

all about **FITTINGS**
by John W. Batts, IV



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Preface

Fittings and tubing comprise a vital, but often ignored portion of the HPLC system. At first glance, the inexperienced eye sees little or no difference between fittings styles and brands. Although these components are not particularly complicated, a solid understanding of their application and use is essential to avoid certain HPLC problems.

As I reviewed the manuscript for this booklet, I realized that I have never seen as simple and straightforward an explanation of the function and use of HPLC fittings. The conversational style makes *all about FITTINGS* read like a novel, yet transmit accurate technical details. The addition of cartoon characters helps to draw the reader's attention to the core concepts. A couple of hours of reading will reward one with a solid understanding of how HPLC fittings work and when to use different fitting products.

Finally, although Upchurch Scientific has been an innovator in HPLC fitting design and is one of the top suppliers of fittings, the information contained here is not a blatant (or even thinly veiled) advertisement for Upchurch products. Rather, this is a truly educational resource that will remain a part of my technical library.

— John W. Dolan, Ph.D.
LC Troubleshooting Editor
LC/GC Magazine

Foreword

For decades, scientists have been using equipment in their labs. Ironically, with all the time and money spent on such equipment — and the education to know what to do with it — very little is spent on understanding how to make a good connection with fittings.

One popular resource Upchurch Scientific offered in the past was the booklet “HPLC Fittings” by Paul Upchurch, the company's founder. Depended upon by scientists in various universities and laboratories around the globe, this booklet provided some basic information on HPLC equipment and related accessories and fittings.

Recent advancements, however, have necessitated a reworking of this popular booklet to address both existing and emerging trends.

With more and more laboratories using capillary-based equipment and hyphenated systems (such as LC-MS), the need for more detailed yet fundamental information about fittings has greatly increased.

The focus of this booklet is to provide essential information often required to identify the correct style and type of fitting needed for most laboratory applications.

We hope you will find this booklet a tremendous resource, something you can use to help you locate just the right fittings to do all sorts of laboratory work.

If you have any suggestions for future editions, or need some additional help in identifying the fitting you need, please feel free to contact us at any time.

Happy Connecting!

— John W. Batts, IV
Scivex, Upchurch Scientific Division

Introduction

Welcome to *all about FITTINGS*! You are about to embark on an exciting journey designed to help you grasp the fundamentals of equipment fittings and accessories, as well as some basics about HPLC as an analytical technique.

You are most likely familiar with some of the topics covered in this manual. We've found, though, that a lot of the fundamental information about fittings and accessories isn't generally known...often because the information isn't covered in most college-level classes.

To make matters worse, most fittings and accessories-manufacturing companies have a vocabulary and "lingo" all their own — often consisting of engineering terms, mathematical measurements, and a large array of material names...and they expect you to understand their terminology.

That's what this book will help you do!

We've built into this handbook information on fittings basics: how to describe a fitting, how to determine where in your system certain styles of fittings are used, the interchangeability of different types of fittings—among many other topics. We've even built in a discussion on the special terminology and skill set needed to work with hyphenated chromatography systems, such as LC-MS.

Through this booklet, you will gain the confidence you need to know exactly what fitting you need!



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Icons

The Icons below are found in the page margins throughout this booklet to highlight extra-important information you'll need to know:



Key Concept

"Key Concept" — Pay extra close attention...the concept covered is important to understand!



This or That

"This or That?" — Draws your attention to extra details you may need when deciding what product to use or where to use a product.



I've Struck Gold

"I've Struck Gold!" — Used whenever a "secret" is revealed—something to help make your life easier or help make something more understandable.



Pardon Moi

"Pardon Moi?" — Denotes those frequently-asked questions that puzzle scientists around the world!



Help Me

"Help Me!" — Highlights information that helps explain a topic or make something easier to understand.



Point of Interest

"Point of Interest" — Indicates there's something so interesting about fittings that we just can't hide it.

Just What ARE Fittings?



Pardon Moi

That's one of the most popular questions never asked! In fact, it's not usually until your system breaks down that you even realize all the places where fittings are used!

Just what are fittings anyway?

According to the dictionary, a fitting is...

...a small part used to join, adjust, or adapt other parts, as in a system of pipes

In other words, anywhere you need to attach, adapt, or adjust piping – or tubing, in this case – there's a fitting involved!

In fact, all kinds of fittings are used in a standard laboratory system: flanged and flangeless; metallic and non-metallic; high-pressure and low-pressure; flat-bottomed and coned; internal and external; wrench-tightened and finger-tightened.

As Paul Upchurch states in his book entitled *HPLC Fittings*:

“...every chromatographer knows, in order to use any HPLC system, you spend a lot of time working with fittings. You must become knowledgeable with the plumbing of an HPLC system in order to do any practical HPLC work.”

Chances are by now, you probably realize just how important it is to really understand fittings.

So, let's talk about some basics...

Actually, what we commonly call a “fitting” in the analytical instrument world really refers to a **system** comprised of a **nut** and a **ferrule** (pronounced “FAIR-ruhl”).

Ultimately, the choice of which nut and which ferrule to use in your system will be dependent upon a number of parameters:

- ① Threads of the receiving port
- ② Geometry of the receiving port
- ③ Size and type of tubing used
- ④ Material from which the port is made
- ⑤ Amount of pressure expected

...and several others. With all these factors, let's see if we can shed some light on this dimly-lit fittings pathway.

AWW, Nuts!

The first of the two major components in a fitting system is called a nut. The nut is responsible for providing the driving force that causes the ferrule to seal.

A nut is typically characterized by some sort of head geometry (e.g., knurled, hexagonal, square) to aid in the tightening process and a threaded portion, allowing it to mate with a receiving port. Let's discuss each of these in further detail to help distinguish what products you might be using, as well as what other products are available.

Threads

Most nuts have “external threads,” meaning that the threads are on the outside of the nut. Some nuts, however, have “internal threads,” where the threads are on the inside of the nut – commonly referred to as a “cap nut” or “female nut.” (See Figure 1 on page 10)



Key Concept



Key Concept



This or That



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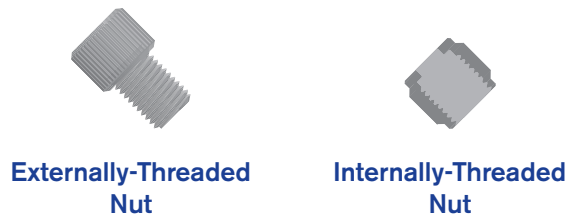


Figure 1 – Threaded Nuts

Because most nuts have external threads, let's focus on this nut geometry...

In order to describe a thread on a fitting, two main numbers are used. The first number tells you about the diameter of the thread, and the second number describes how close together the threads are. Here's an easy example:

One of the most widely-used threads in low pressure fluid transfer is 1/4-28. Notice the two numbers here, separated by a hyphen. Now, let's apply the description above and see if we can determine some basic information about this type of thread.

The first number in the thread call-out is "1/4". Since we know this number tells us about the diameter of the threads, we have our first clue. In this case, the call-out unit of measurement is *inches*, so this denotes a thread diameter of one-quarter of an inch! The diameter of the thread is measured from the crest of a thread all the way across to the opposite crest. In other words, we're finding the **MAXIMUM** diameter of the thread.

The other number in the thread notation is not so obvious. What do you think it means? Remember, the number tells how close together the threads are.

Any ideas? Well, if you thought that it means there are 28 threads on the fitting, you would be in good company...but unfortunately that's not the right answer. What the number tells you, in this case, is how many threads per inch there are on the nut.

Why not just tell you how many threads are on the nut?

Simply because it's not nearly as universal. Every time the length of the nut changed, the specification given the thread would also have to change, and that would make it very difficult to standardize. However, if the thread is measured in something like "threads per inch," then it doesn't matter if the nut is 1/2" long or 5 feet long...it still has the same "name!"



Pardon Moi

Let's look at another thread option – the frequently-used 10-32 thread.

The 10-32 thread is the workhorse of higher pressure chromatography applications, and it is almost exclusively used with 1/16" outer diameter tubing and smaller. So, what does this thread call-out tell us?

Again, we know the first number tells us something about the diameter of the thread. Yet, in this case, it doesn't mean you have a 10 inch diameter thread!

In the "fittings world," when an English thread goes below 1/4" in diameter, then gauge numbers are used when referring to the thread diameter. Therefore, the "10" in the call-out refers to a gauge 10 – which translates to almost exactly 3/16".

So, what about the "32?" As with the 1/4-28 example, this number means *thirty-two threads per inch*. And just like with the 1/4-28, the "threads per inch" is more universal.

So, which of these fittings would you expect to have threads closer together? It would be the 10-32 thread, since it has more "threads per inch" or a finer *thread pitch*.



I've Struck Gold

Food For Thought...

Why are 10-32 threaded nuts used in most high pressure applications instead of 1/4-28 threaded nuts? The denser the threads, the more threads you have to resist the opposing pressure being created inside the port, and the less chance the nut will fail.

...Now You Know!

You probably noticed that the term “English thread” was mentioned a little earlier. That’s because, as with all other major measurements, there are metric-threaded nuts as well! The most common metric thread used in laboratory equipment is M6 x 1 (although you will often only see M6 listed). Let’s try to apply the same principles we learned with the English-threaded fittings to these metric fittings.

First, remember that the first number refers to the diameter of the threads...and since this is a metric fitting, the diameter is 6 millimeters! (One confusing aspect of this thread, though, is that 6 millimeters is very close to 1/4”, making this type of fitting hard to distinguish from a 1/4-28 threaded fitting.)

The “1” portion of the thread name designates the distance between adjacent threads! Therefore, this thread has **1 millimeter between each thread** – the inverse of how an English thread is measured. While an English thread (such as the 10-32 and 1/4-28 we’ve already discussed) measures the number of threads per inch, a metric thread measures the number of millimeters per thread.

Even with the differences between the two systems, tremendous similarities exist...and by studying this information, you should better understand how and why threads are named the way they are!

Let’s Test Your Knowledge...

Now that you’ve learned what the names of threads mean, let’s apply that to another thread and see if you can determine some pertinent information by its call-out.

The thread is...5/16-24.

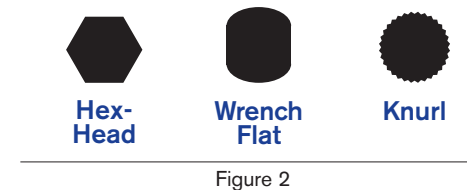
What is the diameter of this thread? How many threads exist in 1/4”?

To see if you’re right, check the answer at the bottom of this page!

The diameter of the thread is 5/16”, and in 1/4” there are 6 threads (24 threads/inch x 1/4” = 6 threads).

Head Geometry

Fittings are described by more than just their threads, as we’ve discussed. Another major factor that helps determine what nut to use is the geometry of the head.



Many nuts can only be properly tightened with a wrench. So, for those nuts, it’s important to note that the nut has a “hex-head” or “wrench-flat” geometry, and then to specify what the diameter is from flat side to flat side. This tells you what wrench you need to use.

Other nuts, however, can be tightened without a wrench; all you need are your fingers to tighten them properly! Unfortunately, it is very difficult to hand-tighten a nut with a “hex-head” or “wrench-flat” geometry. So, to make it easier to tighten this nut style, the head is often knurled to offer more gripping surface and extra friction against your fingers.

Other Items

Besides the thread size and head geometry, there are other factors that influence what nut you need to use.

One such factor is the overall length of the nut. Long nuts are great for use in angled ports, to increase the gap between adjacent fittings. But long nuts may get in the way in some “space-challenged” ports. So, for those ports, shorter nuts are in order.

You should also consider the dimensions of the tubing you are using, as most nuts have a specific-sized hole passing through them (called a “thru-hole”) which often corresponds to a tubing’s outer diameter. Therefore, when choosing the nut for your fitting system, you will often need to reference your tubing’s outer diameter.

Another major factor is what material the nut is made from. Originally, this wasn’t important, as most nuts were made from stainless steel. However, with the advent of nuts that can be hand-tightened, many polymer materials are being used to manufacture nuts. Materials



like Delrin®, Teflon®, Tefzel®, polypropylene, PCTFE, PEEK™, and PPS are all options...and with each new material comes new advantages and disadvantages to consider (e.g. chemical compatibility, thread strength...even *color!*). Refer to the Polymer Reference Chart in the Appendix, or check with your favorite fittings supplier for assistance in choosing the best fitting for your application.

Ferrules...

Now that you've absorbed all this "nut knowledge," don't forget this is only half of the fittings story.

In fact, the nut isn't really the "business end" of a fitting system...it's the ferrule that supplies the holding power!



Key Concept

Most standard laboratory fitting systems work through external compression (or "gripping") onto the tubing's outer wall. And, while the nut provides the driving force for compression, it's the ferrule that compresses against the tubing...and thus holds the tubing in place.

Ferrules aren't nearly as complicated as their nut counterparts, but they have some distinguishing features to help you determine which ones to use.



Figure 3 – Various Ferrules



I've Struck Gold

What They Look Like

While ferrules come in all shapes and sizes (see Figure 3, above), one thing they have in common is their tapered noses...and it's at the end of this nose where ferrules do what they were designed to do—grip the tubing wall!

Where They Are Used

From our discussion earlier, threaded ports can be classified as "high pressure" and "low pressure." While the definition of "high" and "low" is often different depending on whom you're talking to, one distinguishing feature is the geometry of the port receiving the fitting.

Generally, low pressure ports end in a flat-bottom configuration. In other words, as the threads run out, the bottom of the port becomes flat across the diameter, with a small "thru-hole" in the center of the flat bottom. The ferrules used with this type of port will have their tapered noses facing toward the nut and away from the flat bottom of the receiving port.



Figure 4a – Flat-Bottom Port & Fitting

Flat-bottom, low-pressure ports historically are used with flanged tubing. With this type of connection, the tubing wall is flared out – like a little trumpet! – allowing the tubing itself to create the seal in the port. However, since there are pressure limitations with flanged tubing, and because maintaining a flanged connection can be very tricky, many fittings users prefer the convenience and performance of a separate ferrule to create the seal. Because these ferrules allow tubing to be connected to the port without flanging it, they are often called "flangeless!"



Point of Interest



Figure 4b – Coned Port & Fitting

High pressure ports, on the other hand, have an internal taper just past the threaded portion of the port. Then, just beyond the internal taper is a small pocket – often called the "tubing pocket" – which is nearly the same diameter as the tubing that extends into it. In these types of ports, the tapered nose of the ferrule faces away from the nut and toward the port.

A Little Ferrule Ditty...

Ferrules face the port
When pressures are high
But they face the nut
When low pressures apply!

Manufacturing Material

One very important characteristic of any ferrule is the material from which it is made.

Ferrules are often manufactured from materials such as stainless steel, PEEK, Tefzel (ETFE), polypropylene and Kel-F® (PCTFE). Materials such as PEEK polymer (a beige-colored plastic) and stainless steel are often used to manufacture ferrules for high pressure applications. Softer polymers, including ETFE and polypropylene, are used primarily (although not solely) in lower-pressure applications.



Key Concept

Together At Last!

As you might've guessed, both the nut and the ferrule are important components of a fittings system. And that's the crux of the issue...they comprise a *system*, a matched set designed to work together!

In any connection system, you cannot expect to use just the nut alone, as nothing would be present to grip the tubing, and the ferrule cannot grip the tubing wall without the driving force of the nut. The nut and ferrule must work together to provide you, the analyst, with a consistent hold and seal on the outside of the tubing wall.

How Do Fittings Work?

We've taken some time to learn some basics about fittings, and we've even gone over some new vocabulary. Now, let's see if we can figure out how these things work.

But first, we need to lay some groundwork. Whenever we talk about "fittings" from now on, we'll most often be referring to the combined nut and ferrule system. (Be aware that some fittings have both the nut and the ferrule together as one piece...)

Now, let's dig in!

To understand how fittings work, it's important to know what fittings have to DO.

Specifically, fittings have two primary functions:

- ① Preventing liquid (or gas) from leaking out of the flow path;
- ② Holding tubing in place against opposing pressure.

These jobs may sound somewhat mundane; however, when you consider the nature of the chemicals which often travel along a given flow path, coupled with the high pressures that often exist inline, you may begin to realize just what a difficult job fittings have!



Key Concept

So, how do fittings do what they have to do?

Fittings generally work through a process called *external compression*. In other words, as a fitting holds a piece of tubing in place, it compresses the outside (or external) wall of the tubing. Depending on the material the fitting is made from, this external compression either becomes permanent, through a process called *swaging*, or remains temporary, holding the tubing using simple friction against the tubing wall.



Help Me

...which leads us to our next chapter



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Pardon Moi

So, what drives this compression? It has to do with a concept called “interfering angles.” This gets a bit more technical, so let’s delve deeper to gain a better understanding.

We already discussed how ferrules are most often tapered on at least one end, and how the orientation of the ferrule usually depends on the pressure you expect the fitting to withstand (and the internal geometry of the receiving port, of course!). Generally, the primary tapered portion of any ferrule (or fitting, if it is a one-piece style) will face the portion of the receiving connection that is also tapered, resulting in a connection that has two angled surfaces coming together.

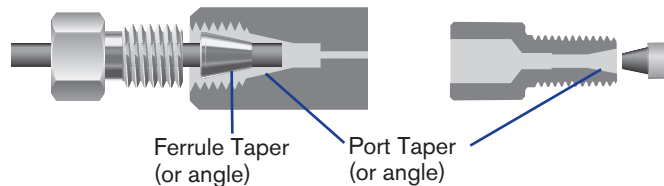


Figure 5 – Ferrule Orientation

Let’s look at the three possible outcomes when two angled surfaces come together, as described above:

❶ Ferrule has a wider angle than the receiving port.

In this scenario, as the fitting tightens into place, the tip of the ferrule’s tapered nose doesn’t actually make contact with the receiving taper at all; rather, contact occurs partially up the ferrule’s wall. This scenario creates ineffective compression of the ferrule’s tapered nose on the tubing wall.

❷ Ferrule and receiving port have identical angles.

In this example, because the angles match, the entire ferrule makes contact with the receiving port at the same time. And, no matter how much you tighten the accompanying nut, the two mating surfaces are simply wedged more tightly into place. The result: virtually no compression on the tubing wall.

❸ Ferrule has a narrower angle than the receiving port.

Here, the tip of the ferrule makes contact with the receiving port first, and as the fitting is tightened into place, the ferrule begins to conform to the port’s angle...and compresses against the tubing wall. Just what you want!

Figure 6 illustrates each of these scenarios.

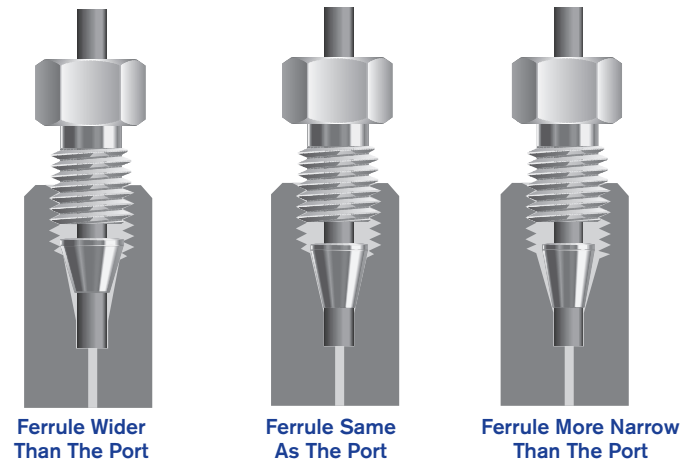


Figure 6 – Three Ferrule Interfaces



I've Struck Gold

Besides the seal and grip created around the outside of the tubing wall, you also need a seal between the outside surface of the ferrule and the tapered wall of the receiving port. The quality of the seal really depends on the surface quality of both the receiving port and the ferrule. Any imperfections on either surface may cause a leak.



Point of Interest

To Swage or Not To Swage...

We briefly mentioned the concept of swaging earlier. Now, let’s look at the process of swaging a little more closely – what it is, how it works, and why it remains so popular today.

First, as a recap, swaging means permanently attaching a ferrule to a piece of tubing, typically in a high pressure application. Often,

both the fitting and the tubing are manufactured from some form of stainless steel, although that doesn't always have to be the case.

In order to swage a ferrule onto a piece of tubing, slip the nut over the tubing being connected. Follow that by slipping the ferrule over the tubing, just below the nut, ensuring the tapered nose of the ferrule is facing away from the nut (remember the little poem?).

Once the nut and ferrule are in place, insert the tubing into the receiving port until the tubing butts up against the bottom of the port. (Please Note: This process works for most ports; however, some ports do not have a solid stop against which the tubing can rest. For these, extra care is necessary to ensure the tubing remains extended past the ferrule's nose...but not too far!)

With the tubing held in place, finger-tighten the nut completely, and then wrench-tighten it another 3/4 turn. This often will complete the swage, crimping the ferrule onto the surface of the tubing. To make sure this has been done correctly, loosen the assembly and remove it from the receiving port. Inspect the ferrule to ensure it will not come off; if it's still loose, reinsert the fitting and tubing assembly into the receiving port and tighten in 1/4-turn increments, checking to see if the ferrule has been fixed onto the tubing after each 1/4-turn.

Food For Thought...

Swaged fittings offer some significant advantages. One is that they permanently attach to the wall of the tubing. This allows swaged fittings to hold to high pressures – almost always well above the pressure rating of the equipment into which the fitting is attached.

The fact that swaged fittings are permanently attached also helps to keep the fittings with a piece of tubing. Since they're attached, they won't come off accidentally. (This is a big help if you tend to be “challenged” with small things!)



Unfortunately, this permanent attachment also becomes a major disadvantage! Why? **Because you can only use the fitting in one port – the one it was swaged into.**

You see, when a fitting is swaged, there is always a length of tubing extending past the ferrule. It has to be this way, or the ferrule would have no place to bite. The problem is that every major manufacturer requires the length to be a little bit different (see Figure 7, below). This means that, once swaged into a receiving port, for the best chromatographic results, a fitting should only be used *with that original port!* Attempting to use any other port may result in either dead volume or a chemical leak – particularly when multiple manufacturers' equipment are being used!

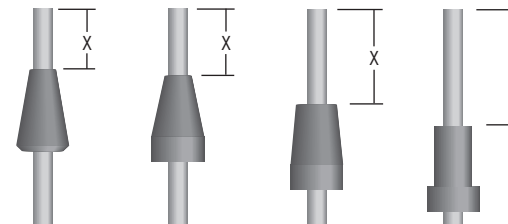


Figure 7 – “Dimension X” from Various Manufacturers

The Problem Fixer

One of the most commonly encountered problems with fittings is a chemical leak (e.g., when switching between different brands of HPLC columns). Initially, when a leak occurs, it's often best to make sure the fitting has been tightened properly. Often, though, fixing the leak involves repositioning or replacing the ferrule. However, with a swaged fitting, the ferrule is permanently attached to the tubing, so it cannot be repositioned or changed. This usually means you throw away the entire piece of tubing – with two ferrules and nuts attached, which makes this option very expensive and not very flexible.





This or That

Is there anything you can do? There **IS** another option...the fingertight fitting. This style of fitting offers many advantages over a swaged, metal fitting, and the disadvantages are very few!

One major hassle with swaged fittings involves the use of tools. Wrench-tightening one or two connections works fine, but in an environment demanding lots of tightening and loosening, using tools all the time can be cumbersome and time consuming – time better spent on your analyses.



Point of Interest

The stumbling blocks were always the pressure the fitting needed to withstand and the amount of tightening required to get the fitting to hold to that kind of pressure. During a time when there seemed to be no way around using wrenches with metallic fittings, fittings pioneers Paul Upchurch and Hans Schick spent most of 1982 and into 1983 developing the world's first fingertight – a fitting to hold high pressures without wrenches or permanent swaging. This new fitting was made from a durable and chemically-resistant plastic you could tighten with just your fingers!

In addition to the obvious benefits, other advantages have helped the fingertight fitting become a laboratory essential:

- **biocompatible** – Many biological samples are known to interact with iron, a chief component of stainless steel...but not a component of most polymers.
- **universal** – While many customers are forced to use system-specific metal fittings, in most cases just one style of fingertight fitting can be used to make connections throughout a system.
- **interchangeable** – Because fingertight fittings do not permanently attach to the tubing wall, they can be repositioned as needed...unlike metal fittings, which are locked into place by swaging.

What Fitting Do I Have?

Once you understand how fittings work, one of the biggest frustrations can be figuring out what fitting you have, so you can replace it with a usable alternative.

We've already discussed some defining characteristics for fittings, all of which will help you figure out what fitting you already have... and then help you decide which fitting(s) would best suit your needs:

- 1 Threads on the fitting
- 2 Geometry of the fitting (coned or flat-bottom)
- 3 Size of tubing being connected
- 4 Material from which the fitting is manufactured
- 5 Length of the fitting

A Thread By Any Other Name...

We talked earlier about the thread on a fitting, and what that thread call-out tells you specifically. Reviewing, each thread call-out consists of two main numbers – both telling you something about the thread. For instance, looking at a thread call-out such as 1/4-28, the “1/4” portion of the name tells us that the thread has a maximum diameter of 1/4”, and the “28” tells us how many threads per inch exist along the shaft of the fitting.

But, how do you tell what type of fitting you have? If you don't have any description for the fitting, then determining its thread description is one of the most critical pieces of information you can use.

Fortunately, you can usually determine what thread you have with a common ruler and a good eye. First, line up the threaded section of your fitting against your ruler's edge, as shown in Figure 8a on the next page. It's most helpful if you use at least a 1/4” section of threads for performing this measurement.



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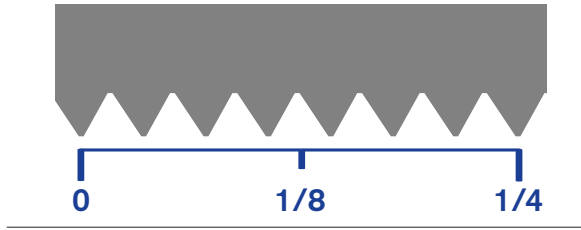


Figure 8a – Counting Your Threads

Next, count the number of threads along the barrel of the fitting, until you get to the 1/4" mark on your ruler.

REMEMBER: THE FIRST THREAD IS YOUR ZERO MARK...DON'T INCLUDE IT IN YOUR THREAD COUNT!

If you have an English thread, one of the threads on your fitting should line up with the 1/4" mark (In Figure 8a, there are 7 threads in 1/4"). Then, all you have to do is multiply your result by four to get the number of threads per inch – otherwise known as the **thread pitch!** (If you have a metric thread, then this won't work! We'll go through that in a moment...)



Once you determine the number of threads per inch on your fitting, the diameter of the threaded portion is much easier. Simply place the threaded barrel of your fitting on top of a ruler and measure the widest distance, from the thread crests on one side of the nut to the thread crests on the other side of the nut, as illustrated in Figure 8b, below, where this distance is 1/4".

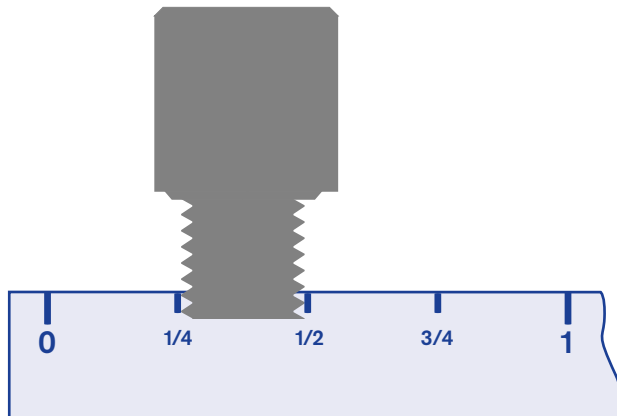


Figure 8b – Thread Diameter

For most English threaded fittings used in chromatography, the diameter often corresponds with the thread pitch according to this chart:

Threads Per Inch	Diameter
20	1/2"
24	5/16"
28	1/4"
32	3/16" (Gauge 10) and 9/64" (Gauge 6)

Of course, there are variations which exist, but this chart is a good starting place.

When working with metric-threaded fittings, manual measurement is somewhat different but uses the same concepts.

Remember that the most common metric-threaded fitting used in chromatography and related applications has the designation "M6." Technically, this is incomplete; the real designation for the thread is M6 x 1. If you recall, this means that the thread has a six millimeter diameter (the "M6" part), and has a thread pitch of one millimeter per thread (the "x1" part). Notice this is inverse from English threads, which tell you how many threads per inch. (See page 12 for more information...)



To measure the thread pitch of a metric-threaded fitting, hold the threaded barrel of your fitting against a ruler with millimeter increments. Line up the crest of a thread near the head of the fitting with one of the millimeter markings on the ruler. Then, examine where the rest of the thread crests fall. The most commonly used metric threads will have each thread crest line up EXACTLY with each millimeter mark on your ruler. Other metric-threaded fittings may not have their threads spaced one millimeter apart; however, they will follow a pattern (like ten threads in five millimeters, for instance – used for a M3 x 0.5 thread).

To measure the diameter of your metric-threaded fitting, use the same method described for English threaded fittings.

In Case You Want It Easy...

Now that you've gone through the thread-measuring exercise, you can use the diagrams in the figure below to help you more easily determine the threading of your fitting.









To use this resource, simply hold your fitting over each of the silhouettes to see if your fitting matches any of them. Each shadow drawing is an exact replica of the thread given; therefore, the diagrams can be used either to quickly verify a thread or as a check against your measurements.

WHAT THREADS DO I HAVE?

Hold your fitting over the thread silhouettes below to identify the threads.

U.S. CUSTOMARY THREADS

6*32	
6*40	
10*32	
1/4*28	
5/16*24	
1/2*20	

METRIC THREADS



M6	
M8X1	

Figure 9 – Thread Silhouettes

Plastic or Metallic – Which Do I Choose?

One of the most common dilemmas a fittings user faces is deciding between plastic and metallic fittings. These and many other questions are often asked:

- “My system already uses stainless steel fittings, so I have to keep using them...right?”
- “Will my chemicals or my sample interact with the fitting?”
- “If I'm using metal tubing, I can't use plastic nuts and ferrules...can I?”
- “Which type of fitting will actually hold up to the pressure I need?”

Here, we'll address the most common questions and concerns.**Can I Really Change My Fittings?**

Many good analysts don't consider alternative fittings because they think they have to use the same type of fitting originally included with the instrument. In fact, this is the main reason stainless steel fittings have remained as popular as they are!

To avoid this trap, first consider what the fitting is supposed to do. Does it hold high pressure or low pressure? What is the chemical environment? What is the operating temperature? After answering these questions, you'll usually find a polymer fitting can do the job as well as the original stainless steel fitting with more convenience and lower costs.

Regardless of the tubing being connected (whether it be metallic or a semi-rigid polymer), polymer fittings are an excellent choice. Ironically, the opposite does not hold true – stainless steel fittings cannot be used in many places where polymer fittings are being employed...specifically in those applications where a polymer fitting is attaching tubing to a plastic port. In those applications, using a stainless steel fitting will often cause damage to the receiving port!

One other factor to consider when deciding what fitting to use is how the fitting will be used. If you plan to connect a piece of tubing to a receiving port and rarely take it out, then a stainless steel fitting is often a good option. However, if you expect to make frequent connections with the fitting, or if you plan to use the tubing in multiple locations (for instance, with multiple columns in your HPLC system), a polymer fitting is really the best option.

What About The Chemical Environment?

This is a very valid question, and one that must be considered regardless of what type of fitting you choose. If incompatible chemicals contact a fitting, its ability to seal and its thread integrity may be compromised.

ALWAYS CONSIDER THE CHEMICAL ENVIRONMENT!!!

Additionally, since most fittings comprise two pieces – the nut and the ferrule – you should consider chemical compatibility with both the nut and the ferrule material. For example, you may have a fingertight fitting with a PEEK polymer ferrule and a Delrin nut. In any application where a low-level of trifluoroacetic acid is used, the PEEK ferrule will perform adequately. However, if the solution came in prolonged contact with the nut (e.g., if some of the chemical solution got on the port's threads when the fitting was previously loosened), eventually the polymer would erode away at the point of contact. The fitting's integrity would then be compromised – resulting in a leak!

There are many sources for up-to-date information regarding chemical compatibility (a summary of chemical compatibility information is provided in the Appendix of this booklet on page 60). Other sources are also available on the internet. We highly recommend you refer to one of these sources before finalizing your fittings selection.



Point of Interest

What Material With What Tubing?

As we've already discussed, you can nearly always use a plastic fitting on any type of tubing – polymer or metallic. As long as the fitting will hold more than the pressure you expect with your application, then you should not have any problems with the connection.

On the other hand, it can be dangerous...or even impossible... to use a metal fitting on plastic tubing. When a metal fitting crimps down on plastic tubing, because the metal is so much stronger than the tubing material, it very firmly squeezes the tubing wall. In fact, it might squeeze the tubing wall so hard that it damages the tubing or collapses the inner diameter to the point of being unusable in your application.

TABLE 1 – FITTINGS APPLICATIONS

Fitting	Tubing	Port	Recommended?
Plastic	Plastic	Plastic	Yes
Plastic	Steel	Plastic	Yes
Plastic	Steel	Steel	Yes
Plastic	Plastic	Steel	Yes
Steel	Steel	Steel	Yes
Steel	Plastic	Steel	No
Steel	Plastic	Plastic	No
Steel	Steel	Plastic	No

Which to Choose – a poem

Steel on plastic
Way too drastic!
Plastic on steel
What a deal!

What About The Pressure?

One of the stigmas associated with using a polymer fitting instead of a metallic one has to do with the system pressure. In fact, most metallic fittings will hold to higher pressures than their polymer fitting counterparts. Yet, the REAL question to ask is just how much pressure must the fitting withstand? If your application only calls for 1,000 pounds per square inch (psi), and you have a choice between a stainless steel fitting that holds to 10,000 psi and a polymer fitting that works to 6,000 psi, it doesn't matter which fitting you choose because both will work for that application.

For super high pressure applications, most analysts prefer to stick with metal nuts and ferrules. But for low, moderate and standard high pressure applications, a polymer fitting option usually exists. Be sure to consult the manufacturer's data on a fitting's ability to hold to the pressure your application demands.

Summing Up

Overall, plastic fittings should be the connection of choice for a vast majority of tubing connections. The following reasons show why:

- A wide variety of polymer fittings is available.
- Polymer fittings frequently offer superior chemical compatibility.
- Polymer fittings can hold to most required pressures.
- Nearly universal functionality is possible with polymer fittings.
- Polymer fittings are reusable over and over again.


The Bottom Line:
Polymer fittings are simply superior to similar ones made of stainless steel or other metals.



This or That


What is HPLC?

With all this talk of fittings, it only seems appropriate to shed some light on HPLC – the main liquid transfer application using the fittings we've discussed.

HPLC is an acronym that stands for High Performance Liquid Chromatography. (Many people think the "P" stands for "Pressure," as the operating pressures for many HPLC applications is quite high; however, the "P" really does stand for "Performance"...) 

Point of Interest

HPLC began to be used in the 1960's. The technique allows analysts to separate a sample of known or unknown composition into its components, and then to quantify how much of each component is present in the sample. And, because this technique is generally non-destructive, HPLC is a very useful tool in the laboratory, as it allows the scientist to continue performing other tests on his or her sample after it has been analyzed using an HPLC system.

Separation occurs by introducing a sample into a liquid chemical stream known as the **mobile phase**, which in turn carries the sample to a specialized tube called the **column** packed with small, chemically-active particles known as the **stationary phase**. Inside the column, the sample interacts with both the mobile phase and the stationary phase and begins to chemically separate into its components. Other equipment in the system create and collect data from the analysis of those separated sample components. Those data are then summarized in a printed graphical plot called a **chromatogram**. (WHEW!) 

Key Concept

What Makes Up An HPLC System?

Before we can go any further, it is important to understand what components are part of a standard HPLC system.



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An HPLC system includes seven basic components, each with a vital function:

solvent reservoir – The solvent reservoir holds the chemical solution that moves through the system. Because this solution is moving throughout the analysis, it is called the **mobile phase**.

pump – The pump pulls the mobile phase out of the reservoir and pushes it out through the rest of the system. The most common pump in use today is the dual-piston pump – capable of delivering stable flow rates at high pressures.

injection valve – The injection valve introduces the sample into the mobile phase. The most common injection valve is a six-port, two-position valve (see Figure 10, below). This type of valve allows a controlled amount of sample to be reproducibly introduced into the mobile phase pathway with little or no disturbance to the rest of the system.

The invention of the injection valve truly revolutionized chromatography, as it automated the way for samples to be introduced and analyzed. What originally began as an analysis technique with the capacity of working with 20-40 samples per day, HPLC systems (when coupled with the proper equipment) can handle hundreds of samples per day, allowing for rapid drug discovery as well as high throughput genomics and proteomics work.

column – Often called the “heart” of the HPLC system, think of the column as a chemical “filter,” of sorts. As mentioned, the column is a tube of some specified length and inner diameter and usually filled with small beads. Typically, the beads are coated with a chemical substance designed to interact with the sample components and enhance separation. Most frequently, the beads – described earlier as the **stationary phase**, because they don’t move in the system – are very small diameter silica particles and typically have octadecylsilane (C18) chemically bonded to their surfaces. Other polymeric materials are sometimes used instead of silica, as are other more specialized packing materials. Also, in addition to the C18 bonded to the surfaces of the beads, other, more specialized molecules can be bonded to the base material to further enhance the separation achieved in the column.

detector – The detector is responsible for “seeing” the sample components, separated in the column, as they are being carried along by the mobile phase. The most commonly-used detector passes a beam of ultra-violet light through a special window (known as the “**flow cell**”). As the sample components pass through the flow cell, the amount of light transmitting through the flow cell changes. The electronics of the detector then convert the change in transmitted light intensity into a signal.

Other detector options are being used more frequently as the cost of technology continues to drop. Detectors such as mass spectrometers (MS) and nuclear magnetic resonance (NMR) detectors are finding increased use in many laboratories. With improved detection options now available to the chromatographer, HPLC – already a mainstay in many laboratories – is finding increasing use as an analytical technique.



Point of Interest

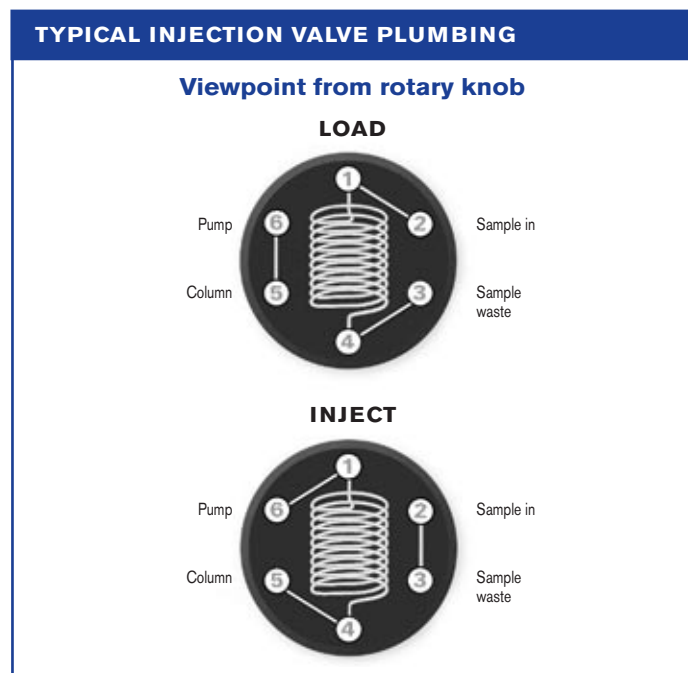


Figure 10

recorder — The recorder translates the signal generated by the detector into a plot, creating a **chromatogram** (see the diagram of signal versus time below.)

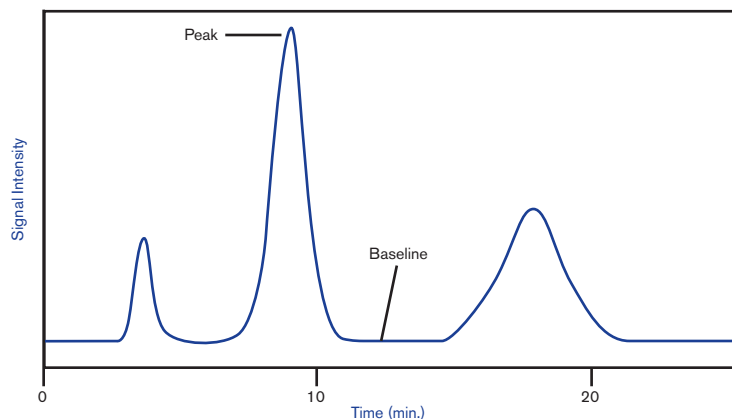


Figure 11 – Example Chromatogram



In the early days, this device was nothing more than a strip chart recorder with a pen that moved in response to the signal created by the detector, writing on a piece of graph paper moving at a controlled speed. Then, the peaks would be carefully cut out and the paper weighed (yes...*weighed*) on a scale to obtain semi-quantitative data. Nowadays, the recording device is usually a computer, equipped with software designed to not only translate the signal from the detector, but to also process the data digitally. This allows much more reproducible information to be generated and has facilitated the use of smaller and smaller samples.

waste reservoir — The last component in a basic HPLC system, the waste reservoir safely collects all the mobile phase and sample components after they pass through the system.



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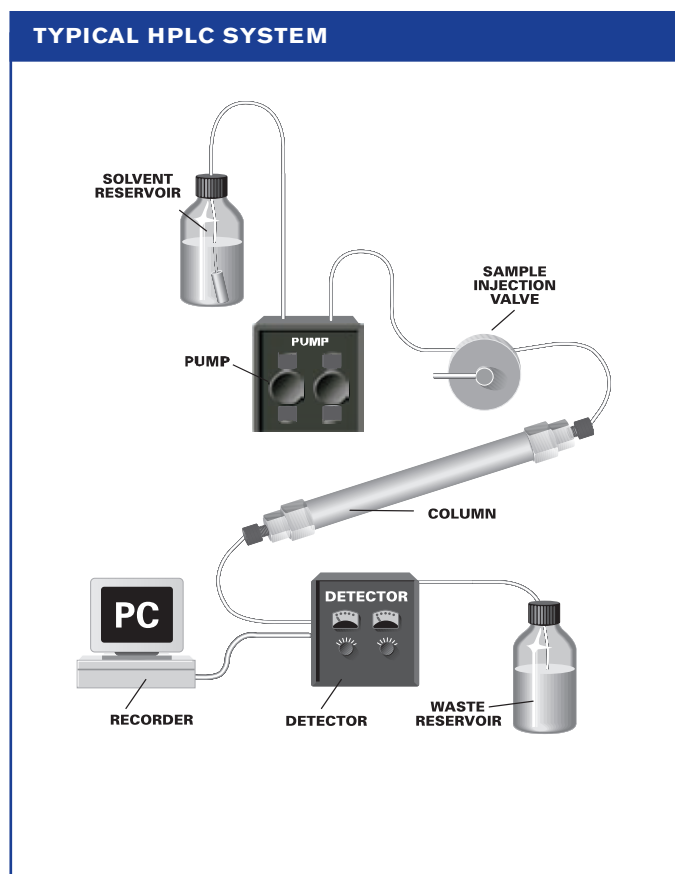


Figure 12

Looking At The Components As A System...

Now that you understand the basics of the key HPLC system components, let's run through the whole process again. Once a mobile phase is prepared, the pump then moves it through the system, where it encounters the sample and carries it through the column. Inside the column, the sample components selectively interact with the mobile phase and the stationary phase, separating into groups of like molecules as they move through the column. Leaving the column, the sample component groups then pass through the detector, which "sees" the components and sends a signal to the recording device, which in turn collects and processes the data. The waste reservoir then collects the fluid for disposal.



Key Concept

Other System “Stuff”

While an HPLC system cannot function without the basic seven components, it most certainly can function with more than those seven. In fact, there are many commonly-used accessories which help enhance the performance of a standard HPLC system:

filters – Found in the solvent reservoir and along the flow path in numerous locations, filters play a vital role in maintaining system performance. Filters prevent solid particles from passing through the system; failure to use filters can result in damaged system components, increased down time, and poor chromatographic results. (For more information on filters, check out the Appendix, beginning on page 51.)

guard columns – Guard columns do exactly what their name implies...they guard the main analytical column. They are typically small versions of the analytical column, used to trap sample components that would otherwise irreversibly bind to the inside of the analytical column. The guard column acts like an insurance policy for the primary analytical column, helping to delay the often-costly replacement of the primary column.

back pressure regulators – A back pressure regulator (BPR) is most often placed inline between the detector and the waste reservoir. It creates additional pressure inline, to prevent any gases which might be dissolved in solution from outgassing and creating bubbles along the mobile phase flow path. (If any bubbles pass through the detector’s flow cell, a phenomenon known as “noise” can show up on the chromatogram’s baseline and limit the sensitivity of analysis.) (For more information on BPRs, see the Appendix, pages 55-56.)



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What Goes Where?

Of course, fittings are standard items used to connect the components together, using tubing as the flow path bridge between components. Yet, one of the most commonly-asked questions regarding fittings is, “Where do all these fittings go in my system?” In other words, how do I know what to use where?

One common way to distinguish between fittings is by classifying them based on the pressure they can hold. This narrows the fittings choice based on where in the system they will be used.

Generally, fittings are classified as either “low pressure” or “high pressure” fittings. Correspondingly, “low pressure” most often refers to applications or areas in your system where the flow path pressures do not exceed 1,000 psi (~70 bar). “High pressure” areas can have flow path pressures that exceed 6,000 psi (~400 bar)! (Much of the differences between high pressure and low pressure fittings has already been discussed earlier in this manual.)

In an HPLC system, there are generally three pressurized zones, each with its family of fittings. The first zone exists between the reservoir and the inlet of the pump. This is typically a low pressure zone, as the pump is pulling the mobile phase from the reservoir in this area, usually creating some negative pressure. The fittings used in this zone are generally low pressure, low-cost fittings, most often having a 1/4-28 flat-bottom geometry, and are generally for 1/8” outer diameter (OD) tubing.

The second zone in the system exists from the outlet of the pump through the injection valve and through the HPLC column. In this zone, the fluid flow is being resisted by the stationary phase inside the column, and as such, the pump experiences higher back pressures...as do the fittings.



Pardon Moi

In this second zone, fittings are generally classified as high pressure fittings and most frequently have a 10-32 coned geometry for use with 1/16" OD or 1/32" OD tubing. Because performance demands of the fittings are greater in this area of the system, the cost of the fittings are generally higher.

Lastly, in the third zone – from the tubing exiting the column through the detector and on to the waste reservoir – the system pressures are generally near ambient pressures, with slightly elevated pressures being experienced when extra components are used (like back pressure regulators, for instance). Because the system pressure in this third zone is typically low, the fittings once again assume a standard, 1/4-28 flat-bottom geometry, and most often are intended for use with 1/16" OD tubing.

Even though this is a lower pressure zone, many OEM ("Original Equipment Manufacturer") companies realize that most laboratories have more fittings with the 10-32 coned for 1/16" OD tubing geometry than any other type of fitting. Therefore, many of the receiving ports available on equipment in this zone may require the use of "high pressure" fittings, even though the inline pressures are low.

The diagram on the next page shows an "accessorized" HPLC system, to help you understand how a system is put together – including what fittings to put where.

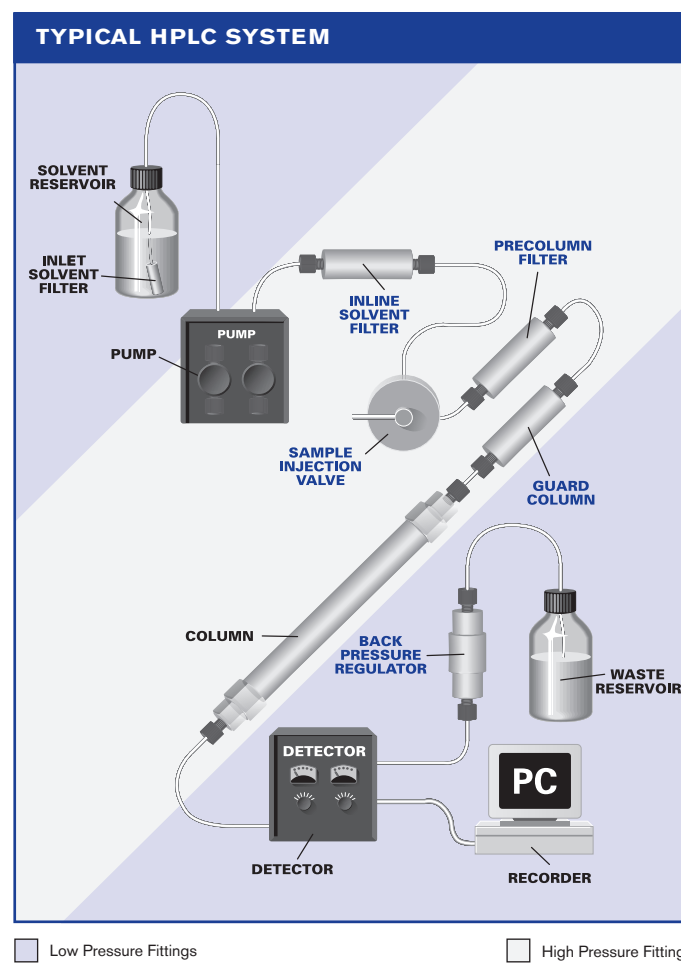


Figure 13

How Do I...?

No fittings manual would be complete without the “practical application” section...answering those longing questions about how to do specific things, like adapt a piece of 1/16” OD tubing and a piece of 1/8” OD tubing together in one connection, or how to successfully make connections with capillary tubing.

Here, we’ll take some time to look at some of the “biggies” often asked, or often misunderstood, when it comes to making proper connections.

How Do I Adapt?

With the long list of manufacturers creating equipment, and with all the different variations on tubing size and threaded port configuration, scientists will frequently need to find adapters to make a special tubing connection.

Or will they?

Ironically, while many people might approach making a connection with an adapter, frequently adapters are unnecessary. To better understand this, let’s distinguish between adapters and their counterparts – unions.

Basically, adapters “adapt” between two different types of thread/port configurations, while unions have the same thread/port configuration on both sides.

To illustrate this point, Figure 14 shows various connectors and classifies them as either adapters or unions.

Before continuing, it’s important to establish one general “rule of thumb”: Unions are typically less expensive than adapters while performing equally as well. Therefore, wouldn’t it make sense to use a union wherever possible?

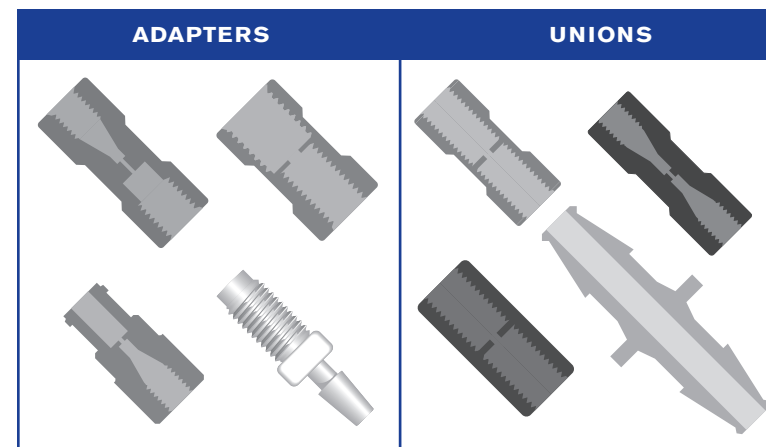


Figure 14 – Adapters vs. Unions

The real question to consider is this:

How do you know when you can use a union?

First, examine the connection and determine if it will be low pressure or high pressure. Sometimes this may not be so obvious, but you can at least make some assumptions.

For instance, if you want to connect 1/16” OD PEEK tubing to a piece of 1/8” OD Teflon tubing, then it would be safe to say you are making a low pressure connection. In fact, it doesn’t really matter what pressure the PEEK tubing will hold – the connection is limited by the amount of pressure the Teflon tubing can handle.

Once you know the pressure classification for the connection you need, find out what’s available in that classification from your fittings supplier. (For example, for low pressure connections, a number of low pressure unions with 1/4-28 internal flat-bottom geometry on both sides are available. You’ll also find available other options with matching M6 and 5/16-24 internal threads.)

Armed with this information, now all you need to do is find fittings that work with your tubing sizes to mate with opposite sides of an available union. If such fittings exist, you can use a union to connect them – even if your tubing outer diameters are different!



Key Concept



I've Struck Gold

Let's continue with the previous example: a low pressure connection between 1/16" and 1/8" OD tubing.

Most people confronting this connection will look for an adapter. Of course, many suppliers manufacture adapters that can be used for that purpose. However, most manufacturers and suppliers carry 1/4-28 flat-bottom fittings for both 1/16" OD and 1/8" OD tubing.

This means an inexpensive union can be used, even with different tubing sizes, without compromising performance.

Of course, there are still a number of cases where only an adapter will do. However, it is often worth the exercise of looking through available products to determine if a union will work for your needs.

Making Capillary Tubing Connections

Another important topic to cover is the rapidly growing field of capillary tubing connections.

As analytical techniques and applications force fluid volumes lower and lower, the size of the tubing used for the flow path must also shrink to accommodate the methodology. Special guidelines are useful in selecting and using the best connectors for capillary tubing applications.

First, we will define capillary tubing as any tubing with an outer diameter smaller than 1/16" OD (chosen because it is the primary size of tubing used historically with most HPLC systems). As flow rates decrease, often the tubing OD and ID will also decrease. Tubing sizes like 1/32" OD and 360 micrometer (μm) OD are being used with increasing frequency in chromatography and related disciplines, and using these tubing sizes requires some special skills normally unnecessary with 1/16" OD tubing. This is especially evident when using the smaller tubing in receiving ports normally designed for the larger, 1/16" OD tubing.

Frequently, analysts will attempt to adapt existing hardware (e.g. pumps, injection valves, even columns) to accommodate capillary tubing. Issues abound, however, because of the geometry of the receiving ports.

Generally, the high pressure receiving ports in most of the equipment on the market have an internal geometry supporting 1/16" OD tubing. This means that the port has an internal pocket into which the 1/16" OD tubing extends. As long as tubing with this same diameter is used, then potential dead volume resulting from the connection is kept to a minimum. However, when the tubing you're using is smaller than what the receiving port was designed for, it's easy for dead volume to be introduced in the connection...which, with capillary-based applications, can be extremely detrimental (see Figure 15).

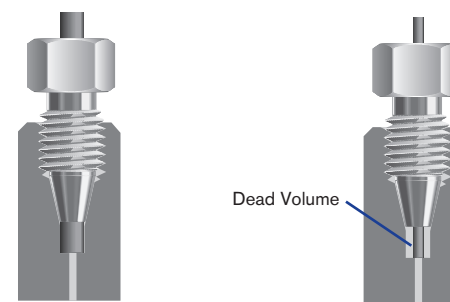


Figure 15 – Potential Dead Volume in Capillary-Based Applications

Numerous manufacturers have developed ways to adapt smaller, capillary tubing into the receiving ports meant for larger OD tubing. Of the options available, two stand out as the most popular.

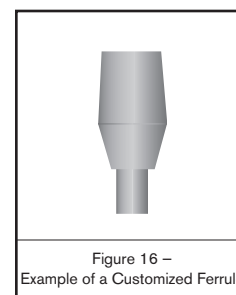


Figure 16 –
Example of a Customized Ferrule

The first option involves customized ferrules which look similar to those used for the larger tubing, but which feature smaller holes drilled through them to better accommodate the capillary tubing. Additionally, most ferrules used for this purpose also offer a fixed-length nose extending beyond the tapered portion of the ferrule to reduce dead volume in the connection. Ferrule systems such as these

generally hold the tubing well and have a long usable lifetime. However, there are drawbacks to consider.



I've Struck Gold



Point of Interest

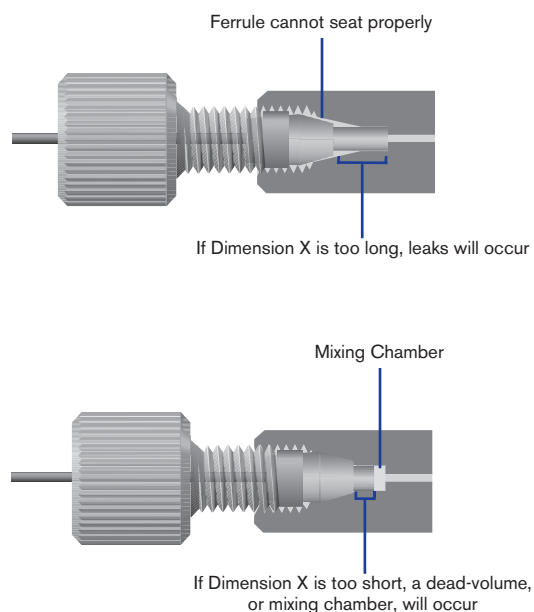


Figure 17

First, custom-drilled ferrules are “economically-challenged.” (In other words, they ain’t cheap!) These items are generally manufactured from expensive materials and are typically machined versus injection molded...both of which drive up the cost.

Second, and more importantly, their fixed geometries limit where they can be used. As most ports have internal geometries that vary from one to another, using ferrules with fixed geometries can either prevent the ferrule from sealing properly or leave empty pockets in the receiving port where mobile phase and/or sample may collect. These dead volume pockets can lead to poor chromatography, including issues with carryover, split peaks, and band broadening. And with such small sample sizes and flow rates being used in capillary-based applications, dead volume pockets play a much larger role in the quality of the results.

Fortunately, the second option – the use of a special tubing sleeve – overcomes both of the disadvantages exhibited by the customized ferrules.

Tubing sleeves generally have a controlled outer diameter (usually 1/16”) which allows them to fit into standard threaded ports. And, since the sleeve will slide through a ferrule until it bottoms out in a receiving port, the tubing pocket beyond the ferrule will be completely filled to help avoid dead volume in the connection.

Tubing sleeves also offer several side benefits. One is the structural support sleeves provide to the outside of the capillary tubing, helping prevent damage to the tubing as it leaves the fitting. Also, because the tubing sleeves come from extruded material, the sleeves can be offered in a variety of materials. Furthermore, the extrusion process generally allows a more concentric connection than precision drilling and machining can provide, allowing for more accurate tubing-to-thru-hole alignment and decreasing inline turbulence and mixing.

For all these reasons, using a tubing sleeve to connect capillary tubing into a receiving port is the method of preference – unless the receiving port is specifically designed for use with the size of tubing you are using!

But what about instances where there isn’t even enough room for a threaded port to exist, such as Lab-on-a-Chip methods? Many people using techniques like this are forced to glue their tubing in place in lieu of using a fitting for the connection. This causes a number of problems, including a weak hold on the tubing and interaction between the sample being analyzed and the glue (often epoxy) securing the tubing in place.

One available solution to this problem involves bonding a threaded port directly to the substrate’s surface. By doing this, and by isolating the adhesive away from the mobile phase or the sample, you can make a reliable connection with your tubing using a traditional fitting rather than epoxy. Products, such as Upchurch Scientific’s NanoPorts™, were developed specifically for this task.



Key Concept



I've Struck Gold

Cutting Fused Silica and Polymer Capillary Tubing

Besides connecting the tubing with the proper fittings, other issues should be considered when you use capillary tubing, to ensure good connections and good chromatography.

One of the major issues centers on how well you cut the tubing. Depending on the type of capillary tubing and the cutting tool used, the effect can be dramatic.

Let's take fused silica capillary tubing as a first example. One of the most popular methods for cutting this tubing employs a ceramic scoring stone. This "tool" is really just a piece of sharp-edged ceramic that is pulled across the surface of the fused silica tubing. This process scores the surface of the tubing and allows it to be pulled apart easily at the score mark.

In theory, this will allow for reasonable cut quality. However, in practice, the results are usually different. Often, as the ceramic stone is pulled across the tubing, the polymer coating is shredded and the fused silica underneath the polymer coating is shattered under the force of the blade. Initially, you may think the tubing looks acceptable; however, once in use, the coating material or pieces of the fused silica tubing can break off and clog important internal passageways. Also, with damaged ends on the tubing, using standard compression fittings to hold that tubing in place can cause the tubing to completely shatter and result in a leak, a clogged system component, or a combination of the two.



Key Concept

It is almost always a better idea to circumscribe the tubing you are cutting, going all the way around the circumference of the tubing, if you want to achieve a good, reproducible, quality cut on fused silica tubing. While the tools available for making this type of cut are fairly expensive, the results (and lack of a daily headache!) usually far outweigh the cost of the cutter. And, these tools are very easy to use.

Interestingly, the concept of circumscribing the tubing applies to polymer capillary tubing as well...but for different reasons. Often, when polymer capillary tubing is used, the inner diameters are very small – as low as .002" or even smaller! With inner diameters this small, it is vital to keep the hole in the tubing open and centered with the passageway in the receiving port to avoid over pressurizing the connection. That's where accurate cutting comes into play.

If you cut the tubing by simply slicing it with a blade, the force of the blade pushing through the plastic will frequently cause the inner diameter to shift off center or collapse. While cutting this way won't technically cause damage to polymer tubing like it would to fused silica tubing, it can render it near-useless, depending on the degree of inner diameter shift. To avoid this risk, it is best to cut the polymer capillary tubing all the way around its circumference as well.



Help Me

Again, the tools to do this are available, and they are a bit expensive; however, the benefits far outweigh the costs.

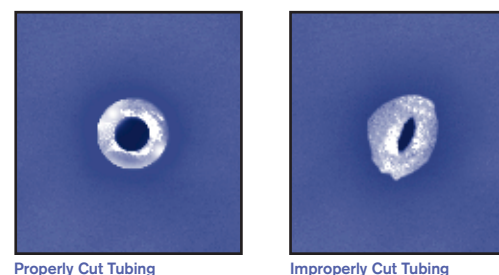


Figure 18 – Cut Polymer Tubing

What's The Big ID?

One of the other main problems you might face is choosing the right inner diameter for your applications. With low flow rates and incredibly small amounts of sample available, there is a general push to reduce the inner diameter to the smallest possible size. While these miniscule pathways allow the fluid to travel as fast as possible from point A to point B, analysts often fail to consider the back pressure along the tubing pathway. If system pressures become too high where polymer fittings are used, the tubing may pop free from the fitting's friction hold, causing a leak and other undesirable consequences (e.g., system shut-down, sample loss). While many factors can contribute to system pressure, the ID of the tubing has the greatest impact.

Please refer to the "Speaking of Pressure" section on page 57 in the Appendix for a useful formula that allows you to calculate expected back pressure in your fluid pathway.

Void, Dead and Swept...An Interesting Concept

Often, when making connections, people want more information on the amount of “dead volume” in the connection. However, this is usually the wrong question to ask.

Most people who inquire about the dead volume really need to know just how much internal volume exists within a connection, not how much of that internal volume is considered “dead.”

But what do these terms mean?

Specifically, three terms describe the internal volume of a product: void volume, dead volume and swept volume.

Void volume is simply another way of describing the total internal volume. It is defined as any space within a connection into which fluid can flow.

Dead volume is that portion of the void volume that is out of the flow path. (See Figure 19 below.)

Swept volume is that portion of the void volume in a connection directly in the fluid pathway. (Again, see Figure 19.)

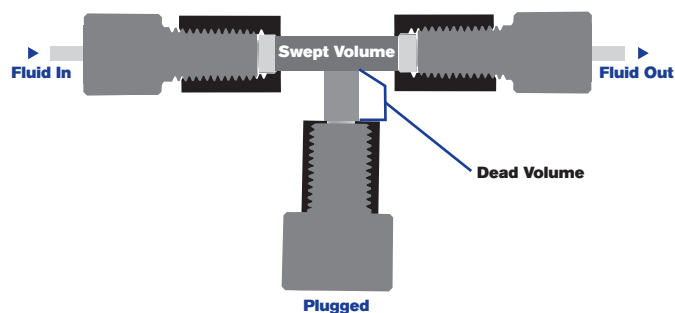


Figure 19 – Internal Volume Defined

Mathematically speaking, when dead volume and swept volume are added together, the total equals the overall void volume.

$$\text{Void Volume} = \text{Dead Volume} + \text{Swept Volume}$$

Dead volume, however – particularly in capillary connections – can cause some very undesirable chromatographic effects, including:

- ① analysis delays
- ② broadened peaks
- ③ poor resolution
- ④ sample carry-over
- ⑤ split peaks
- ⑥ gas collection



In addition to keeping the volume inside the connection to a minimum, all dead volume should be removed from the connection if possible. Therefore, in capillary connections, the preferred relationship could be expressed as follows:

$$\text{Void Volume} = \text{Swept Volume}$$

(and should be REALLY small!)

A Helpful Note: In order to keep most of the void volume truly swept volume, match the tubing ID as closely as possible with the diameter of the holes in your equipment. This helps ensure that the fluid, once it leaves the tubing, runs completely through the entire internal passageway. This diameter matching also helps reduce the amount of turbulence the fluid experiences as it passes through the connection.

Some Small Volume Notes

We've already discussed ways to keep the dead volume in a connection to a bare minimum. But be aware there are practical limitations to how small the swept volume in a connection can truly be, in part due to mechanical limitations which often prevent holes from being drilled much smaller than .005" (125 μm). (Note: The limitations of machining standard connections has been partially responsible for driving the use of Lab-on-a-Chip techniques, which utilize special chemical etching and other tools to form the smaller passageways needed for capillary flow applications.)



The End of the Story

While it's hard to capture all you need to know about fittings in one compact reference, hopefully this resource has proven to be helpful already... and will continue to be something you return to over and over again in the future.

Please feel free to contact the Customer Service staff at Upchurch Scientific if you have any further questions or if you have suggestions for future editions of this guide. Many of the improvements in this current edition are due to suggestions from interested people just like you!



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Appendix

I Know It's Not A Fitting, But...

While this resource is all about fittings, there are other useful accessories that can be used in many applications. Two of the more common accessories are filters and back pressure regulators (or BPRs).

Filters, Filters Everywhere!

"Why would I ever really need a filter?" you might ask...and you wouldn't be alone. Many people assume their fluid pathway is clean when frequently it really isn't, and there are areas where using filters is very important.

Particles of all sorts can be suspended in a fluid stream. (Just because you can't see them doesn't mean they aren't there!) Things like dust, solids originating with a sample, and seal wear particles are all reasons why filters are important!

For example, let's examine where you might need to filter in an HPLC system.

- ❶ "In the beginning..." One of the best places to use a filter is the very beginning of the system – that is, in the reservoir on the end of the solvent inlet tubing. (Recall the basic system components from our earlier discussion of HPLC, starting on page 31.)

In this case, a large, very porous (meaning: "has a lot of little holes") filter is used to ensure particles are trapped inside the reservoir.

Now, you might be thinking, "But I use HPLC-grade chemicals, and I vacuum filter my solutions before I even put them into the reservoir. Isn't this enough filtration?" To illustrate, here's a real-life example of why a filter is needed at the beginning of the process.



Pardon Moi



A story has “filtered” down about a laboratory which ran various HPLC methods, all requiring the use of water. Rather than use bottled, HPLC-grade water, the lab used a multi-stage, reverse-osmosis filtering system to treat the water and completely remove any contaminants.

The water went into an ultra-clean reservoir (sterilized and triple-rinsed, just like chemistry professors teach!), but the standard method stated the water could only be used for two weeks, and afterwards it had to be thrown away and replaced.

It didn’t take long to realize why two weeks was the maximum lifetime for the solution.

Once, the water was left in the reservoir for longer than two weeks. It wasn’t being used...the lab technicians just forgot to empty the reservoir before going home for the weekend. On Monday morning, the water was no longer worthy of being called water. What was clear and inviting days earlier became gross and cloudy, full of bacteria and other garbage. Of course, it had been growing all along...except no one could see it. After two weeks, however, it had grown large enough to be seen.

Fortunately, though, a large filter was on the end of the inlet tubing, so none of the “growing blob of goo” made it to the expensive HPLC pump!

So, what might a filter as important as this one be called?

It’s called a “solvent filter” – because it goes in the solvent reservoir on the end of your solvent inlet tubing. As an added benefit, it also helps hold your tubing down into the reservoir.

Solvent filters are often large in size and in porosity. They keep solid particles (like bacteria or even dust) out of the fluid path without restricting the flow. Like most filters, they are considered consumable and will need to be replaced on a scheduled basis.

(Please note: Another useful place for a solvent filter to be used is on the end of a helium sparging delivery tube. Sparging is a technique used to remove gas from a mobile phase prior to it being pumped through the system. In this case, the solvent filter does two things... it disperses the helium more effectively while also preventing particles, stemming from the gas source, from entering the mobile phase. For a more detailed description of the sparging technique, please see “Mobile-Phase Degassing – Why, When, and How” by Dr. John W. Dolan, *LC-GC Volume 17 Number 10, October 1999*.)

- ② “Keep it inline...” The next most popular place for a filter is along the fluid pathway – called an “inline filter.”

The first location an inline filter is placed is immediately following the pump.

“But why? We just filtered the solution in the reservoir...why filter it again?” you may be asking.

The only reason another filter is needed is due to particles introduced into the fluid after the fluid was filtered. And that is exactly what often happens.

Most HPLC pumps use a piston moving back and forth through a plastic seal. Because the seal has to withstand high pressure environments, it has a very strong grip on the piston’s surface which creates a lot of friction along the inner wall of the seal. This friction can cause the seal to slough off particles – particles which may clog sensitive system components downstream of the pump.

In order to prevent damage to the system and minimize system down-time, use an inline filter immediately following the pump.

- ③ “One good filter deserves another...” You can also place an inline filter immediately after the injection valve and before the column. This filter is often called a “pre-column filter.”

Filtering immediately before your column protects the column from becoming clogged with particles coming from the sample or from the plastic seal inside most injection valves.



(The most common porosity for a pre-column filter is 0.5µm, which generally protects the analytical column really well.) Without this filter, the frit at the head of the column can clog. When this happens, the inline pressure increases dramatically, and the column often has to be replaced, because changing the frit at the head of the column disturbs the stationary phase inside the column and frequently leads to poor chromatographic results. Pre-column filters, on the other hand, can be safely changed without disturbing the packed bed of the column. They are also typically much less expensive than columns to replace and are a good investment for the protection of your column and your system.

How Long Will They Last?

This is one of the most frequently asked questions regarding the use of filters.

Unfortunately, there is no standard filter lifetime. You may go for months with very clean samples and mobile phase and not have to change your filters, or they may only make it a few days. It all depends on how clean your fluids are and on the condition of your pump and injection valve seals.



Point of Interest

A good lab practice tip is to make the replacement of your filters or frits part of your system's preventative maintenance routine, which typically occurs every six months to one year.

There will be times when you will need to change your filter or frit in addition to the regularly-scheduled changes. But how do you know when it's time to change them? Here are some hints that may help:

- * If you notice your chromatogram peaks coming out later than normal, it may indicate that your pump isn't dispensing the right volume. If everything on the pump appears to be functioning properly, and if there is no evidence of a leak, then the solvent filter is likely clogged, restricting the solvent flow to the pump.

To test this quickly, temporarily remove the solvent inlet filter from the inlet tube and re-run a few standard samples. If the peaks return to their normal position, then the pump was not getting enough fluid with the filter in place. Replace the filter with a new one, and begin your analyses again. *Do not operate for long periods without an inlet filter, as damage to your system may occur.* (Remember this saying: "If in doubt, throw it out." Solvent filters are generally inexpensive, making it easier to be safe than sorry!)

- * If you notice the system pressure increasing, that's a good indicator that either your inline filter or your pre-column filter needs to be changed.

As your inline filter and pre-column filter do their jobs, the frits inside will collect particles from the fluid pathway. As more particles are collected, less room is available for fluid to move through. Eventually, the pump has to "push harder" to get the same amount of fluid through, causing the system pressure to increase.

To best determine if an inline filter needs to be replaced, start at the end of your system and, moving backwards toward the pump, disconnect the fluid pathway fittings one-by-one, monitoring the system pressure. Watch for the pressure to drop suddenly; when it does, look for the restriction to be in the component last removed from the flow pathway. If this happens just before a filter, replace the frit inside the filter housing. (Remember to be careful and not create a chemical spill when performing this test.)



I've Struck Gold

Regulate Me!

When it comes to system pressure, inline pressure regulation is often a necessity. And that's where Back Pressure Regulators (BPRs) come into play.

A BPR is a device that creates a static inline pressure, relatively independent of the fluid flow rate or viscosity.

But what's a BPR for?

There are two main applications that call for BPRs to be used in HPLC systems.



Key Concept

The first application is to help prevent noise on a chromatogram's baseline. (Noise is the term applied to the erratic fluctuations on the baseline.) As fluid passes through your system, it will go through some terrific pressure changes in a short period of time. If gas is dissolved into solution, bubbles will form in the fluid pathway as the mobile phase decompresses. As these bubbles float into the detector's flow cell, they can cause the detector's signal to bounce around.

This "noise" makes it very tough to analyze small peaks on the chromatogram.

If the noise you are experiencing comes from bubbles in the flow cell, there are two solutions: remove the gas from the mobile phase, or prevent bubbles from forming. Using BPRs won't remove the gas, but they can help prevent the bubbles from forming.



I've Struck Gold

To use a BPR for this purpose, simply place it between the detector's flowcell and the waste reservoir. When the fluid leaves the column, the BPR will help ensure the mobile phase remains under pressure as it flows through the detector, inhibiting bubbles from forming. *Keep in mind your detector flow cell's pressure limit as you are selecting the correct BPR for your application.*

A BPR can also help your pump's check valves operate more efficiently. Many standard check valves are gravity-fed and rely on system back pressure to work well. If your application doesn't cause sufficient back pressure to be developed, your pump's check valves may not work correctly. However, a BPR right after the pump (but before the injection valve) often causes the pump's check valves to operate more quickly and therefore more efficiently. (Note: For most HPLC applications, it is unlikely a BPR would be needed in this location, as the column used in most HPLC systems will create sufficient back pressure to ensure efficient check valve operation.)

Speaking of Pressure...

Sometimes it may be necessary to predict how much pressure might be created inline by your tubing. Here's a formula we have found to be very accurate (answer in psi):

$$\Delta P = ((F \times 8 \times L \times V) / (\pi \times r^4)) \times 2.42 \times 10^{-9}$$

Where:

- F = flow rate in mL/min
- L = tubing length in cm
- V = viscosity in centipoise
- r = tubing inside radius in cm

Conversion Factors

Here are some other useful formulas:

Inches to Millimeters:	Inches x 25.4mm/inch
Inches to Centimeters:	Inches x 2.54cm/inch
Inches to Microns:	Inches x 25.4mm/inch x 1000µm/mm
Diameter in inches to linear volume (µL/inch)	((Inches/2) x 2.54cm/in) ² x π x 2.54cm/inch x 1000µL/cm ³
Diameter in inches to linear volume (µL/cm)	((Inches/2) x 2.54cm/in) ² x π x 1cm/cm x 1000µL/cm ³
Celsius to Fahrenheit	(Celsius x 9 / 5) + 32
Fahrenheit to Celsius	(Fahrenheit - 32) x 5 / 9
psi to bar	psi x 0.06894757
psi to atmospheres	psi x 0.06804596
psi to MPa	psi x 0.00689476
psi to torr	psi x 51.7150733



Polymer Information

Polymer fittings, tubing and accessories have proven superior to stainless steel in many analytical applications. The following performance information has been compiled for many of the polymers commonly used in analytical analyses.

Delrin® Acetal Resin. Delrin has excellent chemical resistance to most organic solvents as well as to most neutral-pH aqueous solvents. However, it is not suitable for use with acids, bases, or oxidizing agents. This polymer's high tensile strength yields superior, highly wear-resistant threads. Delrin's use is generally limited to threaded products; it is not typically used for tubing or ferrules.

Halar® ECTFE (ethylene-chlorotrifluoroethylene). Halar is another member of the fluoropolymer family. It offers excellent chemical resistance coupled with a mechanical strength superior to Teflon. Halar also outperforms Teflon in its ability to withstand radiation, making it an attractive alternative for medical applications. Its exceptionally smooth surface enhances optical clarity while also helping prevent the shedding of micro-particles into the fluid stream.

Kel-F® PCTFE (Polychloro-trifluoroethylene). Kel-F has excellent chemical resistance. In general, only THF and a few halogenated solvents will react with it. This resilient fluoropolymer is ideal for fittings and sealing surfaces.

PEEK™ Polyetheretherketone. PEEK polymer has excellent chemical resistance to virtually all commonly used solvents. However, the following solvents are usually not recommended for use with PEEK: nitric acid; sulfuric acid; halogenated acids, such as hydrofluoric acid, hydrobromic acid, and hydroiodic acid (hydrochloric acid is approved for use in most applications); and pure halogenated gases. Additionally, due to a swelling effect, be cautious in using the following solvents with PEEK tubing: methylene chloride, THF, and DMSO. PEEK polymer is used extensively to manufacture tubing, fittings, filters, and other accessories.

Polypropylene Polypropylene is a relatively soft polymer commonly used in low-pressure applications. Polypropylene is excellent for aqueous solutions; however, it should not be used with chlorinated, aromatic, and some organic solvents. Polypropylene is used primarily for adapters, low pressure fittings and accessories.

PPS Polyphenylene Sulfide. PPS is a resilient polymer known for its high tensile strength and excellent chemical resistance. PPS may be safely used at room temperature with most organic solvents and neutral-to-high pH aqueous solvents. However, it is not recommended for use with chlorinated solvents, inorganic acids, or any solvent at elevated temperatures. PPS is used sparingly to manufacture fittings; it is not normally used to produce tubing or ferrules.

Radel® R Polyphenylsulphone. Radel is an amorphous thermopolymer that is mechanically strong and offers good chemical resistance. This polymer withstands repeated autoclave sterilization cycles without suffering thermal breakdown. This property, coupled with its optical clarity, makes Radel R an excellent choice for medical applications as well as applications where visual monitoring is essential. Radel R is also a readily wetted material, minimizing air bubble accumulation on the inner walls of tubing manufactured with this polymer.

Teflon® FEP (fluorinated ethylene-propylene) and PFA (perfluoroalkoxy alkane). Both forms of Teflon are inert to virtually all chemicals used in HPLC. However, because of their relative softness and low durability, Teflon polymers are generally used for low pressure applications. Teflon is most often utilized for low pressure fittings, tubing, and accessories. Choose PFA for high purity applications, or choose FEP for general, low pressure applications.

Tefzel® ETFE (ethylene-tetrafluoroethylene). As a member of the fluoropolymer family, Tefzel has excellent solvent resistance. Its relatively high tensile strength makes it ideal for demanding sealing applications. While most commonly used solvents do not interact with Tefzel, take caution when using some chlorinated chemicals. Tefzel is used extensively to manufacture low pressure fittings, tubing, and accessories.

UHMWPE Ultra-high molecular weight polyethylene. UHMWPE is a well-known and durable manufacturing polymer. This polymer's physical properties make it ideal for general, aqueous-based environments. Take caution when using this polymer in heavily organic-based applications.

For more Polymer information see the Chemical Compatibility Chart on the next page.

Chemical Compatibility Chart

CHEMICAL FAMILY	DELRIN®	HALAR®	KEL-F®	PEEK™	POLYPROPYLENE	PPS ¹	RADEL® R	TEFLON®	TEFZEL®	UHMWPE
Aromatics	R	R ¹	R	R	NR	R	M	R	R	NR
Chlorinated	M	R	M	M	NR	M	M	R	R	M
Ketones	R	R ¹	R	R	M	R	M	R	R	M
Aldehydes	R	R ¹	R	R	R	R	M	R	R	R
Ethers	R	M	M	M	NR	R	M	R	R	M
Amines	M	M	R	R	R	R	M	R	M	M
Aliphatic Solutions	R	R	R	R	M	R	R	R	R	M
Organic Acids	NR	R	R	M	M	R	R	R	R	M
Inorganic Acids	NR	R	R	M	M	M	M	R	M	M
Bases	NR	R	R	R	R	R	R	R	R	R
Sulfonated Compounds	R	R	R	M	M	R	M	R	R	M
THREAD STRENGTH*	Excellent	N/A	Good	Excellent	Fair	Excellent	N/A	Good	Good	Good
Max. Recommended Operating Temp. (°C)								FEP	PFA	
Fittings	60	N/A	80	125**	40	50	N/A	N/A	80	50
Tubing	N/A	50	N/A	100	N/A	N/A	100†	50	80	N/A

¹ Chemical resistance assumes room temperature use. Elevated temperatures may result in a significant reduction in chemical resistance.
² While the chemical compatibility of FEP & PFA is virtually identical, please note the temperature limit differences.
³ Recommended.
⁴ Some solvents in this category are satisfactory, others are not. In addition, maximum concentration can vary with the specific product type and chemical. Please contact your fittings supplier for further information.
⁵ NR: Chemicals in this category are generally not recommended for use with this polymer.
⁶ Shear Strength.
⁷ ** In some cases, PEEK fittings can be used to 150°C. Please contact your fittings supplier for specific information.
⁸ † In some cases, PEEK fittings can be used to 150°C. Please contact your fittings supplier for specific information.
⁹ ‡ Rodel is an amorphous polymer, and as such, its upper limit service temperature is application and chemical dependent, and may be higher than 100° C in some cases.

If Your Fittings Leak

1. Check to make sure your tubing is seated properly. When using fingertight fittings, the tubing must bottom out in the receiving port before the nut and ferrule are tightened. If a gentle tug disengages your tubing after the fittings have been tightened, loosen the fitting, push the tubing to the bottom of the receiving port, and re-tighten the fitting. (Note: Please don't attempt this while the pump is running, to prevent tubing slippage, dead volume, and a chemical spill.)
2. The fitting may not be tightened enough. Stainless steel nuts and ferrules require a wrench to tighten them, even after repeated use. Fingertight fittings also require a good turn; however, don't use a wrench unless instructed to do so, or you may damage the fitting.
3. You may be using incompatible fittings. Make sure you are using a nut and ferrule that are compatible with each other and with the components of your system. To avoid this problem and ensure compatibility, use fingertight fittings. Because the ferrule does not permanently swage onto your tubing, a fingertight can be used over and over again in most systems.
4. Check the condition of the nut and ferrule. After repeated use, nuts (and especially ferrules) will gradually become deformed to the point of being incapable of creating the seal they were designed to make. Always keep an extra supply of all the nuts and ferrules you are using so that you can replace them quickly and avoid unnecessary down time.
5. Check the receiving port for damage. Sometimes a leaking connection has nothing at all to do with the nut and ferrule, but with the receiving port. Ports that have had stainless steel fittings swaged into them are especially susceptible to damage. Check the receiving port for visible burrs or scratches and replace if necessary.
6. Evaluate chemical compatibility. Using fittings made of material that is incompatible with your mobile phase is a sure way of creating leaks. Refer to the Chemical Compatibility Chart on page 60 for general reference purposes.

TELL-TALE SIGNS OF SYSTEM LEAKS

Before you even see the first drip of mobile phase, your system can warn you that a problem exists. The most common signs of system leaks are as follows:

1. No flow or pressure
2. Pump pressures up, but there is no flow or low flow
3. Noisy baseline
4. Baseline drift

While all of these symptoms could also indicate problems completely unrelated to leaking fittings, it is always easiest to start there. Not only are fittings leaks usually easy to repair, they are also the least expensive part of the system that can cause problems.

Glossary

Adapter

A union with different threads or a different geometry on each end; generally used to connect two different types of tubing together.

Back Pressure Regulator (BPR)

A device typically used after the detector to maintain a positive pressure on the flow cell, thus minimizing solvent outgassing problems in the detector.

Biocompatible

Refers to that special quality of some materials allowing them to come into contact with biological materials without changing the materials' bioactivity.

Capillary Tubing

Often refers to tubing smaller than 1/16" OD; frequently used in hyphenated analytical systems, such as LC-MS.

Check Valve

A device inserted into a moving liquid stream that allows flow of the stream in only one direction; often built into the HPLC pump.

Chromatogram

A graphical plot representing the change in signal intensity from a detector and often used in the numerical analysis of a sample's components.

Column

A specialized tube, packed with small, chemically-active particles called the stationary phase, in which the separation of a sample takes place.

Cross

An x-shaped union used to connect four pieces of tubing.

Dead Volume

That portion of the volume within a connection that is not part of the flow path; opposite of swept volume.

Detector

A primary analytical system component that "sees" sample components as they are being carried away from the column by the mobile phase.

End Fitting

Used with most standard columns in the market today, it is the fitting at the end of the column that allows commonly used tubing to interface with the column tube. Additionally, the end fitting often holds the frit in place on either end of the column tube, thus retaining the packing material within the column tube.

Ferrule

A tapered conical ring used to make the seal between a piece of tubing and a receiving port. Ferrules almost invariably must be used in conjunction with a nut of some sort.

Fingertight

A special fitting invented by Upchurch Scientific that can be tightened to normal working HPLC pressures without the use of a wrench.

Filter

A system accessory which helps maintain overall system performance by preventing solid particles from passing along the flow path and potentially damaging sensitive components.

Fittings

Refers to the connectors that join tubing, columns and various LC modules together.

Flanged Fitting

A fitting used in low to moderate pressure applications. The fitting requires flanging - or spreading the end of the tubing - before use. Often used with 1/4-28 or M6 threaded flat-bottom fittings.

Flangeless Fitting

A specialized fitting designed to replace flanged fittings, where through the use of a nut and unique ferrule, a seal can be made on tubing in areas where flanged fittings were traditionally necessary.

Guard Column

A system accessory designed to protect the main analytical column from being damaged by sample components that might irreversibly bind to the stationary phase inside.

HPLC

An acronym for an analytical technique known as High Performance Liquid Chromatography, used in many laboratories around the globe.

Injection Valve

A specialized valve used to introduce a controlled amount of sample into the mobile phase for analysis, while causing little to no system disturbance.

Mobile Phase

Chemical solution pumped throughout an analytical system for the purpose of analyzing samples.

Nut

A common term used to describe the threaded portion of a fitting system.

OEM

An acronym that stands for Original Equipment Manufacturer.

Port (or Receiving Port)

The threaded hole into which or the threaded post onto which a fitting system attaches tubing. Low pressure ports typically have a flat-bottom geometry and high pressure ports most often have a coned geometry.

Pump

A primary piece of an analytical system that pulls mobile phase out of the reservoir and pushes it out through the rest of the system.

Recorder

A primary analytical system device that translates the signal generated by the detector into a plot of signal versus time; most often, this is usually a computer.

Reservoir (or Solvent Reservoir)

Often a glass or plastic bottle that holds the mobile phase.

Stainless Steel

Any variety of steel alloys designed for corrosion resistance. The different varieties - primarily 316 grade - are used to manufacture high-pressure, chemically resistant HPLC fittings and tubing.

Stationary Phase

The small, chemically-active particles densely packed into a specialized tube known as a column and often offering an opposite chemical environment to that which is offered by the mobile phase.

Swaging

The process of permanently attaching a ferrule (usually manufactured from stainless steel) to a piece of tubing.

Swept Volume

That portion of the volume within a connection that is part of the flow path; opposite of "dead volume."

Tee

A T-shaped union used to connect three pieces of tubing.

Thread Pitch

Standardized numerical value describing how close together threads are on a fitting; English threads have a thread pitch that describes the number of threads per inch, while metric threads are described by the distance in millimeters between threads.

Tubing Sleeve

A short length of tubing designed to slip over capillary tubing, allowing the capillary tubing to be successfully connected to a port designed for larger tubing.

Void Volume (or Internal Volume)

The sum total of the dead volume and the swept volume in a connection.

Waste Reservoir

The last component of a standard analytical system that safely collects the mobile phase and sample components after they have been analyzed.

