Maximise the Productivity of Your Process with the Correct Pore Selection

Abstract

Selecting the most suitable stationary phase is the key for efficient and productive purification processes. In addition to selectivity and particle size, pore size is the third important criterion that must be taken into account during resin selection. The pore size determines the surface area of the stationary phase and therefore the retention of the target as well as the loadability. In addition, the accessibility of the pores is an important factor for efficient separation.

Important aspects that can help when choosing pore size:

- ✔ **High loadability / optimal interaction:**
  The smaller the pore size, the larger is the overall surface area.
- ✔ **Sufficient interaction guaranteed / good accessibility:**
  The pore size should be adjusted to the size of the target molecule.
- ✔ **Loadability studies can help determine the best pore size.**
- ➜ **Well selected pore sizes lead to good chromatographic performance.**

This Technical Note helps to determine the most suitable pore size based on the size of the target molecules and additionally gives practical examples as well as tips for scale up.

Correlation between Pore Size and Surface Area of Stationary Phases

The pore size of a stationary phase correlates directly with the specific surface area: The smaller the pores, the larger the total surface area. Therefore, the ligand loading is also higher for smaller pores because a larger surface area is accessible for modification. Consequently, the carbon loading of RP phases is higher for smaller pores. This affects the loadability and the retention behaviour of the target on the stationary phase.

Here, you can find an example of a correlation between pore size, specific surface area and carbon load of the RP stationary phase YMC*Gel ODS-A-HG:

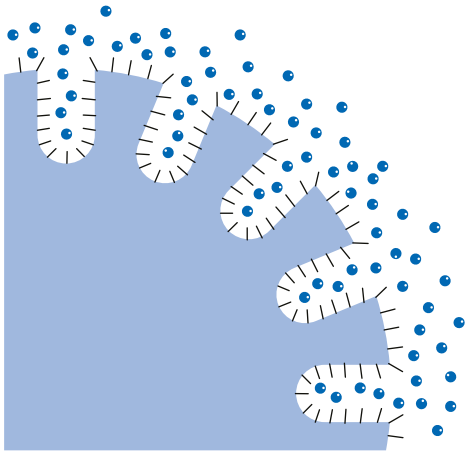
YMC*Gel ODS-A-HG				
Pore Size [nm]	8	12	20	30
Surface Area [m^2/g]	510	330	175	100
Carbon Load [%]	20	17	12	7

With increasing pore sizes, the specific surface area decreases. Consequently, the overall ligand load (in this case the carbon loading of the C18 phase) decreases with larger pores.

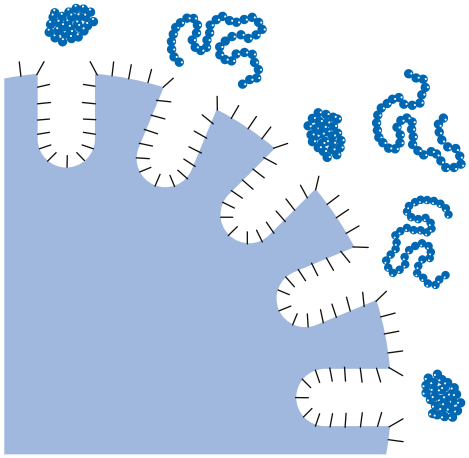
The Relationship between Pore Size and the Size of the Target Molecule

The pore size of the stationary phase has a massive impact on the chromatographic separation because this strongly depends on the size of target molecule that has to access the pore as illustrated in the following scheme:

Small organic compounds



Larger peptides and polymers



Small molecules can easily access the pores of this stationary phase whereas larger molecules like large peptides, proteins or polymers are excluded from the pores. For these types of molecules, a larger pore is needed. Too small and too large pore sizes lead to decreased resolution and peak broadening.

Generally, the pore size should be selected as the smallest possible but the largest necessary.

Selection of the Pore Size based on the Molecular Weight

For optimal separation, the pore size has to be adjusted to the molecular weight of the target. With the YMC selection tool you can immediately find the most suitable pore size at a glance, depending on the size of the target molecule. As a rule of thumb, the larger the target molecule, the larger the pore size of the stationary phase should be and the less hydrophobic the modification of the stationary phase should be. Of course, these values are only recommendations and the limits are guidelines. It therefore makes sense to test different pore sizes with increased loadings.

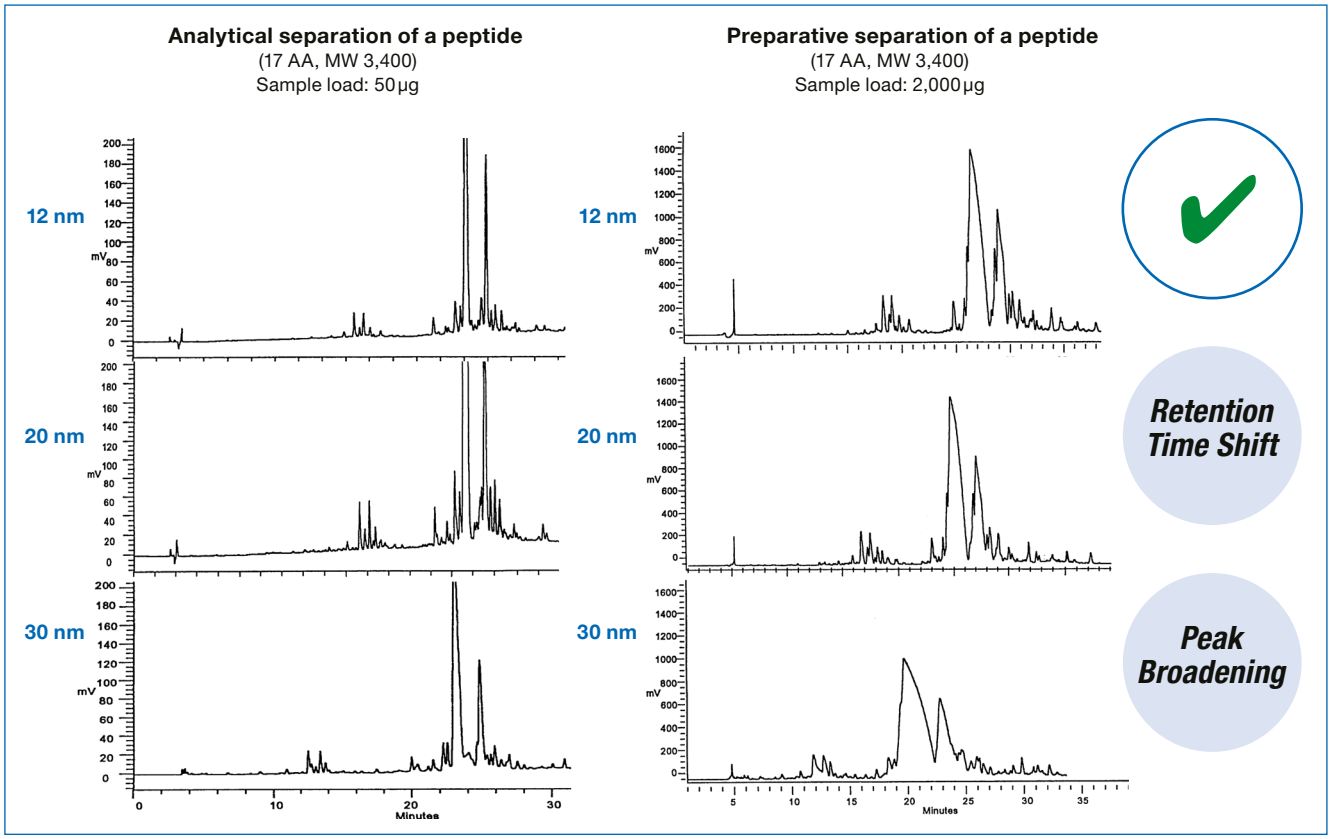
MW		C18	C8	C4
5,000	12 nm	+++	++	+
20,000	20 nm	++	+++	++
100,000	30 nm	+	++	+++

Small molecules: the recommended pore size varies between 8 nm and 12 nm. For RP purifications, 12 nm is the recommended pore size
Peptides, proteins and polymers: the favourable pore size varies between 12 nm and 30 nm depending on the size of the molecule.

Practical Example:
Selection of the Most Suitable Pore Size for Preparative Processes

Selecting the most appropriate pore size leads to optimised separation efficiency and therefore enables higher loadings and improves the productivity. To investigate the pore size selection, screenings with different loadings are beneficial to determine the most suitable pore size for a specific purification process.

This example illustrates the effect of different pore sizes on the separation of a peptide sample at analytical and preparative scale. On an analytical scale with 50 µg loading, the influence of the different pore sizes on the separation profile appears to be small, whereas with increased loading amounts (2 mg), the effect becomes clear.



Therefore, the screening of different pore sizes with increased sample loadings is key to identifying the most appropriate pore sizes for an efficient purification process.

The Stationary Phases of YMC: Multiple Pore Sizes Available

The YMC preparative bulk packing materials for RP and NP purifications – **YMC-Triart Prep** and **YMC*Gel** – are available with different pore sizes. With the YMC materials, the most suitable pore size match for various separations can be found.

YMC*Gel for example is available with the following pore sizes:

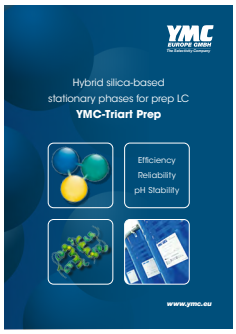
8 – 12 – 20 – 30 nm

Conclusion

The selection of the pore size of the stationary phase is of great importance for efficient separations and therefore for productive purification processes. The pore size determines the **overall surface** area and also the **ligand density**. For best separation results, the pore size should be selected according to the **size of the target molecule**. To identify the most suitable stationary phase, **screening different pore sizes** with increased target loadings is a very successful approach.
Here too, the full flexibility with YMC stationary phase is an additional benefit for every screening: the stationary phases for preparative purifications **YMC-Triart Prep** and **YMC*Gel** are available in many different pore sizes.

Further Information

The YMC packing materials are available in different particle and pore sizes.
Further information including specifications can be found in the product brochures:



Brochure
YMC-Triart Prep

Brochure
YMC*Gel HG-Series

Additional information about the benefits of the YMC bulk packing materials can be found on the YMC website **www.ymc.eu** or please just get in touch with your YMC representative.

Also of interest

You'll find more helpful information for method development and important considerations in the YMC whitepapers:

Whitepaper
Method development strategies

Whitepaper
Strategic Peptide purification!

Whitepaper
Analysis and Purification of Oligonucleotides