1.07
Experimental Methods and Techniques: Basic Techniques

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1.07.1 Introduction

A good deal of the chemistry described in this collective edition of *Comprehensive Organometallic Chemistry* is about organometallic complexes in reduced oxidation states. Such complexes tend to be extremely air-sensitive, and the reader should be aware that sophisticated laboratory techniques were required to prepare and characterize these

compounds. Many of the organometallic transformations needed to be monitored by air-sensitive techniques, and if care was not taken to exclude atmospheric oxygen, the unwanted formation of metal oxides would have prevented a lot of the new and exciting chemistry described throughout this edition from being discovered. Advanced anaerobic laboratory techniques are usually handed down from the experienced user to the novice, but in the absence of lineage of experienced predecessors a written guide is invaluable to getting started in the lab. While there have been entire books and manuscripts devoted to the subject of the manipulation of air-sensitive compounds,[1–7] many of them are now out of print and not easily accessible to the newer student or researcher. The intent of this chapter is not to replace these resources, but to provide an updated and consolidated guide to the first year graduate student or novice in the field.

Note about safety—There are many hazards associated with the use of a vacuum and positive pressures of inert gases with the glassware described throughout this chapter, and anyone attempting the following techniques should thoroughly prepare for all of the hazards that are involved.

1.07.2   High Vacuum and Schlenk Lines

1.07.2.1   High Vacuum Line

Perhaps the most important tool a chemist will need to work with air-sensitive compounds is a double manifold that will permit the introduction of both vacuum and inert gases. High vacuum and Schlenk lines contain such manifolds and can aid in most air-sensitive techniques. There are three major differences between a high vacuum and Schlenk line:

 (i) Ground-glass and/or O-ring joints are used to connect reaction vessels on a high vacuum line whereas flexible tubing is typically used on a Schlenk line.
 (ii) A diffusion pump is commonly used in series with a mechanical forepump on a high vacuum line to achieve lower pressures for more air-sensitive compounds.
 (iii) High vacuum lines are typically used to manipulate volatiles while Schlenk lines are more amenable to the transfer of bulk solutions.

Since high vacuum and Schlenk lines possess double manifolds, many of the operations that are performed on them are similar. However, based on our lab's experience, high vacuum lines are ideal for removing/transferring solvents and drying solids, whereas a Schlenk line is superior for cannula transfers and for working under positive pressures of nitrogen. Therefore, depending on the nature of the research, one or both of the above lines may be needed. General aspects of the two lines are provided below.

The basic components of a high vacuum line include:

 (i) *The double manifold*. A basic diagram of a double manifold is shown in Figure 1. It is constructed so that the ports, or "workstations," are connected to both a vacuum line and nitrogen/argon line via a three-way valve. The valves themselves can be designed for ground-glass stopcocks, which require greasing to prevent leaks, or for poly-tetrafluoroethylene (PTFE) stopcocks that have greaseless Teflon or O-ring seals. A manifold equipped with PTFE valves is shown in Figure 1, and glassware containing these types of valves will be described in more detail throughout this chapter.

Double manifolds can now be purchased commercially through a variety of vendors. However, for heavy users, it is still ideal to have a glassblower customize a manifold to meet individual laboratory specifications and needs. If a custom manifold is built, care must be taken to ensure that the glass walls are thick enough to safely maintain pressures ranging from approximately 10^{-4} to 900 torr. Leaks in a high vacuum line can be detected with the use of a Tesla coil, which provides a high voltage, high frequency spark. The electrical discharge is passed over suspected areas of the vacuum line that contain a pinhole leak, and a bright blue spark is produced at the location of the leak. Tesla coils should not be used on thin glass or on dewars, as punctures may occur from the high concentration of voltage.

One optional, but highly recommended accessory to a double manifold is an active pressure gauge that monitors the pressure in the vacuum line. Since leaks from the line can ruin experiments with air-sensitive compounds, it is critical to be aware of the status of the vacuum at all times. A traditional mercury McLeod gauge can provide accurate readings of pressure, but thermocouple and Pirani gauges can more quickly and

continuously monitor changes in pressure. When incorporating active pressure gauges, it is advisable to have a valve which can isolate the gauge from the vacuum line in case the volatile solvents contain chemicals that could react with and damage the sensors in the gauge itself.

Another optional accessory related to the active gauges is a U-shaped mercury manometer like that shown in Figure 2. Such a manometer is constructed with a long, thin U-shaped column that is filled with mercury and then tacked onto a backing support equipped with a ruler so that one can measure pressures usually handled by standard laboratory glassware (1 atm of gas pushes the mercury 760 mm). Typically, the length of one column is about 850 mm to account for over-pressurizing, and a small reservoir can be added to the columns to minimize mercury spilling. An example of how this simple U-shaped manometer can be used to introduce gases into an NMR tube is described in Section 1.07.4.5.
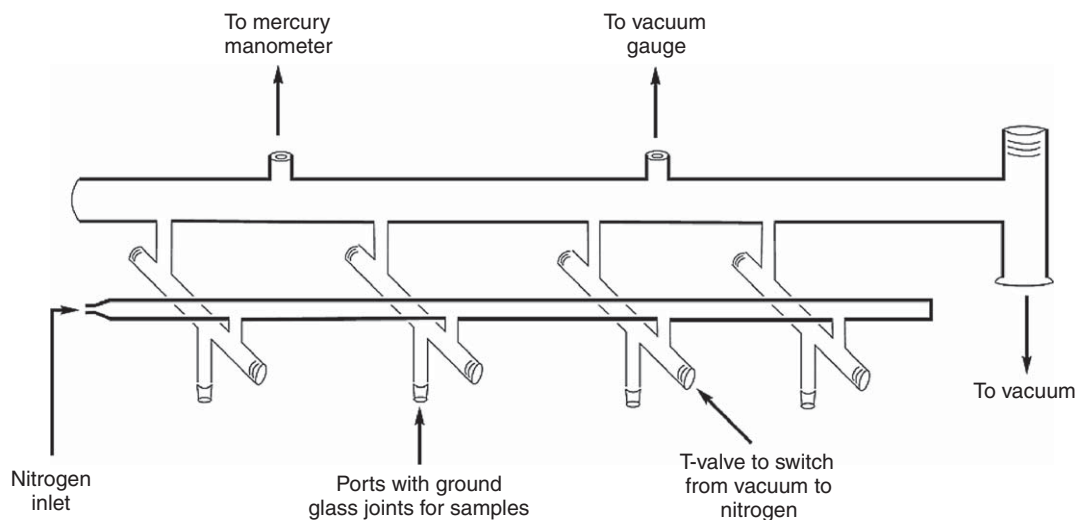
(a)
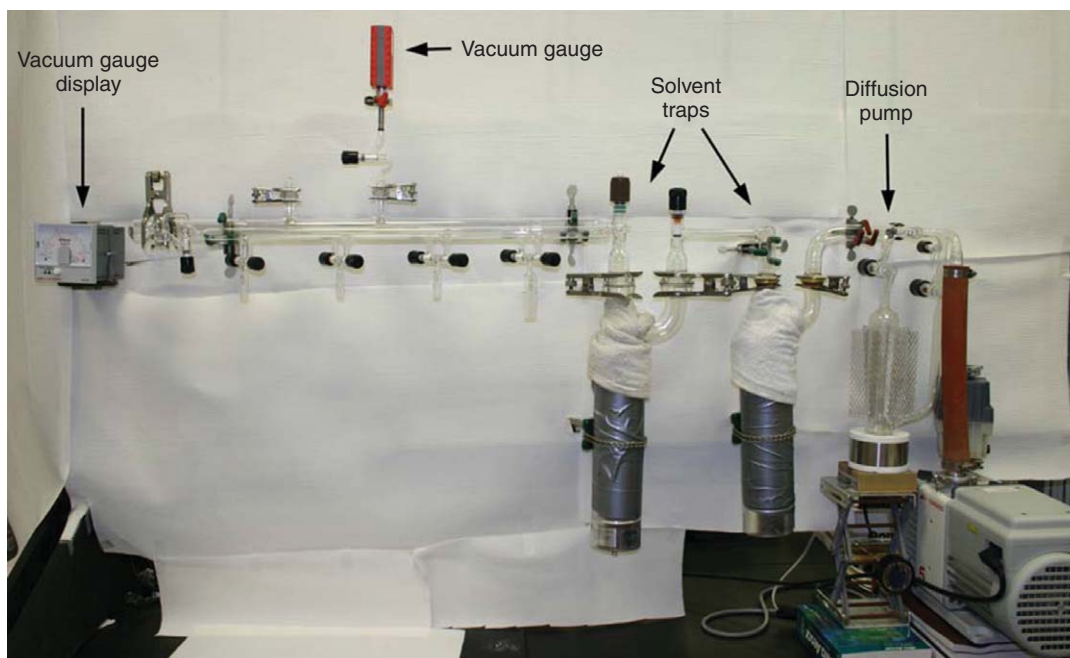
(b)

Figure 1   (a) Schematic diagram of a standard double manifold. (b) Photo of an actual high vacuum line system.
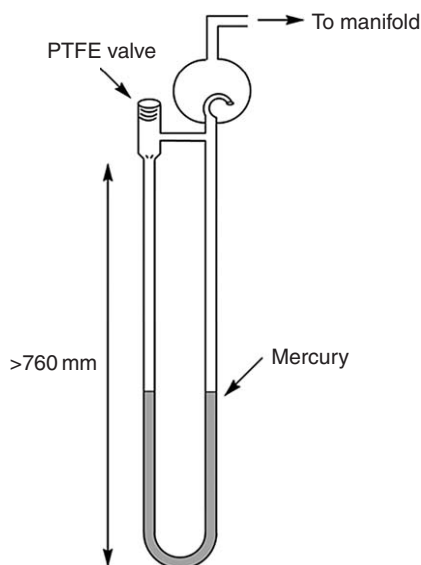
Figure 2   Diagram of a U-shaped mercury manometer for reliably introducing 1 atm of gas into a reaction vessel attached to a high vacuum line. Initially, both sides of the U-shaped column are evacuated along with the rest of the high vacuum manifold. Once a high vacuum has been obtained, the PTFE valve on the manometer is closed, and the high vacuum line is isolated from the vacuum source. Then the gas of choice is introduced into the main manifold until the mercury level advances 760 mm. The manifold is now charged with 1 atm of gas.

The nitrogen/inert gas line of a double manifold can have a number of different designs. The first major consideration is that the inert gas inlet has to be regulated so that pressure does not build up and explode the glass manifold. A dual-stage regulator attached to the inert gas tank is the primary means of controlling pressure. In addition to the regulator, bubblers can be linked to the line to not only provide a pressure release system but also to provide a means to monitor the general flow of nitrogen in the line. Oftentimes one has to introduce the inert gas from a manifold into an evacuated reaction flask. This becomes a tricky process, especially if a bubbler is employed on the nitrogen line, since one has to take care that oil and/or air does not surge into the line once it is opened to the evacuated flask. A common method to avoid such a situation is to use reasonably high positive pressures of nitrogen (being careful not to exceed the safety limits of the glass!) and opening the valve to the evacuated flask slowly enough so that the oil does not flow back into the line. If introduction of inert gas from a manifold into an evacuated reaction flask is a common procedure, then a pressure release bubbler that contains a check-valve (or "firestone valve") to prevent oil or air from surging into the flask may be a good investment.

(ii) *The solvent traps*. Solvent traps are necessary on a vacuum apparatus to prevent organic solvents from ultimately reaching the vacuum pump, where they can mix with and degrade the pump oil. This degradation can be harmful to the pumps, especially direct drive pumps, where the vanes are susceptible to freezing. To prevent these situations from occurring, two, often three, liquid nitrogen traps are placed between the main double manifold where solvents enter the line and the vacuum/diffusion pumps. The traps are designed to be removable, and are separated from the main line and pumps through a series of valves. Figure 3 shows a typical arrangement of two traps in series.

Some solvents have extremely low melting points, so multiple traps are needed to ensure that organic vapors will not reach the pumps. The traps need to be removable so that the condensed solvents can eventually be discarded, and can range in sizes depending on the volumes of solvents being condensed. While the bigger traps are useful in evaporating large amounts of solvents, they also require larger amounts of liquid nitrogen to keep the solvents condensed. Figure 4 shows two sizes of solvent traps. A spare glass trap is handy for rapidly getting the vacuum line up and running while the condensed solvents in the removed trap melt. A spare trap is also recommended to avoid downtime in the event that one trap breaks and needs to be repaired by a glassblower.

(iii) *The vacuum pumps*. A series of two vacuum pumps maintain low pressures on the high vacuum line. The rough forepump usually consists of a direct drive or belt-driven pump which is ultimately exhausted to a fume hood.

Figure 3   Two solvent traps in series. The left trap is connected to the manifold by O-ring joints while the right trap is connected through ball and socket joints.

Because the rough pump can only bring the pressure in a vacuum line down to $\sim 10^{-3}$ torr, placement of a diffusion pump in between the rough pump and the vacuum line helps achieve pressures down to 10^{-4} torr.

Both mercury and oil diffusion pumps are commercially available. The oil-based pumps achieve lower pressures but need to have their oil changed on a regular basis. The mercury-based pumps can go years without having to be changed; however, the health hazards of mercury have led to a general decline in their use. Fan-cooled

Figure 4   Solvent traps can be designed to handle both small (left) and large (right) amounts of solvent.

diffusion pumps are preferred for settings that often require maintenance on the chilled water supply since they do not have to be shut down during any maintenance procedures. The manufacturers of diffusion pumps typically supply instructions with the equipment, so their operational details will not be discussed further in this review.

1.07.2.1.1   Operation of a high vacuum line

Transport of air-sensitive materials to and from a double manifold requires that samples be placed in flasks containing isolation valves which can be linked to the manifold ports. The use of custom-designed one-piece flasks (Figure 5) is perhaps the best way to avoid leaks throughout an air-free manipulation; however, equipping a laboratory with only one-piece flasks is extremely expensive. A more versatile solution is to purchase or design resealable adaptors which can readily attach cheap commercially available glassware to the double manifold. These adaptors are typically designed by a glassblower by means of fusing a resealable PTFE valve to joints that match the manifold and the flask. The adaptors also minimize expenses in the lab, since if a reaction flask breaks, the only piece of equipment that needs to be replaced is commercially available. An example of a round bottom flask attached to a workstation on a high vacuum line via a custom-made adaptor is shown in Figure 6.

1.07.2.1.2   Solvent removal

Now that the basic components of a high vacuum line have been described, it is worth describing in detail the use of a vacuum line for a common laboratory scenario. Take, for instance, a case where a sample needs to be placed on a manifold for solvent evacuation and then later removed without ever being exposed to the air. Such a scenario is common after working up reactions inside a glovebox. The first step in the process is to attach a resealable adaptor to the round bottom flask containing the sample and stir bar (taking care to grease the joints on the adaptor and to close the PTFE valve) in the glovebox. Then, the flask that has been fitted with the adaptor is secured to a port on a double manifold with Keck clips (or rubber bands, or pinch clamps, etc.), and a stir plate is placed below the flask. Next, while leaving the valve on the adaptor closed, the valve on the manifold port is opened to vacuum to remove the air that is trapped between the port and the adaptor valve. Once the pressure on the active gauge display returns to low pressure, the valve on the adaptor can be opened to evaporate the solvent. Evaporation to dryness can be confirmed by monitoring the pressure with the gauge. Once low pressure has again been obtained, the valves on both the manifold port and adaptor are closed. This process seals the flask under a vacuum. The flask with the adaptor can finally be released from the manifold by removing the Keck clip holding the adaptor to the line, opening the nitrogen

Figure 5   (a) Example of a standard taper round bottom flask and its adaptor for use on a high vacuum line. (b) Example of a one-piece flask designed for use on a high vacuum line.

Figure 6   A round bottom flask fitted with an adaptor and attached to a high vacuum line.

valve on the manifold port, and gently pulling the adaptor from the manifold. Since the valve on the adaptor is closed, the sample still remains under vacuum. Now the flask with the sealed adaptor can be brought back into the glovebox (Section 1.07.5.2) and opened under an inert atmosphere for further manipulations. At this point in the process it is advisable to empty the solvent trap of the high vacuum line into the appropriate waste container to ready the line for the next user or application.

1.07.2.1.3   Degassing of a solution

One of the most effective ways to degas a solution is on a high vacuum line using what is referred to as the "freeze–pump–thaw" method. Here a solution containing a stir bar is attached to the vacuum line with the aid of an adaptor as shown in Figure 6. A vacuum is applied up to the PTFE valve of the adaptor, and a dewar of liquid nitrogen is then used to freeze the solution in the flask. Once the solution is frozen, the PTFE valve of the adaptor is opened to the vacuum and any gas that is in the headspace of the frozen solution is evacuated. The gas removal can be confirmed with the use of a pressure gauge. Once a low pressure has been obtained, the PTFE valve of the adaptor is closed and the frozen solution is allowed to thaw. A warm water bath placed on top of a stir plate can be used to facilitate the thawing process. The thawing of the solution allows for any trapped gas bubbles to escape into the headspace of the flask. Repeating this freeze–pump–thaw cycle three times provides quite reliable degassing of standard size reaction flasks.

1.07.2.1.4   Quantitative gas transfers

Another useful feature of a high vacuum line is the ability to transfer precise amounts of gas. For such an operation, a flask of known volume (usually a one-piece flask to prevent leaks) is attached to a workstation and evacuated on a high vacuum line. Then, as described in Figure 2, 1 atm of desired gas is introduced into the system, and the valve of the volumetric flask is closed once the flask contains 1 atm of gas. The number of moles of gas in the volumetric flask can then be determined from the ideal gas law. Introduction of this known quantity of gas into another reaction vessel can then be performed by standard vacuum transfer techniques (Section 1.07.4.4).

At this point it should be mentioned that care must be taken to distinguish between condensable and non-condensable gases. The temperatures involved in gas transfers on a high vacuum line are low enough that many common gases such as methane, ethane, ethylene, O_2, CO, CO_2, can readily condense in a liquid nitrogen cooled

reaction vessel. If the vessel is a closed system, then upon warming of the condensed gas an explosion is likely to ensue. Thus, it is important to only transfer known volumes of condensable gases that are capable of being held safely by the reaction vessel. A rule of thumb is that as long as the volumetric flask is smaller than the available volume of the reaction flask where the gas is being transferred (assuming 1 atm), then pressure will not build up upon warming of the transferred gas. Another common mistake is to forget to remove a liquid nitrogen dewar from a receiving flask or trap once a vacuum has been turned off. Oxygen from the air can condense in such vessels and cause explosions once a sufficiently warm temperature has been achieved.

1.07.2.2   Schlenk Lines

Schlenk lines contain the same double manifold that can be found on high vacuum lines, but are designed to carry out more dynamic techniques such as cannula transfers or operations that require joining multiple pieces of Schlenk ware. Thus, quite often the workstations on a Schlenk line are not standard taper ground-glass joints but rather hose connections for flexible tubing. Since Schlenk lines typically do not have an additional diffusion pump in series with the forepump, they are also more mobile and amenable to fume hood use. Thus, reactions that use low temperature cooling baths or need to be refluxed under a nitrogen atmosphere are typically performed with the aid of a Schlenk line. A huge selection of commercially available Schlenk glassware allows one to perform just about any operation under the exclusion of oxygen, and some basic techniques are described throughout the remainder of this chapter.

1.07.3   Methods of Practical Anaerobic Experimentation

1.07.3.1   Glassware and Preparations

The glassware for air-sensitive experimentation is not only varied but is also constantly evolving. New materials and designs permit safer experimentation and quicker manipulations. Moreover, since double manifolds can easily permit the introduction of inert gases and vacuum to a flask, even standard laboratory glassware can be easily adapted to allow for air-sensitive operations. The Schlenk tube (Figure 7) is the prototypical flask used in air-sensitive operations. It consists of a reservoir to hold samples/solvents and a valve to attach the flask to a source of nitrogen or vacuum. The valves can have greaseless Teflon or O-ring seals or may also be constructed from a ground-glass stopcock that needs to be greased for an airtight seal. The Schlenk tubes also have standard taper ground-glass joints to allow for a variety of connections to other pieces of glassware.

Figure 7   A commercially available Schlenk tube containing a PTFE valve. The valve, tube, and ground-glass joint sizes may vary.

Before introducing an air-sensitive compound into a reaction vessel, the inside of the reaction vessel needs to be placed under an inert atmosphere. On a Schlenk line, this is usually achieved through a number of "pump–fill" cycles. The first step in a pump–fill cycle consists of attaching the vessel to the flexible tubing that is linked to a workstation on the double manifold. The vessel is then sealed with a greased ground-glass stopper. Septa should not be used to seal flasks that must be evacuated because they do not hold a vacuum very well and may even be sucked inside the flask, exposing the compound to air. Septa are generally used only for manipulations that involve positive pressures of nitrogen. A possible exception is the use of inverted joints with septa of the correct size. These combinations lead to sufficiently tight seals that are difficult for the septa to penetrate. Once the flask is sealed and attached to the Schlenk line, the flask is evacuated by opening the workstation valve for the vacuum line on the double manifold. After a substantial amount of oxygen has been removed from the flask, the valve to the vacuum source is closed and the vessel is filled with inert gas by opening up the valve to the nitrogen manifold on the Schlenk line. These pump–fill cycles are repeated at least three times to ensure that the vessel is air-free. A second method for removing oxygen involves flushing an empty flask with an inert gas to displace any oxygen. This method works best with argon, which is heavier than oxygen, but can be employed with nitrogen as long as no part of the apparatus is bypassed by its flow.

Once an inert atmosphere has been achieved in the vessel, the ground-glass stopper can be replaced by a rubber septum so that cannula transfers, syringe additions, or other manipulations involving positive pressures of nitrogen can be facilitated. The exchange of a glass stopper by a rubber septum is achieved by exerting a positive pressure of nitrogen on the flask. The pressure should be large enough to gently force out the ground-glass stopper and prevent any atmospheric gases from entering the flask. To guarantee that the system is producing a strong enough positive pressure, connect a piece of tubing to a vent needle that is plunged into the septum on one end and to an oil bubbler on the other end. When the septum is placed on the flask, bubbles should be appearing in the bubbler. Once the septum has been secured to the flask, the pressure is then adjusted so the septum will not pop off (not a problem when using inverted joints).

1.07.3.2 Additions and Cannula Transfers

Additions to compounds under an inert atmosphere can occur in a variety of ways. Four common methods include:

 (i) rapid addition of air-stable solids under a positive pressure of nitrogen,
 (ii) addition of air-sensitive solids via solid addition funnels,
(iii) liquid or gas additions via syringes, and
(iv) cannula transfer of liquids.

If a solid that is not air sensitive needs to be added to a reaction vessel, then (under a strong positive pressure of nitrogen) the septum or stopper may be removed and the solid can be added directly with a spatula. Often a powder funnel is placed over the opening before the addition to prevent the solids from sticking to the joints or to the sides of the flask.

The addition of air-sensitive solids is trickier and is best performed in a glovebox or glovebag. However, if these are unavailable, or not amenable to the experiment on hand (like variable temperature additions), then commercially available solid addition funnels may suffice. The commercially available addition funnels are not always rigorously air free, however. A simple, more rigorously air-free alternative is a ground-glass sidearm such as that shown in Figure 8 in which one end is fused-closed. With this custom-made accessory, the solids rest on the slanted sidearm and are then added by carefully tapping the sidearm allowing the solids to slowly fall into the flask.
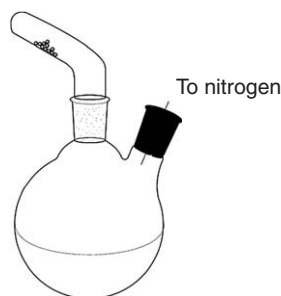

To nitrogen

Figure 8   A simple sidearm extender fused at one end can make a good solid addition apparatus.
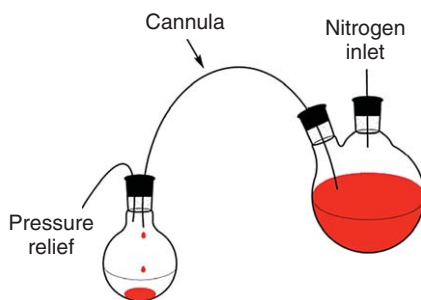
Figure 9   Schematic diagram representing the correct operation of a cannula for the transferring of voluminous liquids.

When using a syringe for solution additions and withdrawals, the syringe is often initially purged multiple times by inserting it into the septum of the flask, slowly drawing up the inert gas of the system, and expelling the gas outside of the system. This needs to be done under a positive pressure of nitrogen, because anytime gas or liquid is withdrawn from a system a partial vacuum is created that needs to be filled with inert gas before oxygen leaks into the system. This fact should be especially kept in mind when withdrawing air-sensitive reagents packed in Sure/Seal™ containers. If a secondary needle to an inert gas source is not inserted into a Sure/Seal™ container while the solution is withdrawn from another syringe, then upon removal of the withdrawing syringe, oxygen will rush into the container and contaminate the reagent. Once a syringe is filled with a liquid, it is usually adequate to quickly insert the syringe into the rubber septum of the reaction vessel and release the contents into the flask. Small volumes of gases may also be added to a vessel under similar conditions, with the only additional requirement of employing gas-tight syringes.

A cannula is especially useful in transferring copious amounts of liquids. It is simply a hollow piece of Teflon or stainless-steel tubing containing double-tipped non-coring ends. In other words, it is a double-ended needle. To transfer solvents or solutions, a cannula is inserted into a solution in one flask that has a positive pressure of nitrogen above it. The other end is then inserted into a receiving flask containing a pressure relief (Figure 9). A cannula transfer operates by forcing nitrogen into the solution vessel through a septum causing a buildup of pressure. Because of this pressure buildup, new septa should be used whenever possible, as older septa tend to leak and could slow down the transfer process. The pressure buildup forces the liquid in the solution vessel through the cannula needle and into the receiving flask in order to relieve the pressure. It is very important to relieve the pressure in the receiving flask through an extra syringe needle vent or bubbler. The rate of addition can be controlled by adjusting the flow rate of nitrogen entering the flask or by adjusting the rate at which pressure is relieved. Adjusting pressure relief is possible through the use of a bubbler containing a pinch clamp attached to its tubing. The transfer can be stopped altogether simply by removing the submerged cannula from the liquid. Cannulation of a solution from a suspended mixture can also be performed by securing filter paper or a sintered filter over one end of the cannula needle.[7]

1.07.3.3   Filtering and Washing

By far, the quickest and easiest method of filtering and washing air-sensitive compounds is by standard benchtop methods inside of a glovebox. For filtration outside of a glovebox, a variety of Schlenk-ware is available to filter reaction flasks without ever exposing the system to oxygen. Filtration can also be performed on a high vacuum line by means of a swivel frit. The swivel frit is composed of a fritted glass funnel with connections for both a reaction flask and a receiving flask. The frit is also constructed with a double manifold connection and a valve that can control the flow of pressure on both sides of the frit. A diagram of a typical swivel frit and its attachment to a high vacuum line are shown in Figure 10.

To perform a swivel frit filtration, the frit is turned upside down (swiveled) and a slight vacuum is applied through control of the manifold workstation valve to "pull" the solution through the frit where it is collected in the receiving flask. The sidearm bypass valve of the swivel frit is closed during the filtering procedure. The solvents may also be "pushed" through the frit by using a positive pressure of nitrogen from the manifold. Alternatively, the solvents may also be pushed through the frit by heating the reaction flask with a heat gun, or they can be pulled through by cooling the receiving flask in a low temperature bath.

(a)

(b)
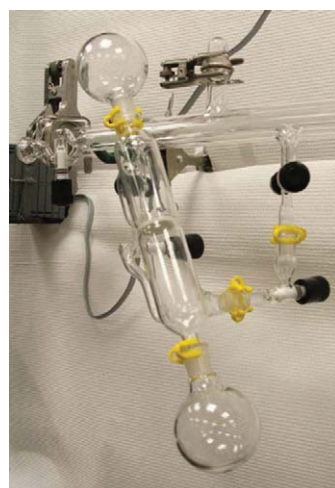
(c)

Figure 10   (a, b) Different views of a swivel frit designed to be used on a high vacuum line with the use of an adaptor. (c) Picture of a swivel frit/round bottom flask assembly attached to a high vacuum line with the use of an adaptor.

The insoluble material collected on the fritted disk of the swivel frit can then be washed using some clever vacuum transfer techniques. First, the solution in the receiving flask is degassed by several freeze–pump–thaw cycles. Then, the solution is warmed with stirring on a water bath, and the sidearm valve of the swivel frit is opened. Next the top flask is swabbed with a dry ice/acetone slurry. A good swab can be constructed from pipe-cleaner wrapped around the tip of a spatula. "Clean" solvent from the filtrate should then condense at the cold, swabbed areas of the top flask. Upon warming, this condensed solvent will run down the sides of the swivel frit and effectively wash the solid material. At this point the sidearm valve can be closed again and the filtration process can be repeated.

1.07.3.4   Crystallization

Schlenk and high vacuum line techniques can be used to perform standard protocols of crystallization such as concentration of solvent, cannula transfer of solvent in which the solute is not soluble, and cooling of a solution to a temperature that leads to precipitation of the solute. In effect, slowing down each of these techniques will produce

larger crystals which could be used for X-ray diffraction experiments. There are advantages and disadvantages to growing X-ray quality crystals with organometallic compounds. One advantage is that most organometallic compounds contain at least one heavy atom, which can aid in recovering phase information in a diffraction experiment. However, the fact that most organometallic compounds are also air sensitive requires some extra care and specialized techniques to both grow and mount crystals. Common methods of crystal growth for organometallic compounds are described below.

1.07.3.4.1 Vapor diffusion

Vapor diffusion is one of the best methods to grow large crystals from a minimum amount of material. This method involves taking a concentrated solution of your sample, say in a small test tube, and placing the test tube inside of a larger Schlenk tube that contains a solvent to precipitate your compound. The Schlenk tube is then sealed tightly and the apparatus is left undisturbed throughout the crystal growth process. Crystals will grow by means of slow vapor diffusion of precipitating solvent from the outer Schlenk tube into the smaller test tube. The same technique can be performed inside of a glovebox using a small vial placed inside a larger one, taking care that the outer vial is equipped with a Teflon-lined or chemically resistant cap. The precipitating solvent should have a lower boiling point than that used in the inner vial. Figure 11 shows a typical setup for vapor diffusion crystal growth inside of a Schlenk tube.

1.07.3.4.2 Solvent layering

This is a simple method by which a precipitating solvent is added slowly enough to a concentrated solution of a sample so that layers form. The vessel is then allowed to stand undisturbed while the two layers slowly mix causing crystal growth. The slow addition of solvent can be achieved in a glovebox by placing a pipette tip on the inner side of the vial containing a solution of the sample and slowly pipetting in the precipitating solvent. With a Schlenk flask, slow addition of the solvent can be achieved by careful cannula transfer of the precipitating solvent from another flask. This method takes practice, and care must be taken so that the flow of the solvent is slow and runs down the side of the Schlenk tube to prevent rapid mixing. The solvent layering method is often combined with the slow cooling process to prevent decomposition of thermally sensitive compounds.

Figure 11   Picture of a vapor diffusion setup for crystal growth inside a Schlenk tube. Solvents are colored for better visual aid.

1.07.3.4.3 Slow cooling

This method works well for compounds that are highly soluble in hydrocarbon solvents. Here, the idea is to cool a concentrated solution of a sample as slowly as possible to produce large crystals. This might be achieved by placing a Schlenk tube containing a solution of the material in a plastic or Styrofoam container before placing in a refrigerator. The only complication with this technique is that care must be taken to ensure the integrity of any valves throughout the cooling process. Grease may freeze and produce channels for oxygen to enter the flask, and PTFE valves can contract causing leaks. Hence, a glovebox fitted with a refrigerator or freezer is ideal for this crystal growing technique.

1.07.3.4.4 Crystal selection and mounting

Once a batch of X-ray quality crystals is in hand, it is usually standard practice to transfer the crystals to a microscope slide in order to choose the best crystal to mount on the diffractometer. With air-sensitive compounds, the easiest way to achieve this is to decant the crystallizing solvent and immerse the crystals in a dense and degassed oil such as Paratone-N, so they can be removed from a vial or Schlenk tube and placed on a slide. The oil forms a protective layer around the crystals that usually prevents oxygen from reacting with the crystals in the amount of time it takes to select and mount the crystal. Paratone-N is an especially nice oil for low temperature crystallography because it is also a cryoprotectant that becomes sufficiently hard upon cooling to $-50\,°C$ that additional glue or epoxy is not needed to mount the crystals to glass fibers throughout the data collection.

1.07.3.5 Chromatography

Under appropriate conditions, column chromatography can be performed on air-sensitive organometallic compounds. Cannula transfer techniques allow chromatography to be done on the more stable compounds without the need for custom-made glassware. For these cases, a standard column is packed dry in the fume hood and then a septum and vent needle are attached. Degassed solvents are subsequently cannulated into the column and the adsorbent is packed tightly under a positive pressure of nitrogen. Eluted bands are then collected under Schlenk-type conditions.

   If access to a glovebox is available, chromatography on the more air-sensitive compounds can be performed quite readily. Gravity elution is often necessary, but if the glovebox is equipped with either a supplemental nitrogen or vacuum inlet, then flash chromatography would be possible. Figure 12 shows one possible adaptation of standard glassware to perform flash chromatography inside a glovebox using a vacuum source.
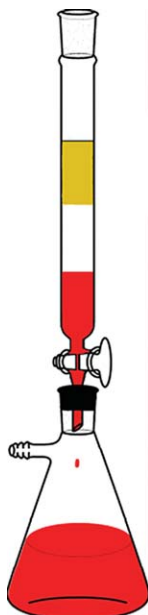

Figure 12   Exploiting a vacuum inlet for flash column chromatography inside a glovebox. The tip of the column is fitted with neoprene filter adapters in order to have a good seal with a standard filter flask. With plenty of solvent on the column and in reserve, a controlled vacuum is then applied to the filter flask in order to rapidly elute the various fractions.

Column chromatography of the highly fragile compounds requires additional care. Sometimes, the adsorbent needs to be prepped before introducing a solution containing an organometallic compound. The author's group has found that, in general, organometallic compounds are quite stable toward activated aluminum oxide (Brockman I, ~150 mesh) that has been heated to 200 °C on a high vacuum line for 2 days before being used. The enhanced stability is perhaps due to the removal of trace amounts of oxygen, water, and/or acid. This rigorous preparation of the adsorbent does not always prevent decomposition, however, and for the extremely sensitive compounds low temperature column chromatography can be performed using columns containing an additional jacket for circulated coolants.[8]

1.07.4   NMR Spectroscopy of Air-Sensitive Compounds

1.07.4.1   Introduction

NMR spectroscopy is routinely used to characterize new compounds, monitor reaction kinetics, measure magnetic susceptibilities,[9] and quantify chemical yields. NMR spectroscopy is also a major technique used to follow exploratory organometallic reactions since thin layer chromatography of fragile, air-sensitive compounds can either be impossible or too tedious. Thus, a wide range of techniques and equipment have been developed to facilitate the use of NMR spectroscopy with air-sensitive compounds. Some essential air-free techniques for a novice in the field are described below.

1.07.4.2   Glassware Involved

The four most widely used NMR tubes used for organometallic chemistry include those equipped with a resealable J. Young valve, those that can be permanently flame-sealed, those containing screw-caps, and standard NMR tubes fitted with a small rubber septum. The most expensive but most versatile NMR tube for manipulating air-sensitive complexes is the one equipped with a J. Young valve (Figure 13). This threaded valve not only forms a tight seal that can be readily opened and closed, but is also designed to attach to a high vacuum line for further manipulations. The high vacuum line capability allows for quantitative transfer of volatiles and gases in and out of the NMR tube.

Permanently sealed NMR tubes are ideal for situations that might cause a J. Young-type NMR tube to leak over time. One such situation involves low temperature reactions in a cold room that may cause the PTFE seal on a J. Young-type NMR tube to contract and leak. Another area of use for a sealed NMR tube is for NMR-scale reactions that take weeks to months to complete. A leak-free environment is thus assured by a permanent glass seal. Sealed tubes also make good NMR reference tubes, as the solvents inside a flame-sealed NMR tube will not evaporate.

Sealing NMR tubes can be done on a high vacuum line using an NMR tube that has been fused to a ground-glass joint assembly (Figure 14). Typically, the NMR tube is loaded with the air-sensitive sample and deuterated solvent inside of a glovebox. Then a high vacuum line adaptor is attached to the ground-glass joint and the whole apparatus is removed from the glovebox and attached to the high vacuum line. Upon cooling the solution to −196 °C, the NMR tube is evacuated. The reduced pressure in the NMR tube causes the walls to collapse upon

Figure 13   Top of a J. Young NMR tube (left), a J. Young valve (center), and a manifold adapter for a J. Young NMR tube (right).

Figure 14 An NMR tube blown to a standard taper ground-glass joint.

treatment of the upper section of the tube with a cool flame from a glass-blowing torch. A torch which uses natural gas (or propane) and oxygen is best, as a hydrogen/oxygen torch produces flames that are too hot. The sample solution is always kept at $-196\,°C$ throughout the flame-sealing process to maintain low pressures in the vacuum line and to prevent ignition of the solvent in the event of a crack in the NMR tube during the flame-sealing process. The flame sealing should also be performed using shaded glassblowing goggles that provide protection against the sodium flare. Once a seal has been made, it is advisable to turn off the oxygen gas and anneal the tube with the natural gas flame. Annealing should be continued until a thin carbon black deposit appears on the surface of the glass. Since the glass of a sealed NMR tube may have additional stress points remaining, the solution should be allowed to warm to room temperature behind proper shielding. Moreover, if the sealed NMR tube is to be subsequently heated in the NMR spectrometer, testing the integrity of the tube by brief heating in an oil bath is recommended to prevent damage to the NMR probe.

Sealing NMR tubes has been made easier by the introduction of commercially available sealing adaptors, which allow one to tip off standard NMR tubes without the need of additional sealing to ground-glass or O-ring joints. Commercially available NMR tubes that are constricted on one end to allow for rapid and symmetrical flame sealing are also available, and a device to conveniently seal and open NMR tubes under air-free conditions has been described elsewhere.[10]

The use of screw-capped tubes (Figure 15) is a rapid way to seal an NMR tube inside of a glovebox, although these tubes cannot be adapted to versatile high vacuum line techniques. Screw-capped tubes contain small seals that permit the introduction of fluids by either syringe or cannula to avoid contamination. The seals on screw-cap NMR tubes are not as tight as for J. Young valves, and should only be used for mildly air-sensitive compounds.

Figure 15 Screw-capped septum NMR tube.

Figure 16   An NMR tube fitted with a small rubber septum.

Attachment of a rubber septum to a standard NMR tube (Figure 16) is the least expensive method for manipulating air-sensitive organometallic compounds. This method works well for sample preparations inside a glovebox. Like a screw-capped tube, one can inject or transfer liquids via cannula liquid into the tube. Caution must be used when attaching a septum to an NMR tube because the thin-walled tube has the potential of breaking.

1.07.4.3   Deuterated Solvent Preparation

Rigorous drying and degassing of the deuterated solvent must typically be performed before exposure to an organometallic compound. Deuterated solvent is placed in a round bottom flask containing a stir bar and the appropriate drying reagent, and multiple freeze–pump–thaw cycles are performed to remove any dissolved gases in the solvent. Stirring the degassed solvents overnight over a large amount of drying agent (or until a color indicator confirms dryness) is usually adequate. Once the solvent is dry, small quantities can be vacuum transferred directly into an NMR tube on a high vacuum line (see below). Alternatively, the bulk dried and degassed solvent can be vacuum transferred into one-piece greaseless ampoules such as those shown in Figure 17 for long-term storage.

1.07.4.4   Transfer of Deuterated Solvents

Although deuterated solvents can be stored in a glovebox and simply pipetted into NMR tubes, there is a risk of contamination from vapors from other solvents that might also be present in the glovebox atmosphere. Purging a glovebox with copious amounts of nitrogen may help reduce contamination of the deuterated solvent. An alternative and cleaner method for transfer of deuterated solvent involves the use of a high vacuum line according to the following basic protocol: an ampoule containing dried and degassed deuterated solvent (like that shown in Figure 17) is placed on a high vacuum line and the manifold is evacuated up to the needle valve of the ampoule.

Figure 17   Air-free ampoules used for deuterated solvent storage and transfer.

Figure 18   Typical setup for transferring deuterated solvents on a high vacuum manifold.

An NMR tube containing a J. Young valve is also placed on the manifold (Figure 18) and fully evacuated. A dewar containing liquid nitrogen is then placed under the NMR tube and the bottom portion of the NMR tube is chilled. Next, the main valve to the vacuum source on the high vacuum line is closed, creating a static vacuum between the ampoule and the NMR tube. Immediately after the line is isolated from the vacuum source, the needle valve on the ampoule containing the deuterated solvent is opened. The temperature difference causes the solvent from the ampoule to be transferred into the chilled NMR tube. Once an appropriate amount of solvent has been transferred, the valves on both the ampoule and the NMR tube are closed, and the manifold can be evacuated once again. The NMR tube is then carefully thawed, usually with organic solvents like acetone, to prevent cracking. The rate of transfer of deuterated solvent will depend upon its vapor pressure, the temperature of the solvent pot (a hot water bath could facilitate transfer), and the ultimate pressure in the vacuum line.

1.07.4.5   Transfer of Gases into an NMR Tube

The transfer of 1 atm of gas into a J. Young NMR tube is most easily accomplished on a high vacuum line with the aid of a U-shaped manometer (Figure 2). During the transfer of gas, the manometer, the NMR tube, and a hose connection to the gas regulator will all be attached to the vacuum manifold. However, before the gas transfer, the solution in the NMR tube must be freeze–pump–thawed to ensure that there is no residual nitrogen atmosphere in the tube. Care must also be taken to thaw the deuterated solution before the gas transfer, so an excess of gas is not condensed into the tube, which may lead to an explosion. Once the vacuum line is charged with 1 atm of gas, the J. Young valve is opened briefly to allow the gas to enter the tube, and then the valve is closed.

1.07.5   Glovebag and Glovebox Techniques

1.07.5.1   The Glovebag

The glovebag allows chemists to manipulate air-sensitive compounds while maintaining an inert atmosphere less expensively than a glovebox. Glovebags are commercially available plastic bags containing hand ports that permit a variety of temporary solutions to many air-sensitive applications. One advantage glovebags have over gloveboxes is the ability to quickly introduce more expensive inert gases such as argon. This feature comes in handy when working with such compounds like lithium metal, which cannot be handled under a nitrogen atmosphere. Glovebags are also more mobile than gloveboxes, and may be employed in small rooms or over various pieces of equipment. For instance, air-sensitive solids can also be conveniently weighed with a glovebag by placing the entire balance inside the bag. This procedure would eliminate the need for storing air-sensitive reagents permanently inside of a glovebox.

Techniques needed for using a glovebag are quite simple. First, make sure there are no holes in the glovebag prior to use. Any wires or tubing that span that plastic membrane should be sealed to reduce the infiltration of atmospheric air inside the bag, which could be potentially dangerous. This can simply be done by wrapping the exit ports with excess Parafilm®. Before opening air-sensitive complexes in the glovebag, purge multiple times to assure an inert atmosphere in the glovebag. Keep a constant pressure of gas to assure no other gases are entering the glovebag. Once one has finished using the glovebag, the air-sensitive compounds should be resealed and the glovebag opened to remove the contents. Glovebags can be reused as long as the plastic integrity of the glovebag is not compromised.

1.07.5.2   The Glovebox

1.07.5.2.1   Introduction

The inert atmosphere glovebox allows one to handle air-sensitive compounds without having to use complicated Schlenk-like glassware. This greatly reduces the amount of time needed to perform anaerobic reactions, especially those involving a lot of manipulations of solids. A wide range of gloveboxes are commercially available, and can come with a custom number of glove ports, refrigerators, cold wells, electrical leads, built-in solvent taps, etc. These amenities further facilitate the workup of air-sensitive organometallic reactions, and the glovebox has evolved from a place to just store air-sensitive solids to a full benchtop replacement. Glovebox work is also ideal for training new students in the field of organometallic chemistry, as chemical fires can be minimized because of the "leak-free," low-oxygen-level environment.

1.07.5.2.2   Glovebox catalyst and atmosphere

Gloveboxes maintain their inert atmosphere either by constant purging of the box with a positive pressure of inert gas, or by removing the moisture and oxygen from the inert atmosphere of the glovebox by recycling an inert atmosphere though a catalyst. If properly maintained, a glovebox can provide an inert atmosphere with oxygen levels less than 1 ppm. For gloveboxes that are not on a constant purge, it is important to realize that any volatile materials that are brought into the box have a certain vapor pressure and can eventually contribute to the glovebox atmosphere. This partial evaporation of solvents inside the box can in turn contaminate other solids and reagents that are stored inside the box. For instance, THF use in a glovebox may interfere with the synthesis of extremely oxophilic and solvent-free lanthanide complexes. However, solvent mixing can be minimized by regular purging of a glovebox under a rapid flow of inert gas, and it is ultimately up to the discretion of the user whether to permit solvent use in a glovebox. Another disadvantage of using solvents in the box is that, over time, solvents can interfere with the ability of the regeneration catalyst to remove oxygen and water and can also limit the lifetime of any oxygen and water sensors that are in use. Regeneration cycles on contemporary gloveboxes do contain extended evacuation cycles which aid in solvent removal from the catalyst bed. Nevertheless, some volatiles absolutely cannot be used in the glovebox because they will permanently poison the catalyst, and the owner should refer to the glovebox manual for a list of chemicals that should be avoided. Typically for gloveboxes employing copper-based catalysts, the following volatiles should be avoided: amines, halogenated solvents, alcohols, phosphines, and thiols. One can also minimize exposure of the catalyst to certain volatiles simply by isolating the catalyst from the atmosphere by closing the circulation valves during solvent use, and then thoroughly purging the atmosphere before the valves are reopened. The term "dry box" is often used for gloveboxes that are set aside to be solvent-free.

1.07.5.2.3   Monitoring the environment of a glovebox

The integrity of the atmosphere of a glovebox can be tested by a few classic methods. The light bulb test estimates the total amount of oxygen inside the box by the time it takes a light bulb filament to burn out when exposed to the glovebox atmosphere. Exposure of the filament to the atmosphere can be achieved by etching a hole in a standard light bulb. If the filament burns out in less than 6 hours, the oxygen/moisture levels are greater than 5 ppm. If the filament burns for days (or weeks!), then the oxygen/moisture levels are between 1 and 5 ppm (see Vac-Atm glovebox instruction manual). For more precise calculation, the filament lifetime relative to ppm oxygen levels can even be calibrated.[11]

A chemical test for the residual moisture levels of a glovebox can be performed with a bottle of titanium tetrachloride ($TiCl_4$). If upon the opening of the bottle a white smoke is immediately produced, it quickly indicates the presence of moisture in the glovebox atmosphere. Chemical tests, while rapid and inexpensive, also may evolve HCl or other impurities that could harm the catalyst. Consequently, purging of the atmosphere with nitrogen should be performed immediately after chemical testing of the glovebox environment. Gloveboxes may also be purchased with convenient digital oxygen and water sensors, and retrofitting older gloveboxes with the newer sensors has become relatively inexpensive. Such sensors can rapidly and continuously monitor the glovebox atmosphere and are highly recommended.

1.07.5.2.4   Sources of impurities

Maintaining an air- and moisture-free box is a constant struggle. Major sources of impurities stem from leaks, normal diffusion of air and moisture into the glovebox atmosphere, and outgassing from porous materials. Leaks can occur anywhere there is a bad seal; however, the major source of leaks in a glovebox is from tears or puncture holes in the rubber gloves. If a small hole is found in the glove, then a simple bicycle repair kit or electric tape can be used as temporary repair. Larger holes in gloves require the gloves to be replaced, so often a set of replacement gloves are kept in the laboratory in case of an emergency. Replacement gloves come as one-piece bonded gloves or two-piece sleeve/glove combinations. The two-piece units provide the convenience of quick, easy glove replacement without having to remove or replace the entire sleeve from the glove box.

Diffusion of air into a glovebox is usually only a concern for gloveboxes containing a large amount of glove ports or a significant amount of plastic construction. Diffusion of air and moisture is accelerated by heavy use, and body heat can increase diffusion of air, and sweat can contribute to diffusion of moisture through the rubber gloves. Typically, there is nothing much one can do to prevent normal diffusion of air and moisture from occurring, so more attention is placed on maintaining an active regeneration catalyst to maintain an inert environment. One can, however, minimize a good deal of contaminants that may arise from the slow outgassing of porous materials. Cork rings, pencils, paper, and other wood-based materials are to be avoided inside a box. If one desires to use Kimwipes or other cloth materials, they should at least be evacuated overnight inside the antechamber before being brought inside.

1.07.5.2.5   Antechamber

The primary disadvantage of a glovebox is that some time is needed to bring materials in and out of a glovebox. The antechamber is the chamber separating the inside of the glovebox from the outside environment. In order to bring items into the glovebox, the outer antechamber door is opened (making sure that the inner door is closed), the contents are placed inside the antechamber, the outer door is closed, and a vacuum is applied to remove oxygen. The amount of time the antechamber is pumped down depends both upon the size of the antechamber and the speed of the vacuum pump, but the time may be shortened by multiple pump–fill cycles using the inert atmosphere inside of the box as the filling gas. Mini-antechambers can usually be found on modern-day gloveboxes, and allow one to bring smaller items into the glovebox more rapidly. Once the antechamber is adequately air-free from multiple pump–fill cycles, a final refill is performed and the contents can be brought through the inner antechamber door.

One complication that arises from the need to use pump–fill cycles to bring items into the glovebox is that any solvent or solution that is brought into the glovebox must be degassed on a vacuum line and/or placed in a thick-walled container to prevent the explosion of the flask from the pressure differential of the evacuated antechamber. An example of a thick-walled flask used to bring bulk solvents into a glovebox is shown in Figure 19. This flask contains two ports which can be connected to both a solvent still and a high vacuum line, allowing the direct transfer of the solvent from a still to the flask under anaerobic conditions. Thus, before this flask is placed in the antechamber, the contents are already under vacuum and the seals are strong enough to survive the pump–fill cycles of the
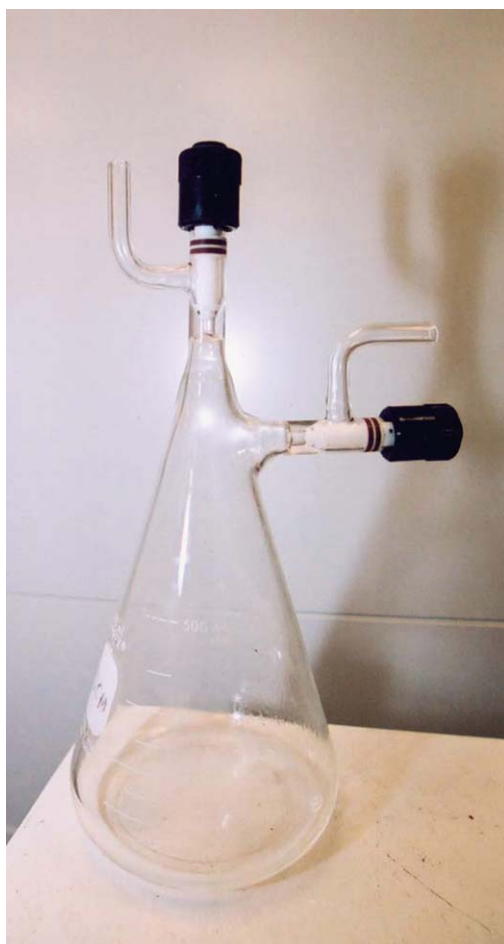
Figure 19 A thick-walled flask used to bring solvents into the glovebox.

antechamber. An additional note is that flasks containing solvent that are sealed with a septum typically cannot survive the pump–fill cycles as the pressure differential will pop off the septum, even if it is secured with wire reinforcements.

1.07.5.2.6 Miscellaneous glovebox techniques

A very useful optional feature of modern-day gloveboxes is the integrated cold well (Figure 20). The cold well contains an external dewar which can be filled with liquid nitrogen or other cooling agents to permit low temperature operations inside of a glovebox. Cold wells are especially nice for low temperature crystallizations, which can be combined with filtration techniques (see below) to rapidly work up reactions under air-free conditions. Combined with a recirculation pump that can handle organic materials, these cold wells can also permit refluxing operations to be performed inside of a glovebox using anhydrous solvent as a cooling fluid. A comparable and inexpensive system that enables the cooling of reactions inside a glovebox without the use of a cold well has previously been described.[12]

Vacuum filtration can be performed in a glovebox after a few minor adjustments. Assuming a port for standard pipe fittings is available on a glovebox, a filtration device can be built that uses a solenoid valve connected to a foot switch on the exterior of the glovebox port, and linked to a safety valve containing a hose connection on the inside of the box (Figure 21). A flexible hose can then be employed for connecting the external vacuum pump to standard filter flasks. Such a setup allows the operator to use both hands inside of the glovebox for performing delicate operations while maintaining control of the vacuum with the foot switch.

Figure 20 Picture of a Vacuum Atmospheres Co. (VAC) glovebox equipped with a cold well.

Figure 21 Picture of a pipe fitting adapted for vacuum filtration use inside a glovebox. The solenoid valve controls an opening to a vacuum source through a foot switch (not shown).

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