as a carrier for the test gas containing argon. Then the valves between the sample vial and the preparation lines (nitrogen and pump) are closed. The leak valve between the mass spectrometer and the sample stays continuously open and the intensities of the ions formed in the mass spectrometer source are continuously recorded, together with the pressure in the mass spectrometer. The liquid sample is introduced into the sample vial using a gas tight syringe (Valco Instruments Co., Texas, US) that includes an open-and-close valve at its tip. The syringe is screwed onto the injection point with this valve closed (early tests used a needle piercing a gas tight septum). The syringe valve is then opened to inject the sample into the glass tube. The injection time appears as a fast increase on the pressure measurement and the headspace gas is slowly drawn through the leak valve into the quadrupole mass spectrometer to detect argon atoms, and eventually exit to the exhaust. The measurement is run (5000 s) until all of the argon in the sample is drawn out of the liquid and passed through the quadrupole.

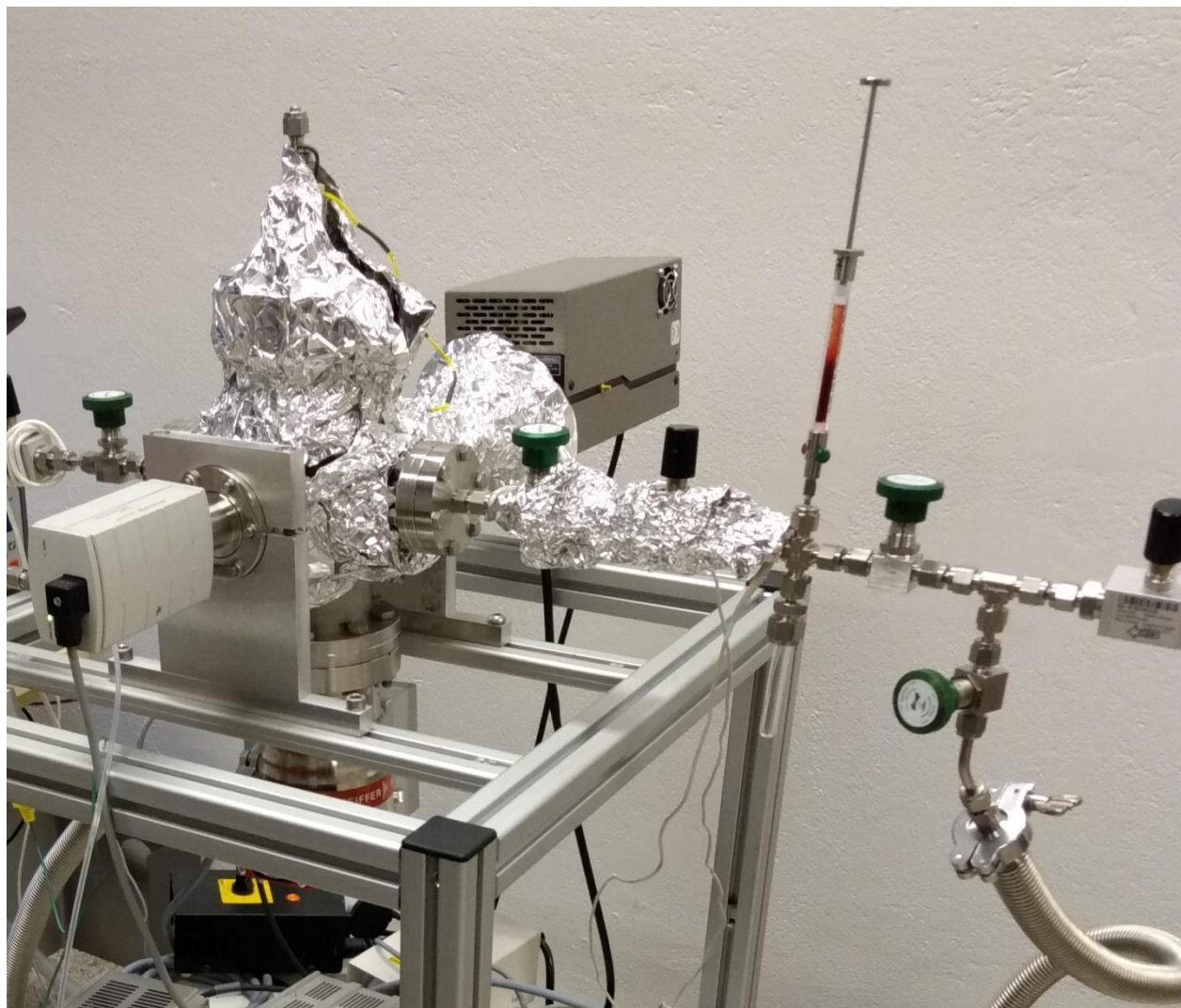


Figure 1. A photograph of the prototype.

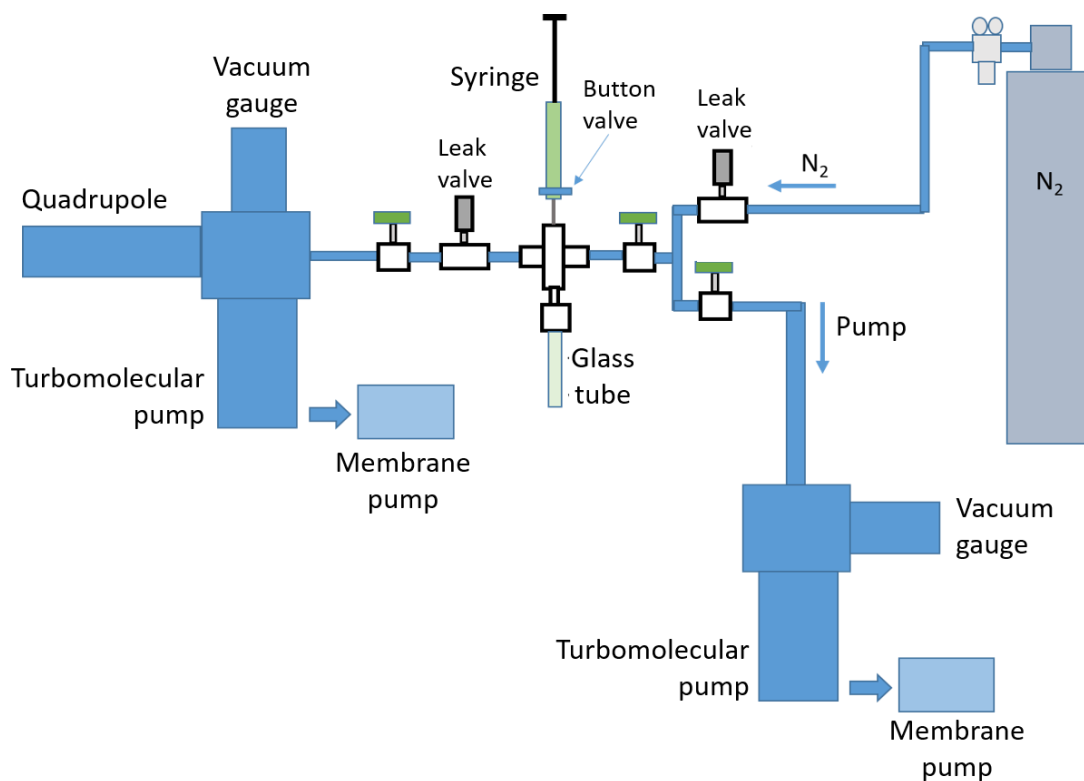


Figure 2. Schematic of the system.

The analyzer samples the gas using a leak valve allowing only a very small amount of the gas into the unit. The pressure in the vacuum chamber of the mass spectrometer is typically set in the range 5×10^{-5} to 1×10^{-4} mbar when the sample volume is at atmospheric pressure. The quadrupole mass spectrometer then analyzes the sample providing ion intensities versus m/z (mass over charge) data. Each m/z originates from a particular neutral atomic or molecular gas. At each time point the ion peak intensity is proportional to the partial pressure of the neutral precursor. For this reason the intensities are given in Torr, however the signals presented do not take into account the difference in ionization efficiency for the different gases. For instance, the intensity measured for the Ar^+ ion will be proportional to the partial pressure of argon. The curve created by this measurement of the Ar^+ intensity over time (see Figure 3) is sensitive to the argon content of the original sample injected into the vial. The integral under this curve is the actual output of the measurement technique and is proportional to the number of moles of Ar initially present in the sample (in units of torr-s). This is similar to a full evaporation technique¹³ in that the amount of a gas species is measured by following dynamically the argon partial pressure

evolution with time, not by measuring the gas concentration in the headspace at equilibrium. Argon volatility is normally sufficient to end the measurement before full evaporation of the liquid solvent.

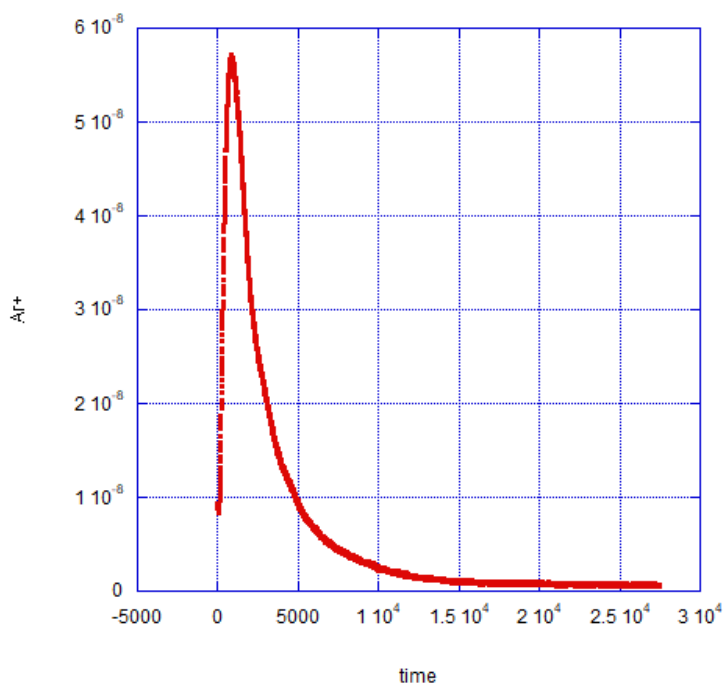


Figure 3. Example of an argon measurement curve. The units for the time, x- axis are seconds. The units of the Ar+, y-axis are the pressure unit torr.

Calculation of the number of moles

Ambient air, water saturated with argon, and blood samples from rabbits who were administered an argon mixture were used to examine the argon sensitivity of the system. The different mediums were employed to obtain a wide range for the number of argon moles.

To calculate the number of moles for air samples we use concentration in the form of (moles/volume). Using the perfect gas law C_{Gas} is

$$C_{Gas} = \frac{p_{Gas}}{RT} \quad (1)$$

Where p_{Gas} is the partial pressure of the gas species of interest, R is the universal gas constant, and T is the absolute temperature. The argon gas concentration is (% by volume=% by mole)

$$C_{Ar-gas} = \frac{(\%Ar/100)p_{Total}}{RT} \quad (2)$$

For ambient air the molar concentration is 0.93%. The total number of gas moles in the air sample is then:

$$\#molesAr = V_{air}C_{Ar-gas} = V_{air} \frac{(\%Ar/100)p_{Total}}{RT} \quad (3)$$

Measurements were performed using $V_{air}=0.25, 0.5,$ and 1 ml air sample volumes.

For the liquid samples, both saturated water and rabbit blood, the number of moles in the sample is calculated based on the partition coefficient. The partition coefficient (PC) is defined as the ratio of the equilibrium concentration of a gas species in a liquid to the concentration in the gas phase.

$$PC = \frac{C_{Liquide}}{C_{Gas}} \quad (4)$$

In this case, C_{Gas} is found using Equation 2, where %Ar was 100% for all of the saturated water tests and ambient (0.93%), 50% or 75% administered to the rabbits. The #molesAr varied based on the temperature of the sample in a controlled bath for water or 37 °C for the blood samples. The total number of gas moles in the liquid sample is then:

$$\#molesAr = V_{Liquid}C_{Ar-liquide} = V_{Liquid} \frac{PC(\%Ar/100)p_{Total}}{RT} \quad (5)$$

where V_{Liquid} is the volume of the liquid sample (1 ml). Note that PC, p_{Total} , and T are the conditions during the gas administration to the liquid. PC values for water¹⁴ and rabbit blood¹⁵ (PC=0.03 at 37° C) were taken from the literature. For water, the tabulated data of PC as a function of temperature were curve fit to the function in Equation (6) using Excel (Microsoft, US),

$$PC_{water} = 7 * 10^{-6}T^2 - 9 * 10^{-4}T \quad (6)$$

Sample preparation

Air samples (0.93% argon) were simply drawn into the syringe in the lab environment at known temperatures. Measurements were performed using different air sample volumes ($V_{air} = 0.25, 0.5,$ and 1 ml) to achieve the variation in the number of moles of argon in the sample.

Saturated water solutions were prepared by bubbling 100% argon gas for at least 30 minutes through distilled water in a flask with septum access for the gas tight syringe. The flask was placed in a temperature controlled water bath to achieve variation in gas solubility.

The rabbit blood sampling was performed at the Ecole Veterinaire Maison-Alfort (ENVA) and the blood samples were analyzed on the quadrupole mass spectrometer gas analyzer located at the Institut de Chimie Physique, CNRS, Université Paris Saclay (RISMAS). Baseline ambient, 50% or 75% argon in mixture with O₂ were administered to the intubated rabbits from premixed gas cylinders through a mechanical ventilator.

Results

Figure 4 shows a composite of all of the sensitivity testing results. Each group of points (color and symbol as shown in the legend) is the result of a set of experiments taken on a particular day over a six-month period using the particular sample medium as also indicated in the legend. The x-axis is the output integral from the quadrupole system. The y-axis is the moles of argon expected to be in the sample based on the calculations using equations 3 and 5 for air, and water and blood, respectively. That is, each sample was prepared to achieve a particular amount of argon moles, and thus, a particular integral output from the system. For air, the variation was achieved by changing the volume of ambient air injected. For water, the variation was achieved by changing the temperature during saturation. For rabbit blood, the variation was achieved by administering different concentrations of argon gas. The overall sensitivity to the argon is apparent in that the general trend is to measure a larger integral output for an increase in argon moles. This result was true for all mediums on any day. However, during all of the sensitivity testing, there was instability in the conversion of integral output to argon moles. Also, note that there is a nonzero output for tests with samples having no argon.

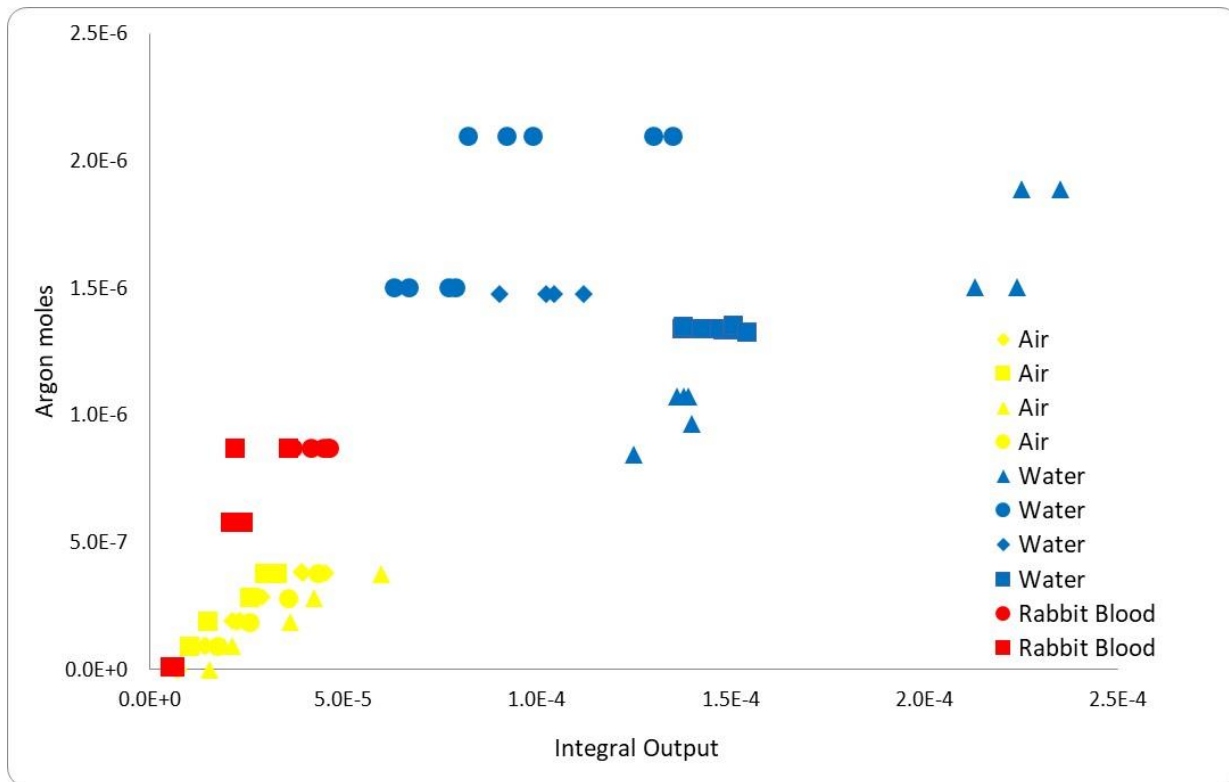


Figure 4. Sensitivity of the quadrupole integral output to the number of argon moles in the sample. Each legend entry represents the result of a set of experiments taken on a particular day over a six-month period using the particular sample medium as indicated in the legend. Variations in all parameters of sample type and preparation are reflected in the number of argon moles. For air, samples the number of moles changes with the volume injected. For water, the number of moles changes with the temperature during saturation with 100% argon. For rabbit blood, the number of moles depends on the concentration of argon administered (50%, 75%, or baseline ambient (0.93%)).

Discussion and Conclusion

A technique for measuring argon solubility in liquids, including blood, based on a quadrupole mass spectrometer gas analyzer has been described. Results from prototype testing of the technique, including rabbit blood, are summarized in Figure 4. The key takeaway from Figure 4 is that the quadrupole system was sensitive to argon during all of the testing. Figure 5 shows the same plot from Figure 4 with only one air and one water test series to highlight this sensitivity. However, the proportionality between the integral output and the number of argon moles was not stable. Also, note that there is a nonzero output for tests with samples having no argon, which implies that not all of the residual ambient argon from the system was cleared or that minute leaks were present.

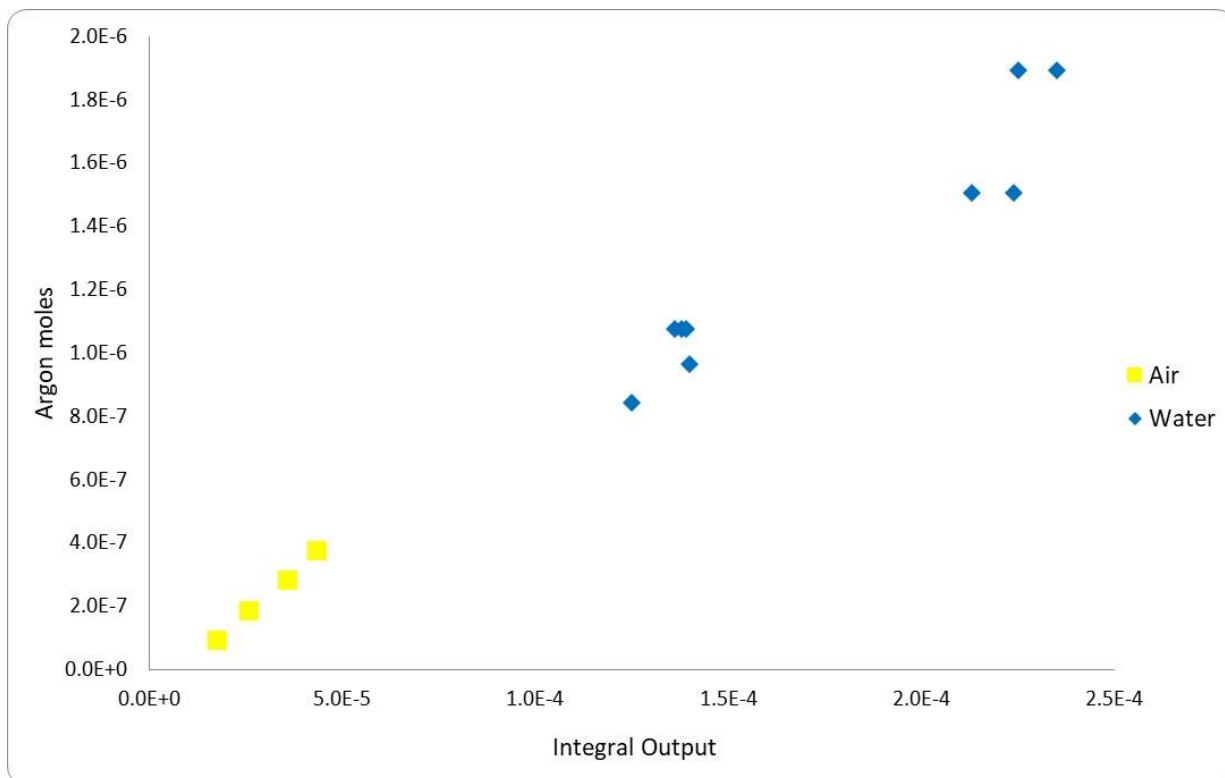


Figure 5. A reduced set of argon sensitivity data from Figure 4 to highlight the stability within each series.

Limitations reflect the development of a prototype, where tweaks of the system may have affected the output. Other limitations of this technique arise from the special precautions

necessary to keep argon-containing samples isolated from the ambient atmosphere and the relatively long duration of the measurement. For blood measurements, it was necessary to transport the samples (on ice) and to store repeat experiment samples for several hours. Preliminary tests indicate some of the instability in blood samples might have occurred due to gas leakage from the syringes. As the goal is to utilize this system for analyzing blood samples taken during pharmacokinetics testing in animals, specifically pigs, a key constraint on using this technique is the time needed to perform each measurement (nearly 2 hours including setup and flushing). Thus, the number of sample points for the PK testing will be limited.

In conclusion, in spite of the limitations described herein, we believe the technique and prototype of the quadrupole mass spectrometer gas analyzer will be capable of inferring argon pharmacokinetics through the analysis of blood samples.

Acknowledgments: Martine Carré for advice on gas analytical methods.

Author contributions: JL, MH, and EL designed the system and experimental protocol, and performed the experiments; IK drafted the paper and designed the experiments; RT performed the gas administration on rabbits, GF, MC and AM designed the experiments; all of the authors read and agreed on the contents.

Conflicts of interest: None GF, MC, IK and AM are employees of Air Liquide

Financial support: This project was funded by Air Liquide.

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