laserchrom
HPLC Laboratories

Technical Tips – Guide to Using Polymer-based RP Columns!

Silica is normally used as the packing material for HPLC columns for a number of reasons. It is very strong, allowing it to be used at high back-pressures, and packed at even higher pressures to give a really stable columns with sharp peaks. It is also relatively cheap, so columns cost between £150-£300. However there are limitations with silica-based columns, which we have all come to accept, but which are a nuisance nevertheless! It dissolves in water, especially at elevated temperature, at higher pH, and in higher buffer concentrations. Hence for many methods, (especially those for basic substances such as amines where we would normally choose to work around pH9-10) we have trade-off between getting a good separation, and not dissolving the column too quickly. This pH limitation can also limit us for column cleaning. Other problems are that the bonded phase can easily be stripped off by pH less than 2 because the Si-O-Si bond is hydrolysed, and the residual silanol sites (Si-OH groups) on the surface can cause peak tailing.

Various approaches are available to minimise the impact of these limitations, but the most popular solution is to use a column with a polymer-based packing material. For many, this is perceived as an expensive and unknown territory. However since the columns work really nicely and last for absolutely ages, it is actually an easy and cost effective way to solve a lot of problems at once! So here is a guide through the maze.

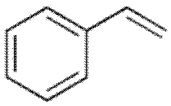
Polymer columns come in a steel tube, just like silica-based columns, but the gel inside is a rigid polymer matrix rather than silica. There are four main polymers which are used, and they each behave a little differently from silica.

Polymethacrylate.
(Shodex DE series columns)

Polymethacrylate is a polymer that was actually developed originally for HPLC, although it now has other uses too. It has good temperature, pH and pressure stability, it is compatible with aqueous and organic solvents, it has well defined pore size, and a large surface area! It also forms spherical particles, and hence is relatively easy to pack efficiently in a column. It is a non-polar material, and is used underivatised (*no C18 chain bonded on*) for reversed phase HPLC (RP-HPLC), just as silica is for normal phase columns. Its polarity is virtually the same as a C18 column, but the selectivity (*the position of the peaks relative to each other*) is a little different.
When transferring a method from a silica-based C18 column, we recommend using a slightly stronger eluent (*a few % more of the organic solvent*).
These columns can be used with a pH range of 2-12, which gives excellent flexibility for all types of samples. Eluents should be made from Methanol:Water or Acetonitrile:Water but not Tetrahydrofuran (THF). The packing is not dissolved in THF, but it causes the gel to swell, disturbing the packing of the column and causing high back-pressure. The maximum pressure is 2250psi, and for best efficiency the flow rate should not be over 1.2ml/min for a 4.6mm id column. If a buffer salt is used, the concentration should be kept below 0.5M, although in HPLC we would hardly ever go anywhere near that anyway.

If you are transferring a method from an ODS column, this is a good one to start with! It is very well mannered, you will have no problems with silanols giving peak tailing or the column dissolving, it is packed with an efficiency of at least 70,000N/m so the peaks will be sharp, and there's no bonded phase to strip off. For comparison, a 150 x 4.6mm columns costs £495, and if you keep your samples free from particles, the column will last for ages!

Styrene/Divinyl Benzene Co-Polymer.
(Shodex DS and RP18 series columns)



Styrene (=vinyl benzene) readily forms a polymer because the vinyl groups link together to form a chain. Divinyl benzene has a second vinyl group (meta or para to the first one) and by adding this to the mixture, it forms part of the polymer and allows the polymer chains to form cross-links between them, forming a much stronger and more rigid polymer. For some industrial applications, very low percentages of DVB are added, but for HPLC much more DVB is used to give a high density of cross-links, and one of the most robust polymer supports available for HPLC. This reaction is well documented and able to be very precisely controlled, allowing the formation of small spherical particles with a very narrow particle size distribution. For reversed phases applications, styrene/divinyl benzene columns are used underivatised.

Columns packed with this material can take over 3000psi, and have a usable pH range of 1-13. Swelling in organic solvents such as THF or chloroform is negligible because of the crosslinking, although it is recommended that the column is not used in totally aqueous eluents. As a guide, it is best to keep a minimum of 5-10% organic solvent with these columns. (For applications requiring 100% water as eluent, use the DE or ODP columns.) The packing is very efficient and efficiencies are guaranteed to exceed 70,000N/m for DS columns and 80,000N/m for RP18, using 3.5u particles.

These columns are more retentive to hydrophilic samples than a silica-based C18 column, which can be a real advantage. This gives a different selectivity to the DE columns, and as with any selectivity change, this can help in some cases and cause co-elution in others. These columns are recommended to complement the DE series when developing methods, and this is the column of choice when using a method developed using styrene divinyl benzene columns from other manufacturers. For comparison, a 150 x 4.6mm column costs £536, and again, they last a very long time!

Polyvinyl alcohol.
(Shodex ODP40 and ODP50)

Polyvinyl alcohol is a polymer which is unusual in that it is not made by polymerisation of vinyl alcohol. Instead it is made by hydrolysis of polyvinyl acetate, replacing the acetate groups with OH. The surface is much more polar than the other two polymers discussed so far, but because the OH groups fit sterically inside the polymer chain, the external surface is actually not very hydrophilic. Nevertheless, column packings based upon PVA almost always have a bonded phase attached. In the case of the ODP range, a choice of C18, C8 and C4 columns are available, offering a wide range of polarity, C4 being the most polar. Shodex PVA columns have a wider pore size than the other two. This means that they can be used with molecules up to a molecular weight of around 200,000, and without the pressure sensitivity that ensues with wide pore silica columns. It does however reduce the surface area a little, so these columns are slightly less efficient than DE or DS columns.

Because the columns have a C18 group bonded on, they have a very similar polarity to a silica based C18 column (and similar to a DE column). This makes the transfer of methods from silica ODS columns much easier. They also have a similar selectivity, so the separation should look very similar. ODP40 is just a smaller particle version of ODP50, giving higher efficiency and sharper peaks. For those wishing to do direct comparisons, the carbon loading is 17%, very similar to most modern type B silica based C18 columns, and the column can be used with eluents up to pH13. It is recommended that the organic modifier should be Methanol or Acetonitrile but not THF. In general, Acetonitrile gives the sharpest peaks with this column. For comparison, a 150 x 4.6 column costs £485 (ODP50) and £495 (ODP40).

Polyhydroxymethacrylate
(NEW - Shodex ODP2 HP)

This material has been developed specifically to make silica columns obsolete! It has all the advantages of a polymer material (wide pH range, no silanol interactions, really long column life) but it also is more efficient than any other polymer based column, giving peaks as sharp as the most expensive silica columns on the market. It has excellent retention of hydrophyllic materials, and good selectivity. But the most important difference now is the price. This column is available for no more than the best silica columns. For comparison, a **150 x 4.6mm column costs just £285!** This material was launched only last week (14th September 2006) and is available now!

FREE sample analysis and comparison on all four columns in our lab:

If you would like to try one of these columns in your lab, Shodex will send out columns on a sale or return basis.

However……

We now have all four of these columns in our lab set up and ready to go. If you would like us to try running a sample for you on each of these four columns, we will do it for free, and with no obligation to buy a column. If we find that a small eluent change would help we will make the change and send you a set of four chromatograms so that you can see what each of these columns could offer you. To arrange this, please call us on 01634-294001, and then send us your sample and method. As soon as we receive it, we'll call you to discuss, and run the separations for you. Once we have the results, we will contact you again to explain what we have done, and answer any question you may have.