

THE EFFECT OF DISSOLVED GAS AND AIR BUBBLES ON THE PRECISION OF VARIABLE VOLUME HPLC AUTOSAMPLERS

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Introduction

The presence of dissolved gas and air bubbles in the Mobile Phase and its effects on the performance of HPLC instrumentation have been well documented¹. Some of these effects are irregular flow rates, nonreproducible retention times, excessive detector noise, decreased sample response, and increased detector background. Air bubbles can be detrimental to certain types of HPLC columns. Although not as well documented, dissolved gas and air bubbles can seriously affect the precision and accuracy of syringe-based, variable volume HPLC autosamplers, and a recent article illustrated the typical problems encountered².

The purpose of this presentation is to discuss the effects of air and dissolved gas in syringe-based, variable volume HPLC autosamplers, and to describe a few simple techniques to eliminate this source of problems.

Theoretical

The three different plumbing architectures typically used in the design of syringe-based, variable volume HPLC autosamplers are shown in Figure 1. Note that these plumbing schemes are not all inclusive, but represent a majority of the designs. Certain manufacturers vary the plumbing scheme by using unique sample pump designs while some of the more expensive instruments have sophisticated bubble detection and elimination systems built into the autosampler. Still others use pneumatics to assist in the sample transfer process and while others do not include a rinse reservoir.

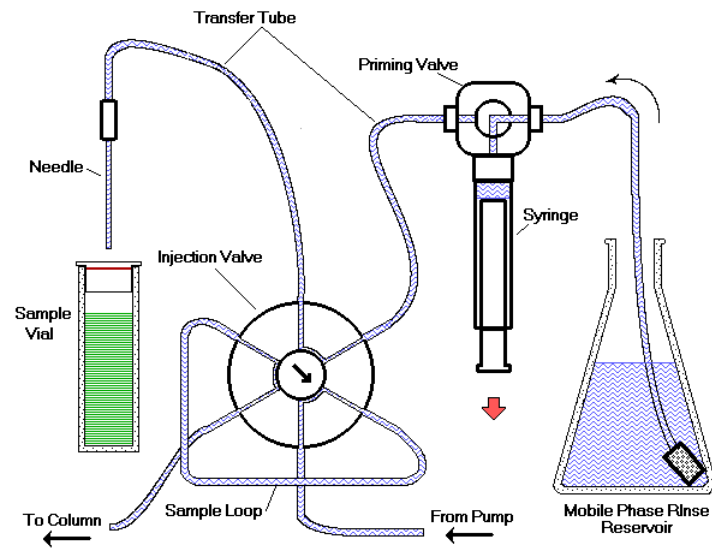


Figure 3A

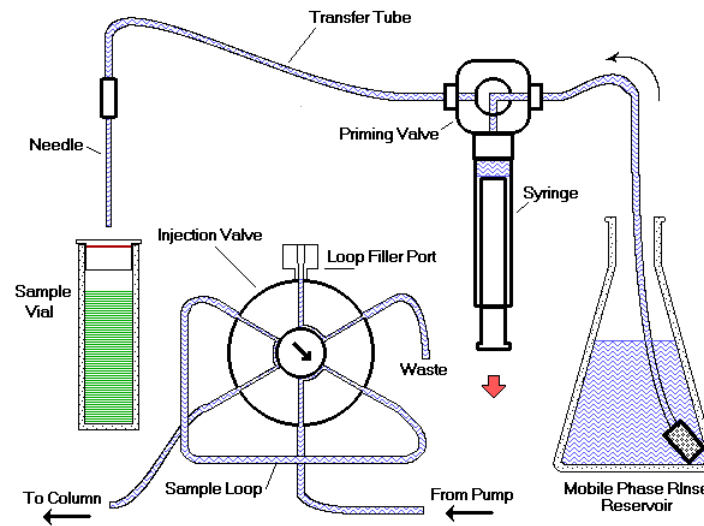


Figure 2B

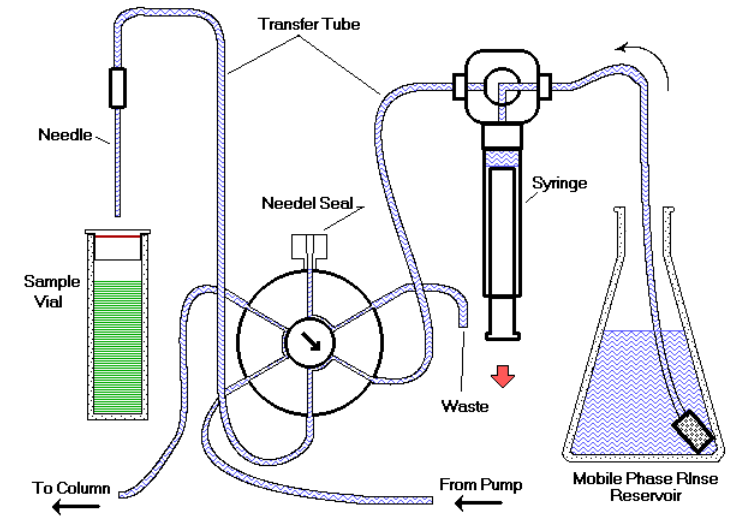


Figure 1C

Note that in each of the designs in Figure 1, the syringe is connected to the sample by means of a long tube which will be called a transfer tube in this discussion. In many designs, the transfer tube is a capillary with an internal

diameter of 0.76 mm (0.030 inches), or less, with lengths ranging from a few centimeters to almost a meter in length. This transfer tube creates a large resistance to fluid flow as the syringe plunger is retracted to withdraw sample. Most autosamplers provide both adjustable sample withdrawal rates and equilibration times to allow compensation for different solvent viscosities and compressibilities. Furthermore, all of these architectures incorporate a solvent reservoir filled with a solution used for priming and rinsing the syringe and its associated plumbing.

Dissolved gases in the sample and the rinse solvent contained within the syringe and transfer tube change the compressibility characteristics of the liquid, usually in an inconsistent manner. For example, if the solution in the rinse reservoir is not degassed daily, the compressibility of that solution will change as air is absorbed. This change in the solvent compressibility affects the consistency in sample metering as the syringe pulls the sample and transfer (rinse/priming) liquid through the long transfer tube. The result is inconsistent injections. In addition, as the syringe plunger retracts to pull liquid through the transfer tube, it causes a vacuum to be applied to the liquid. If dissolved gas is present, it can be pulled out of solution, usually collecting in the syringe barrel. As this gas collects in the syringe, a bubble is formed which grows each time a sample is withdrawn. Again the result is irregular sample injections. The effect that dissolved gas has on the normalized peak area for 30 consecutive 10 μL injections by an autosampler employing Architecture B in Figure 1 is illustrated in Figure 2.

It should be noted that two different effects, plugging or partial plugging of the needle or transfer tube, and the vacuum resulting from a tightly sealed sample vial septum will result in cavitation by the syringe pump. It is not unusual, in these instances, to observe air being withdrawn into the syringe at the plunger seal! When this is observed, all plumbing should be inspected for plugs. If no plugs are found, a slower sample withdraw rate should

be used. To minimize this effect, some instrument manufacturers specify special septa for use with their instruments.

Degassing techniques for HPLC autosamplers are no different from those used for HPLC solvent delivery systems. Reference 1 lists several methods, among which are boiling the solvent before use, exerting a vacuum on the solvent, and inert gas sparging. Most texts on basic HPLC operation list these techniques, however, recent studies have shown that continuous techniques provide the best results^{3,4,5}. Note that using ultra sonics for degassing liquids does not work⁵. The results of continuous Helium degassing and continuous vacuum degassing solutions used with HPLC autosamplers are describe below.

Experimental

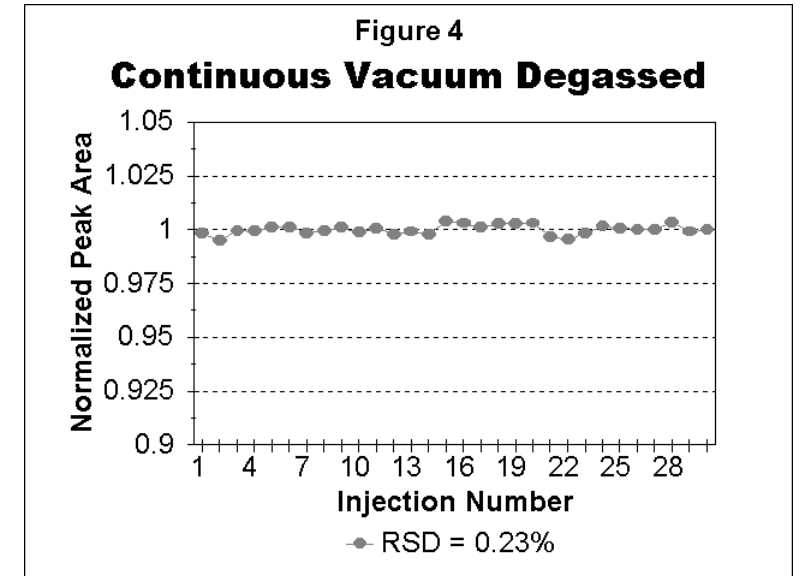
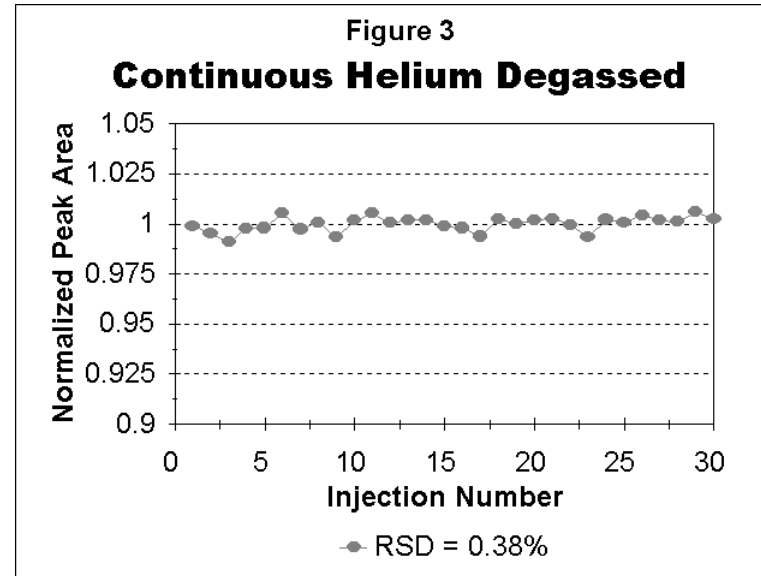
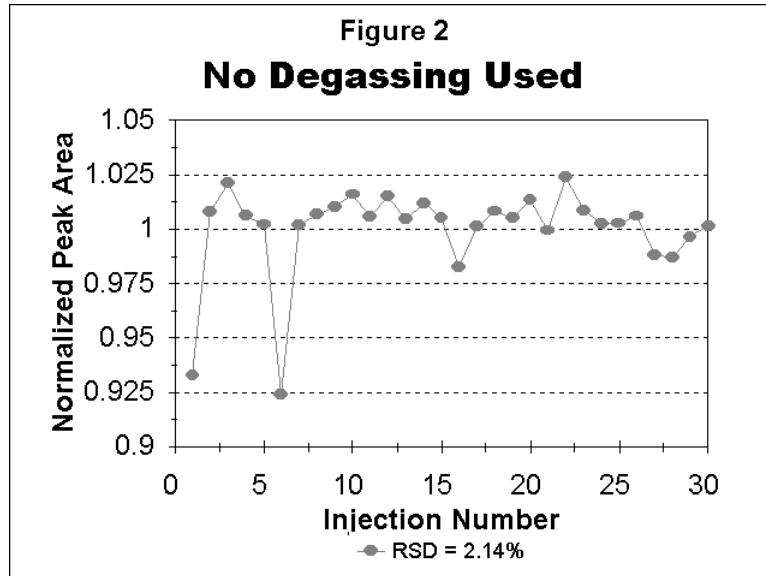
The HPLC system used for the degassing techniques comparison consisted of an Alcott Model 765 HPLC Pump (Alcott Chromatography, Norcross, GA), a Linear Model UVIS 200 Variable Wavelength HPLC Detector and a Spectra Physic Model 4400 Integrator (both from ThermoSeparations, Riviera Beach, FL). The Autosampler was an Alcott Model 718 (Alcott Chromatography, Inc., Norcross, GA) which uses Architecture B in Figure 1. The chromatography was conducted using a 150 mm × 4.5 mm ID column packed with Spherisorb ODS II, 5 μm dp (Phenomenex, Torrance, CA) using a 70/30 Methanol/Water Mobile Phase (HPLC Grade, Fisher Scientific, Norcross, GA). The sample was a Propyl Paraben solution (n-Propyl *p*-Hydroxy Benzoate, Sigma Chemical, St. Louis, MO) with concentration of 0.015 g/L in the 70/30 Methanol/Water Mobile Phase. The Mobile Phase was degassed using a continuous Helium Degassing Accessory (Michrom BioResources, Auburn, CA).

The autosampler Rinse/Prime Solution Reservoir was filled with the Mobile Phase. This solution was used without degassing for the generation of the results shown in Figure 2. The syringe and transfer tubing was primed with 5 mL of this solution (an equivalent of 200 autosampler syringe volumes). For the data shown in Figure 3, the autosampler Rinse/Prime Solution Reservoir filled with fresh Mobile Phase was fitted with a continuous Helium Degas Accessory (Michrom BioResources, Auburn, CA) and the solution was sparged with Helium for 30 minutes at 5 psi. After the sparging, the syringe and transfer tubing was primed with 5 mL of the degassed solution. For Figure 4, the autosampler Rinse/Prime Solution Reservoir filled with fresh Mobile Phase was fitted with a Shodex, Single Channel Continuous Vacuum Degasser (JM Science, Inc., Grand Island, NY). After 30 minutes of vacuum treatment, the syringe and transfer tubing was primed with 5 mL of the degassed solution. Thirty 10 μ L injections of the Propyl Paraben test solution were made under each set of degassing conditions. The Relative Standard Deviation (RSD) for each test is printed in the respective figures.

Results and Discussion

The sample and chromatographic conditions used in these experiments constitute the standard chromatographic checkout procedure used by Alcott Chromatography in the manufacturing of their autosamplers to insure that each instrument meets the design specifications. The peak are precision RSD specification for this instrument under these conditions is better than $\pm 0.50\%$ for 10 μ L injections. As Figure 2 shows, the use of an undegassed Rinse/Prime Solution results in very poor performance (RSD = 2.14%). Use of either of the two continuous degassing systems provides excellent performance as shown in both Figures 3 and 4 showing that the instrument is working well within the specification.

The use of continuous Helium degassing is well documented^{3,4,5} and has been widely used for many years. However, its use is limited in many corporate laboratories due to safety regulations of either the company or the local municipality. In some countries, the cost of Helium is prohibitively expensive. The continuous vacuum device provides a good alternative, however, the cost of these devices may be prohibitive in some laboratories. Nevertheless, either of these techniques work well.



Conclusion

The importance of degassing the samples and solutions used in variable volume autosamplers has been demonstrated to be as important as degassing the Mobile Phase used with the HPLC Solvent Delivery System. The continuous degassing systems provide the best results, although the choice of continuous Helium Sparging and continuous Vacuum devices are left up to the chromatographer. Our experience indicates that both work equally well.

References

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