

Tips & Tricks: GPC/SEC

GPC/SEC Columns – Dos and Don'ts

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The GPC/SEC column is the key component for the separation of macromolecules by size. The column enables a conventional HPLC instrument to be used for GPC/SEC analysis. GPC/SEC columns, in general are mechanically and chemically very stable. They are packed with either porous highly cross-linked polymer particles or porous silica particles.

GPC/SEC columns, like all LC columns, have a finite life span, and to get the best performance and a long life the columns have to be treated properly. However, there are many ways to damage or destroy a column, or influence the column properties and column performance. The polymer network can break down, the pores can partly or totally collapse, the polarity of the column surface can be modified, the column hardware, for example, the metal of the column body, can be damaged or oxidized and the frits can be damaged or blocked.

To run a sample in a pure GPC/SEC

mode, a balanced system of solvent, stationary phase and sample is required (see Figure 1). The column performance can also be described by the plate count, the separation power and the resolution. The pore volume and the pore size distribution influence the separation power and the resolution. The plate count depends mainly on the particle size, the packed bed density and the interstitial volume. The better the value of these three parameters, the better the overall column performance.

Column back pressure is important too. The back pressure is mainly affected by the particle size and the solvent viscosity. As the particle size decreases and the solvent viscosity increases the higher the detected back pressure for a column.

The major question is how to operate a column correctly?

Solvent Considerations

In GPC/SEC the solvent plays a key role in determining the success of the analysis.

The solvent not only has to dissolve the sample but also has to be “compatible” with the column packing material. Don't use a “non-solvent” for the analysis. For polymeric packing materials, a non-solvent is one where the packing material can't interact with the solvent.¹ In this instance the pores can collapse and the column material can be irreversibly damaged. The compatibility of the solvent and the stationary phase of the column is optimized when the polarity of both is comparable (Figure 1).

How to Store the Column Properly

Columns used for GPC/SEC analysis in an organic solvent should be stored in a pure organic solvent but be aware of the vapour pressure. Columns stored in solvents having a high vapour pressure have to be kept cool.

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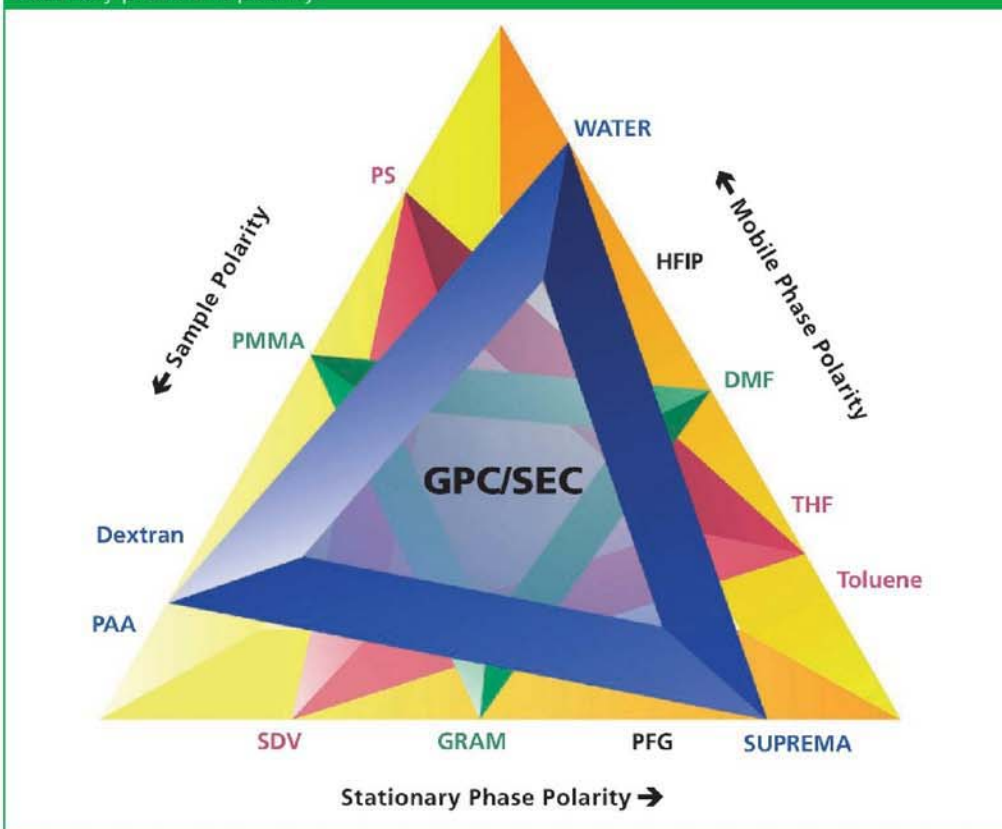


If columns are not going to be used for weeks or months it is good practice to keep them in volatile or unstable solvents in a refrigerator (4 °C) to prevent evaporation or degradation.²

Aqueous columns should be stored adding a small amount of NaN_3 to prevent algae or bacterial growth.

Don't store a column in a salt solution. A salt solution is a potential cause of rust and

Figure 1: "Magic triangle" showing that GPC/SEC requires a balanced system of sample, stationary phase and polarity.



can precipitate in the column if the solvent evaporates during an incorrect storage process. Reinstalling a column that has been stored in a salt solution can also damage the packing material if the salt precipitates because of a solvent evaporation.

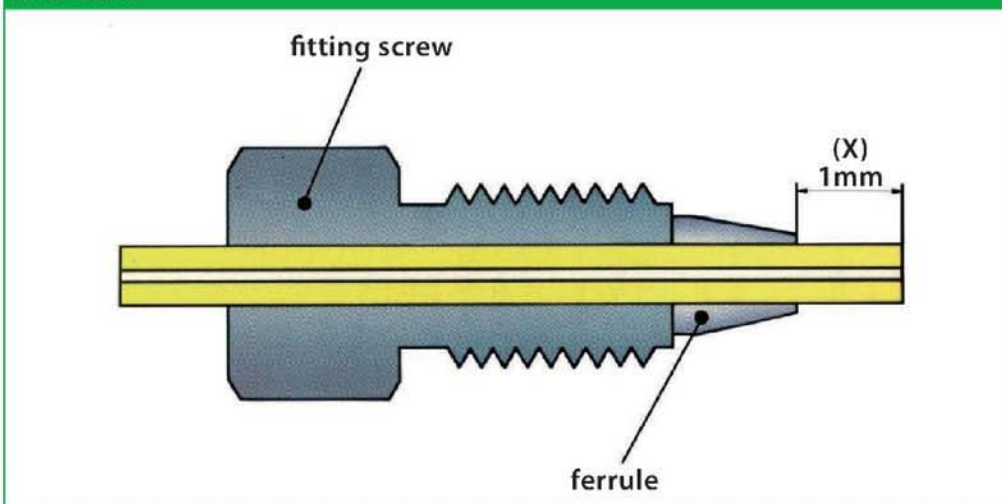
How to Install a Column Properly

As mentioned before, the column is filled with porous particles (see Figure 4). The porosity of these particles is in the range of nm up to several 100 nm. Particle size, itself, is in the range of μm . An efficiently packed GPC/SEC column has a very large

total surface area. The solvent not only covers the external surface of each particle (a relatively small surface area) but also the internal surface (a relatively much larger surface area) as well. This has to be taken into account when installing a new or stored column as the solvent in the pores makes it difficult to get a complete solvent exchange.

The major requirement for an efficient solvent exchange is that the solvents have to be miscible and the exchange solvent has to be a "good solvent" for the stationary phase so that the particles can be wetted.

Figure 2: Depending on the manufacturer, the capillary fittings and capillary end distance will be different.



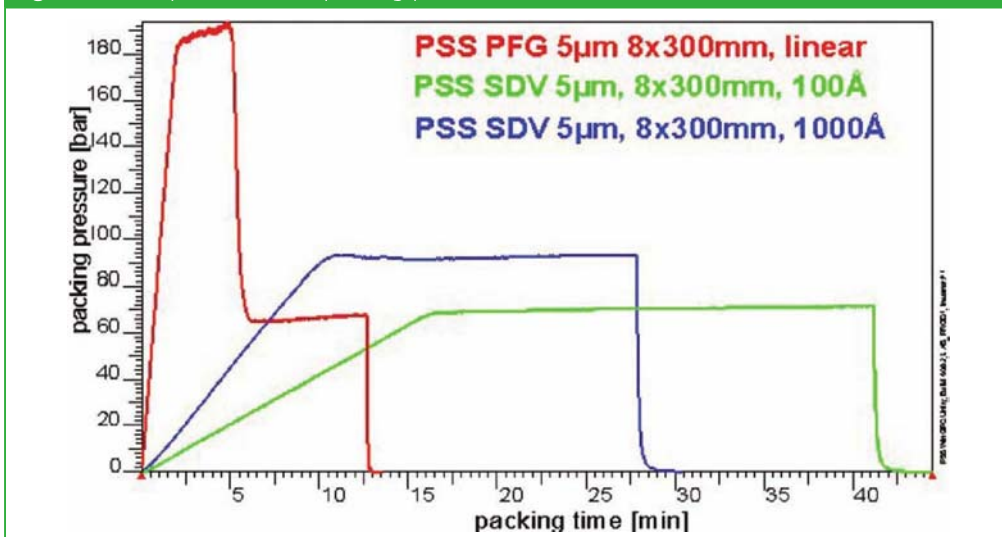
As a rule of thumb, for a complete solvent exchange the amount of solvent should be equal to five times the overall column volume. Also the solvent viscosity influences the performance of the exchange. As the viscosity of the exchange solvent increases, the longer it takes for a complete exchange. It is also recommended that during the exchange process, the flow-rate should be set to approximately 10–30% of the operating flow-rate. It should also be noted that during the first phase of solvent exchange there will be a solvent mixture, so there is the possibility that a short pressure increase can take place.

Finally, if the column has been stored in a refrigerator don't install the column immediately. Allow sufficient time for the column to equilibrate to the operating temperature.

Column Fittings and Flow Direction

Don't mix fittings and capillaries from different suppliers because each supplier has its own specially designed column end fittings, nuts and ferrules, therefore, only the original suppliers fittings and capillaries will fit perfectly into the column end fitting. Using the wrong fittings can either damage

Figure 3: Comparison of the packing pressure for various column materials.



the frit inside the column head or create an additional void volume and will lead to additional band broadening (see Figure 2).

The flow direction along the column axis is normally marked by an arrow. Always install the column according to the indicated flow direction. The flow direction also indicates the direction of the packing of the column. During the packing process the particles and the packed column bed will be oriented along the flow direction, which means that the particles packed in a column have a preferential direction according to the flow vector. Turning the column around and using the column in the opposite flow direction can create a

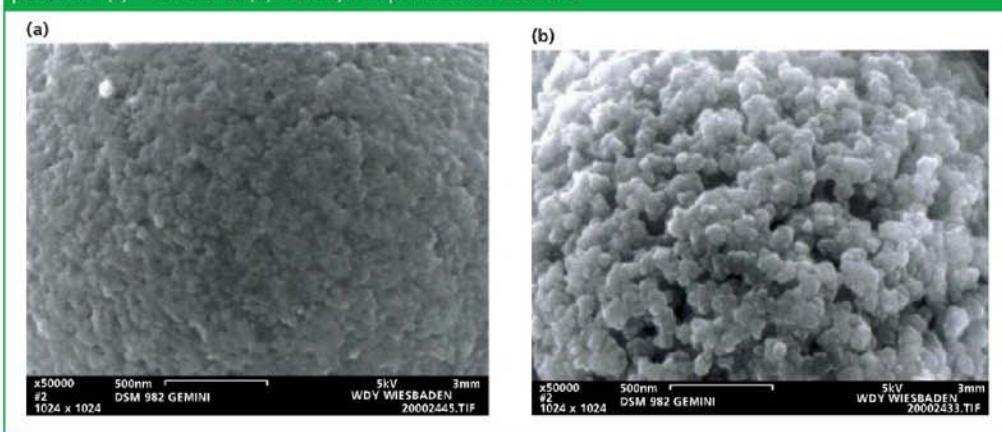
disruption of the packed particles increasing the interstitial volume and so the band broadening.

Watching the Back Pressure

The GPC/SEC columns are packed with a certain flow-rate under high pressure. This high pressure leads to a preferential packing of the particles and forces them into a desired orientation inside of the column (see Figure 3).

The pressure applied for packing the column can be considered as the upper limit for the operating pressure of the column. However, it is recommended to operate the GPC/SEC column up to 60–70% of the pressure limit given on the column certificate. This will

Figure 4: Electron microscopy picture for various pore sizes of PSS SDV 5 μm particles with pores of (a) 100Å and (b) 105Å; Amplification: 50000.



ensure stability of the packing material as well as for the pore structure and extend the life of the column. If the back pressure increases significantly above the packing pressure of the column for any extended period of time, the preferential orientation will break down and the pore structure will tend to collapse. As the pore size increases, the pore structure becomes more fragile and tends to fracture easier. This means that a 10 nm pore on a 5 μm particle is almost twice as stable as a 10^4 nm pore on a 5 μm particle.

However, for a short period of time the column pressure can be increased up to the given limit and even slightly above.

How Can the Column Performance be Tested?

The recommended way to test the column performance is to perform the calculation of the theoretical plate count per metre, N_{th} [m^{-1}] and the specific resolution, R_{sp} .¹

These values will accurately indicate the overall performance of a column. Note; if the plate count given on the supplier's certificate is compared with the plate count calculated for a column in use, the measuring conditions have to be taken into account. Don't compare values generated under different measuring conditions such as sample, injection volume or concentration.

References

1. *Introduction to Modern Liquid Chromatography*, L.R. Snyder, J.J. Kirkland and J.W. Dolan, Wiley, Ch. 5., p. 199ff (2010).
2. G. Reinhold, *The Column*, **5**(17), 7–9 (2009).

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