“Air is Everywhere and Vacuum Sucks” – the Heartache of Headspace Sampling

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Headspace analyses mandate that the operator be encumbered by the adage “air is everywhere and vacuum sucks”. Special precautions are required to prevent room air from contaminating the gas sample during preparations for injection into a gas analyzer.

Gas chromatography, by its very nature, requires the entire sample analytes be gaseous to pass through a column and on to a detector. With naturally gaseous samples, such as natural gas or room air, a simple gas sampling valve or gas tight syringe can work fine for sample introduction into the system. With liquids, we usually employ a heated injector to vaporize the sample at injection. However, if the sample has nonvolatile components or major constituents that can interfere in the chromatographic process, we must process that sample by solvent extraction, or use a flash injector with glass wool, to collect this debris before it enters into the column.

Headspace techniques can frequently solve some of these intractable problems. Sample matrices can be left behind by transferring the analytes into a gas volume above the matrix. In these applications, often we can get a gaseous sample by generating a headspace above the sample, allow the gaseous analytes to partition into this headspace and then sample the headspace. This process is quite appropriate for measurement of volatile alcohols in blood, for detecting dissolved gases in water, gases in soil, and gases in transformer oil. These headspace methods often entail collecting samples into vials with septum caps, with the sample filled to the brim without any air gap. Then they transported back to the laboratory for analysis. After the original collection, samples ***cannot*** be transferred to any other container without risk of losing much of the target analytes when exposed to room air.

One approach to create a headspace is by insertion of two appropriately-sized syringes through the vial septum (Figure 1). One syringe has its plunger at zero to allow for the upcoming liquid displacement and its needle penetrating into the liquid. The other filled with a displacement gas, typically helium, to a volume needed for the headspace. By pushing the plunger in the helium syringe into the sample vial, a headspace is created when the liquid flows into the other syringe. The vial is then agitated to generate the partitioning into the gas phase, according to Henry’s Law.[1]

For solid samples, the headspace must be created during the sample collection process, as removal of solids after the initial sample collection to create the headspace risks the serious loss of analytes when the vial is reopened at the laboratory.

One typical headspace method is the US EPA Protocol RSKSOP-175 – “Sample Preparation and Calculations for Dissolved Gas Analysis in Water Samples Using a GC Headspace Equilibrium Technique”.[2] Here the analytes are hydrogen, methane, ethene (ethylene), ethane, propane, butane, ethyne (acetylene), nitrogen, nitrous oxide and oxygen. If the sample vials are 60 ml, this method suggests creating a headspace of 10%, or 6 ml., shaking it vigorously for 5 minutes and then, with a gas-tight syringe with a valve lock on its end,[3] take out a 2 ml sample of the headspace for GC analysis.

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[1] Henry’s Law states that at a constant temperature, the amount of a given gas dissolved in a given type and volume of liquid is directly proportional to the partial pressure of that gas in equilibrium with that liquid. A large compilation of Henry’s constant for water matrices can be found at www.mpch-mainz.mpg.de/~sander/res/henry.html.

[2] www.epa.gov/region1/info/testmethods/pdfs/RSKsop175v2.pdf.

[3] Examples of this type of syringe can be found at www.vici.com/syr/a2.php.

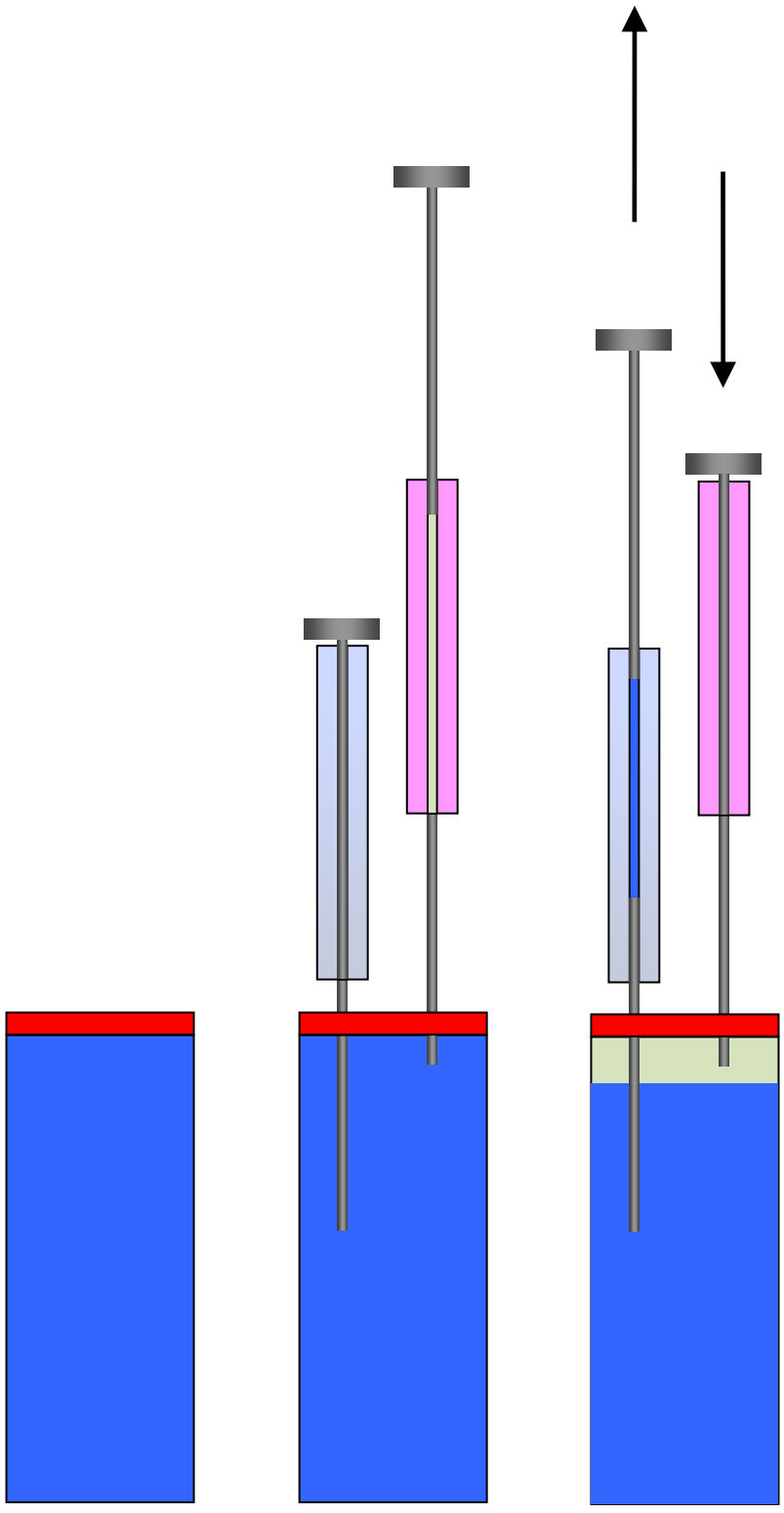

Figure 1. Generation of headspace over liquid in a vial can be accomplished by injecting a gas volume into the septum of the vial, with a second syringe of similar volume set to accept the removed liquid displaced by the gas.

The stopcock action to close off the syringe prior to its removal from the vial is critical because the original 6 ml headspace in the vial has now expanded to a total 8 ml (6 ml in the vial and 2 ml in the syringe), with a corresponding decrease in pressure in both the syringe and the vial headspace. If the syringe were removed without closing off before the needle is removed, the created vacuum would then allow room air to be sucked into the syringe, and effectively alter the composition of the sample.

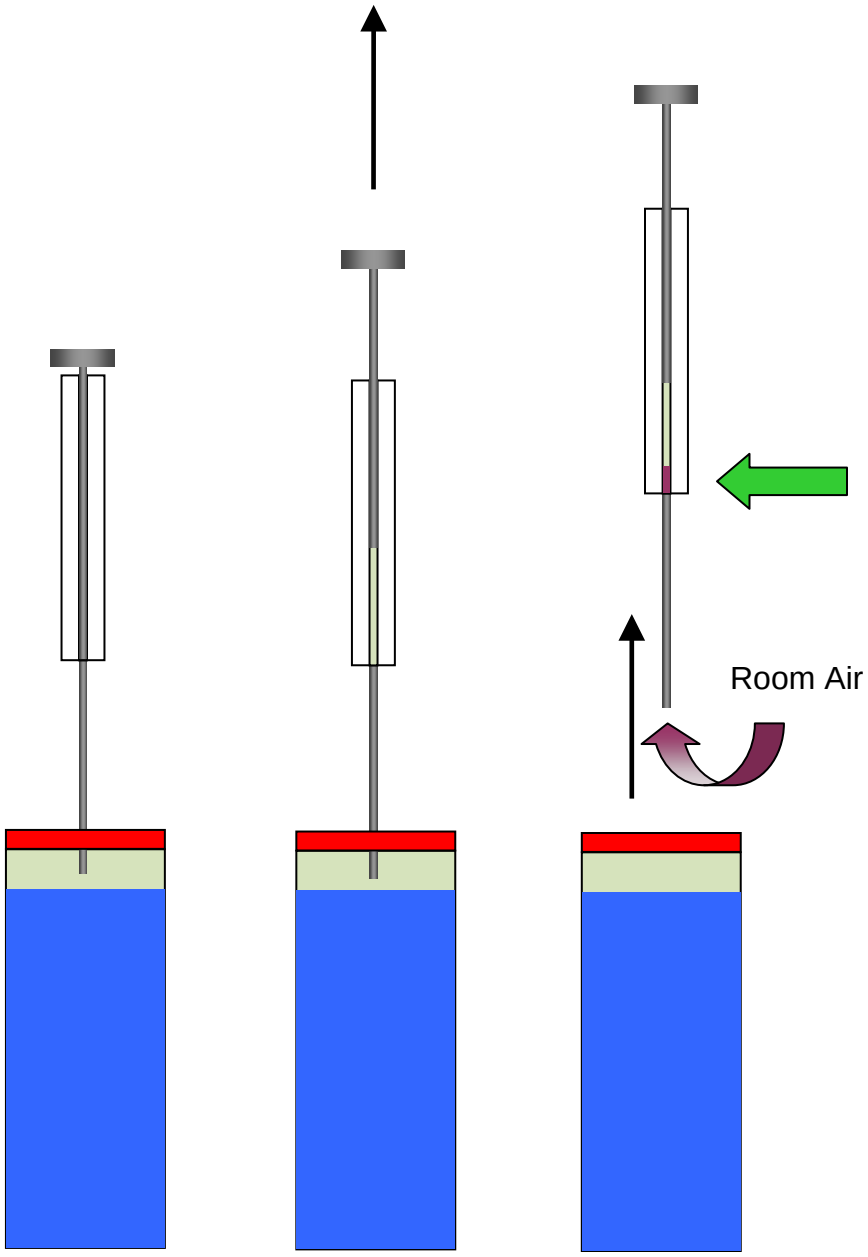

Figure 2. If an aliquot of sample is removed from the headspace of a vial, the contents of the syringe usually will be below atmospheric pressure. When the syringe is removed from the vial, room air will be sucked into the syringe barrel to bring its pressure to atmospheric. The sample is thus diluted and contaminated with components from ambient air.

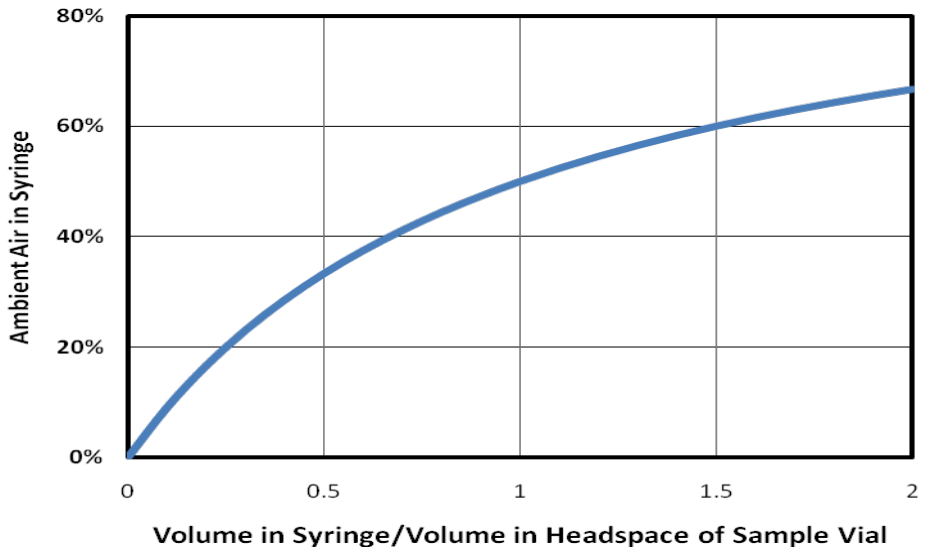

Figure 3. The amount of ambient air sucked up into the syringe is dependent on the volume of the headspace and the extracted volume by syringe. For example, it the headspace volume is 1 ml, and the extracted volume is 0.5 ml, the contents of the syringe will now have 33% ambient air.

The following examples demonstrate the possible systematic errors that could occur with improper processing of a headspace sample.

If a 6 ml headspace is created in a vial and 2 ml of sample are loaded into a syringe for analysis, the pressure in the syringe is now at 0.75 atm. If the syringe were to be removed from the vial without closing off the syringe tip, room air would be sucked back into syringe to equalize the pressure to atmospheric, altering its contents' net concentrations as shown in Table I.

Analyte	Original Concentrations	Ambient dry air Concentrations[4]	Net Result Concentrations[5]	Error
Oxygen/Argon[6]	1.71% V/V	21.88% V/V	6.75% V/V	295%
Nitrogen	6.46% V/V	78.08% V/V	24.4% V/V	277%
Methane	10,240 ppm V/V	1.7 ppm V/V	7,680 ppm V/V	25%
Ethane	34.2 ppm V/V	< 0.1 ppm V/V	25.7 ppm V/V	25%
Ethene	23.6 ppm V/V	< 0.1 ppm V/V	17.7 ppm V/V	25%
Nitrous Oxide	0.530 ppm V/V	0.326 ppmV/V	0.479 ppm V/V	10%

Table I. Errors generated from improper handling of a syringe when extracting 2 ml sample from a 6 ml headspace.

[4] Values obtained from: nssdc.gsfc.nasa.gov/planetary/factsheet/earthfact.html

[5] Net results are computed by :

[Original Conc] *0.75 + [Ambient Conc]*0.25

[6] Oxygen and Argon are extremely difficult to separate without severe chromatographic conditions, including very long columns and very cold column temperatures. Instead, the two concentrations are usually reported together, or oxygen alone can be measured by TCD with argon as carrier, thus cancelling out the contribution from argon.

If instead a 4 ml headspace is created in a vial and 0.25 ml of sample is loaded into a syringe for analysis without closing off the syringe tip with a valve, the pressure in the syringe is now at 0.94 atm and room air will be sucked in to equalize the pressure to atmosphere. Using the same starting points as above, the results would be impacted as shown in Table II.

Analyte	Original Concentrations	Ambient dry air Concentration[3]	Net Result Concentrations[7]	Error
Oxygen/Argon[5]	1.71% V/V	21.88% V/V	2.92% V/V	69%
Nitrogen	6.46% V/V	78.08% V/V	10.76% V/V	65%
Methane	10,240 ppm V/V	1.7 ppm V/V	9,626 ppm V/V	6%
Ethane	34.2 ppm V/V	< 0.1 ppm V/V	32.1 ppm V/V	6%
Ethene	23.6 ppm V/V	< 0.1 ppm V/V	22.2 ppm V/V	6%
Nitrous Oxide	0.530 ppm V/V	0.326 ppmV/V	0.518 ppm V/V	2%

Table I. Errors generated from improper handling of a syringe when extracting 0.25 ml sample from a 4 ml headspace.

To make certain measurement of the injection volume is performed correctly, the best process involves ensuring that the sample syringe achieves a predicted pressure, usually atmospheric pressure, just prior to injection. If this pressure varies, then the amount of sample will be altered by Boyles' Law.[8] When a sample is pulled out of a vial at or below atmospheric pressure, the contents of the syringe will be below atmospheric due to the increase in total volume. By closing off the syringe before it is pulled out of the vial, and allowing the plunger to adjust to an equilibrium pressure, the sample will then be at a constant pressure, at least consistent to a degree under the accuracy of the measurement. In the example illustrated in Table I, 2 ml of sample is initially pulled out and then will collapse to 1.5 ml when allowed to come to pressure equilibrium. With the conditions in Table II, the volume will reduce from 0.25 ml to 0.235 ml. If standards are injected at normal vial pressures and full volume, then the sample results will be in error by significant amounts. A second loading from the same vial will compound the error further.

Atmospheric pressure can vary over the day and especially with approaching storms. Atmospheric pressure has cycles twice daily from global atmospheric tides, with typical variations of 0.27% in a summer day in Long Beach, California (Figure 4). This magnitude can change with weather, with recorded extremes of 7% above average (1968, Agata, Siberia) and 14% below (1979, Western Pacific).[9] Normally this error is minor, but can impact results if system performance can achieve reproducibility below this level.[10]

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[7] Net results are computed by:

[Original Conc] *0.94 + [Ambient Conc]*0.06

[8] Boyles' Law states that for a fixed amount of an ideal gas kept at a fixed temperature, sample pressure and volume are inversely proportional.

[9] www.usatoday.com/weather/resources/askjack/wfaqpres.htm.

[10] Bramston-Cook, R., "Ultimate Performance in Gas Chromatography", Lotus Flower, Inc, Long Beach, Ca, 2007.

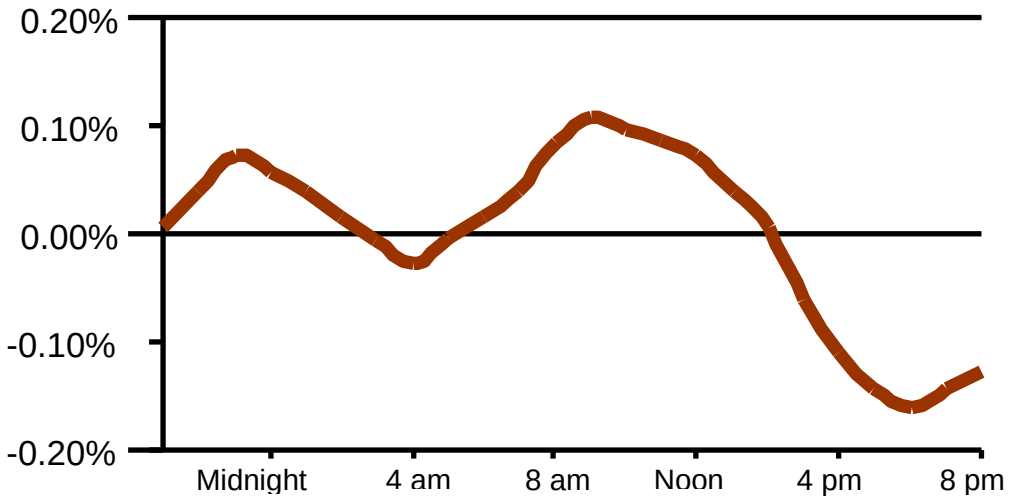

Figure 4. Hourly barometric pressure readings of a typical summer day at Long Beach Airport illustrate routine variations realized over a 24 hour period. Average pressure over the day was 1011 hPa, with the two extremes of 1013 hPa and 1009 hPa. Percentage change in sample loop volume over the day, due to the change in barometric pressure, becomes 0.27% at the extremes.

Another condition that can influence sample loading from a headspace in a vial is the atmospheric pressure at the sample collection point. For example, the relative pressure at Long Beach is 0.999 (2 meters elevation), at Denver airport 0.828 (1,655 meters), and at Berthoud Pass, Colorado 0.644 (3,807 meters). If the sample had been collected and sealed in Denver and transported back to Long Beach for analysis, the sample volume errors in Figure 3 will be compounded by a factor of 1.21, due to the difference in the headspace pressure of the vial. For Berthoud Pass, the factor becomes 1.55. On the other side, if the sample had been collected and sealed in Long Beach and taken to Denver for analysis, the vial will be under pressure, and the syringe will need to expel this excess pressure to yield accurate results.

Temperature of the sample at collection can have an effect on the headspace volume, especially if this temperature is radically different from the laboratory's environs. For example, if the sample were collected at 30 ^{o}C, and then analyzed in a laboratory at 20 ^{o}C, the pressure in the vial would decrease 3%.

Several commercial manufacturers of automated samplers for gas chromatography claim to be able to measure headspace with a simple syringe by just pulling an aliquot of the headspace from a vial. Some systems allow the vial to be first agitated and even heated. However, most do not account for the vacuum generated from removal of some of the headspace volume in the vial, and results will be disrupted as discussed above, with introduction of significant systematic errors and contamination that are very unlikely to be corrected with standards. Applied heat can expand the headspace volume by Charles' Law,[11] but in order to generate an effective total volume of 8 ml for the case in Table I, the vial and syringe temperature would need to be both raised to 125 ^{o}C, with any sample water in the vial now fully aboil.

[11] Charles' Law states that for a constant pressure, the volume of a given mass of an ideal gas increases by the same factor as its temperature.

Suggested Protocols for Extraction of Sample from Headspace in a Vial –

Case 1. Vial sample collected and sealed at atmospheric pressure, with a known-volume headspace. Sample extracted from vial by syringe.

A sample is pulled out with a gas tight syringe with a valve attached (as shown on the right). The valve is closed just prior to removal of the syringe, and the plunger is allowed to adjust to yield atmospheric pressure in the syringe. The sample volume in the syringe is now noted and entered into the sample calculation as a "divisor", with the volume of the standard entered as a "multiplier" to correct for the differences in injection volumes. The sample is then injected for measurement. Standards should be processed in close proximity to samples to minimize possible effects of changes in atmospheric pressure at the time of analysis, especially with approaching or clearing storms.

Case 2. Solid sample collected and sealed at atmospheric pressure with total headspace volume unknown. Sample extracted with headspace vial sampler.

Initial pressure of vial is recorded and entered as a "divisor" into the sample calculation. The vial is then pressurized to a preset value and this value is also noted and entered as a "multiplier", to correct for the effective dilution of the headspace. The sample in the vial is vented through a fixed-volume sample loop and then the loop is injected into the instrument for measurement. Gas standards must be handled by the same process. Volume of the vial is not critical in this computation, other than it should be consistent for both samples and standards; volumes of the sample and standard aliquots are monitored here by pressure readings. The sample should not take up a sizeable portion of the vial content, as its volume cannot be accurately determined readily to effect an adjustment. The vial integrity must be assured when performing this pressurization, as any generated leaks from poor seals can destroy the reliability of the sample contents.

Case 3. Assure that the sample is collected into the vial with sufficient positive pressure and adequately sealed at collection to prevent loss of pressure prior to analysis.

With care at sample collection, the sample vial can be maintained above atmospheric pressure at capping. Then when an aliquot is removed from the vial with a syringe, excess pressure will also be achieved in the syringe. This surplus can then be vented out when the syringe is removed from the vial. The syringe contents should then be injected promptly into the instrument to prevent back diffusion of ambient air into the syringe. A danger here is that the excess pressure could be depleted in the vial without warnings, and the sample can then be diluted as discussed earlier.

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