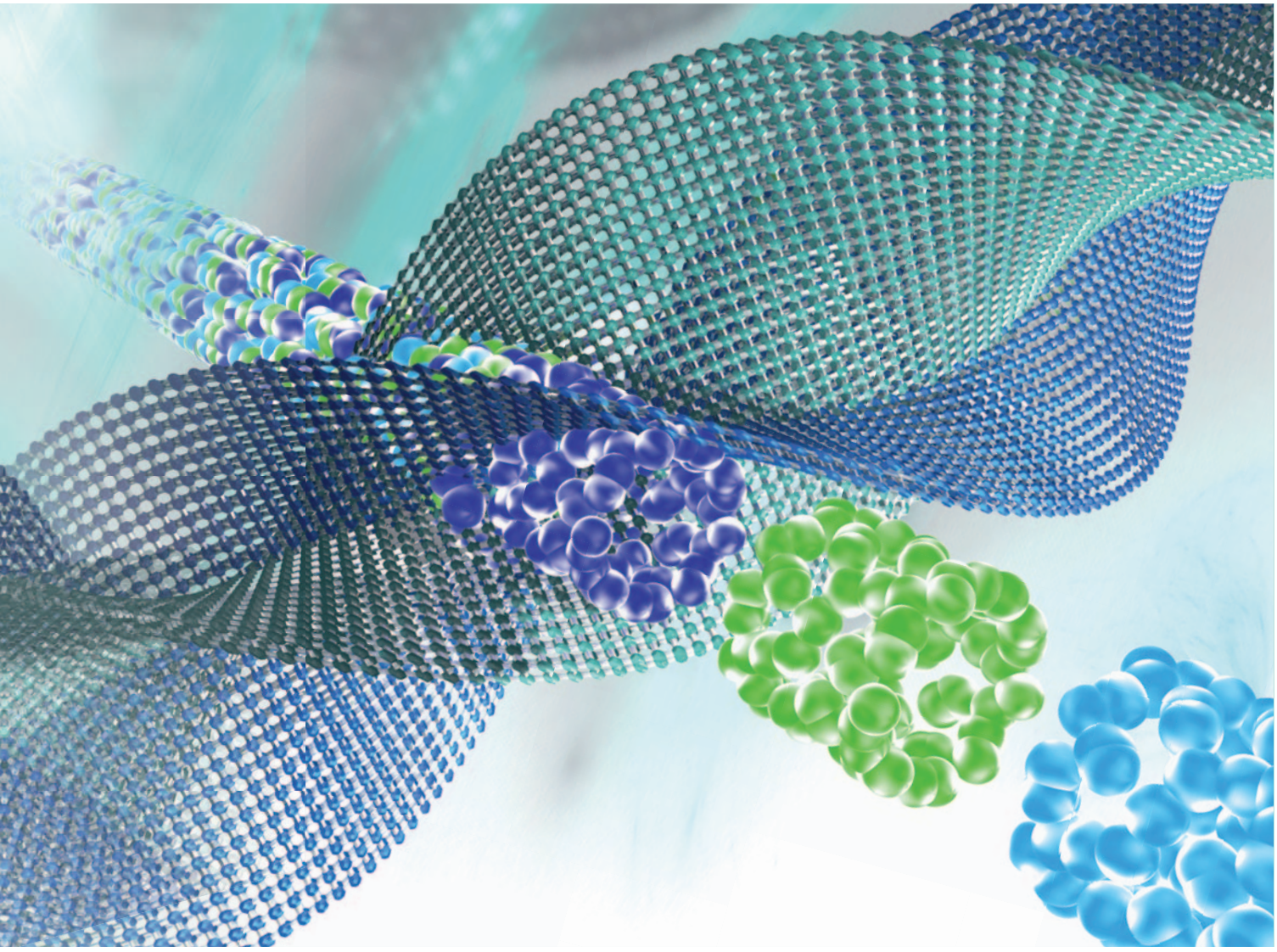
Separation Technologies: our work – our life – our passion.

ZEOCHEM®



ZEOsphere® DRP

Doped Reversed Phase Chromatography Gels

Faster, better, cheaper than traditional Separation Technologies

- ✓ Simplifying Process
- ✓ Increased Selectivity
- ✓ Higher Purity & Yield
- ✓ Lower Costs

ZEOsphere® DRP – High Quality Swiss Production

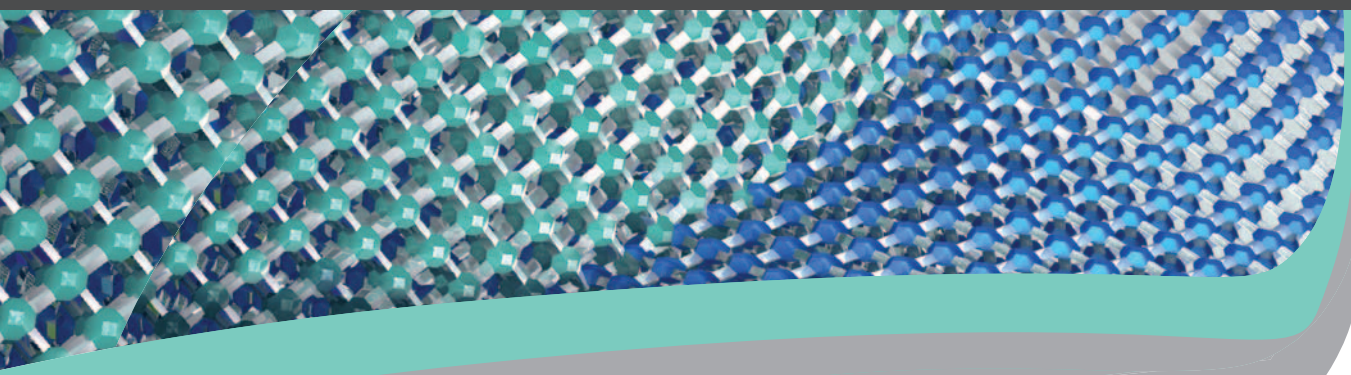
Zeochem is a member of the CPH Chemie + Papier Holding AG, a Swiss Company listed at the SIX Swiss Exchange.
With manufacturing facilities in Switzerland and the US, Zeochem develops, manufactures and sells high-performance chromatography gels for the pharmaceutical and biotechnology industry as well as molecular sieves (zeolites) and silica gels for industrial applications. Zeochem can draw on an industrial tradition dating back almost 200 years. Its headquarters is still based at the original location in Switzerland.

Reversed Phase Chromatography is a major purification tool for pharmaceutical peptides. However, the constant need for improved chromatographic performance calls for better, higher performance stationery phases.

ZEOsphere® DRP (Doped Reversed Phase Chromatography) is a new powerful tool in peptide separation. Developed in collaboration with the Morbidelli group (ETH Zurich), the DRP can be used in the purification of any API currently processed via reversed phase chromatography. By adding limited amounts of ion exchangers onto a reversed phase base (i. e. doping a reversed phase base), both ionic and hydrophobic functionalities can be obtained. These simultaneous functionalities can be controlled by the mobile phase properties and the doping giving a significant performance increase with respect to conventional available chromatographic materials.

Only one phase needed:
Zeosphere® DRP is based on merging high performance reversed-phase with ion-exchange (anion or cation) material. This newly created material exhibits multiple mechanisms of interaction which allows the use of one stationary phase for a much wider range of applications. Moreover, the ZEOsphere® DRP was designed with usability in mind: while exhibiting increased performance, these phases can be used in the same manner as any traditional reversed phase material. Combining unequaled performance and traditional method development only leads to an increase in productivity and a decrease in cost!

NEW FRONTIER IN SE



Faster, better, cheaper than traditional Separation Technologies and with one single phase!

At first glance, Zeosphere® DRP and mixed mode phases seem to be the same, however, differences exist. First, mixed mode materials tend to display different interaction types through only one ligand. This leads to a very stiff system where the overall mixed mode behavior is entirely dependent on the ligand chemistry. On the other hand, ZEOsphere® DRP has different ligands (one ion exchanger (IEX) ligand type and one reversed phase (RP) ligand type). This allows each interaction mechanism to be precisely tuned, depending on the doping concentration. Second, mixed mode materials usually rely on two attractive interaction types. While this is also a possible mode of operation of the ZEOsphere® DRP, it can also be used in attractive-repulsive mode, where the ion exchanger groups are repulsive to the analyte (same charge sign). In fact, increased performance is achieved in attractive-repulsive mode!

The ZEOsphere® DRP is an answer to the need for faster, better and cheaper phases, all the while displaying higher efficiency (productivity, yield and purity) through controllable and reproducible multiple interactions.

PARATION SOLUTIONS

ZEOsphere® DRP – Proved Technical Advantages

ZEOsphere® DRP Standard line up

Strong Anion Exchanger	Anion exchange ligand %*	RP ligand %
ZEOsphere® DRP 120 A2.5 / 10 µm	2.5	97.5
ZEOsphere® DRP 120 A5 / 10 µm	5	95
ZEOsphere® DRP 120 A7.5 / 10 µm	7.5	92.5
ZEOsphere® DRP 120 A10 / 10 µm	10	90
ZEOsphere® DRP 120 A15 / 10 µm	15	85

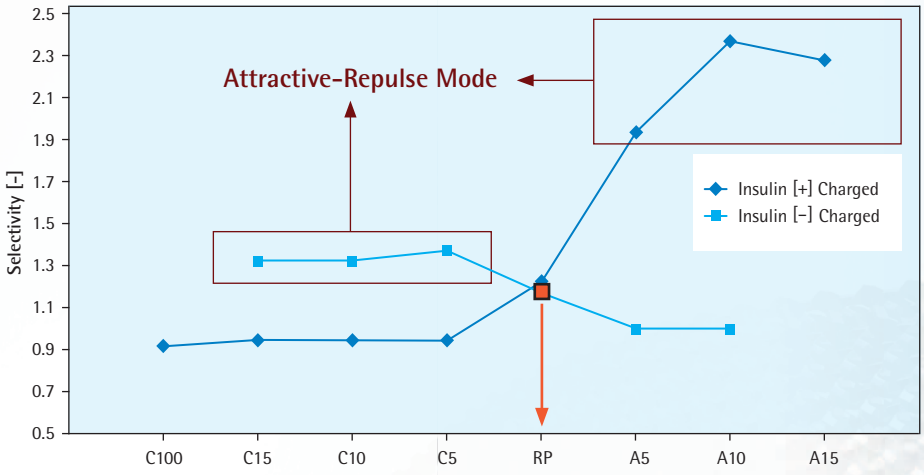
Strong Anion Exchanger	Cation exchange ligand %*	RP ligand %
ZEOsphere® DRP 120 C5 / 10 µm	5	95
ZEOsphere® DRP 120 C10 / 10 µm	10	90
ZEOsphere® DRP 120 C15 / 10 µm	15	85
ZEOsphere® DRP 120 C50 / 10 µm	50	50
ZEOsphere® DRP 120 C100 / 10 µm	100	0

* % in the reaction mixture. This value is directly proportional to the real ligand density on the stationary phase.
For process optimization, other ratios and other pore sizes are available on request.

Test Data Insulin Separation

Higher Selectivity:

A typical separation challenge in RP chromatography is the separation of insulin from its deaminated variant. As both the insulin and the impurity are extremely similar, this separation is difficult to achieve! However, by using the ZEOsphere® DRP phase in attractive-repulsive mode, selectivities up to twice as high as in traditional RP phases can be achieved!

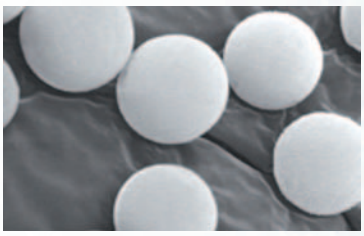


Chromatographic conditions:
Mobile Phase: 240 mM sodium acetate buffer pH=3.5 (left plot); 240 mM ammonium acetate buffer pH=6.5, isocratic, different ACN concentrations to achieve the same tr on all materials. T=25 °C.

Test Data API Separation

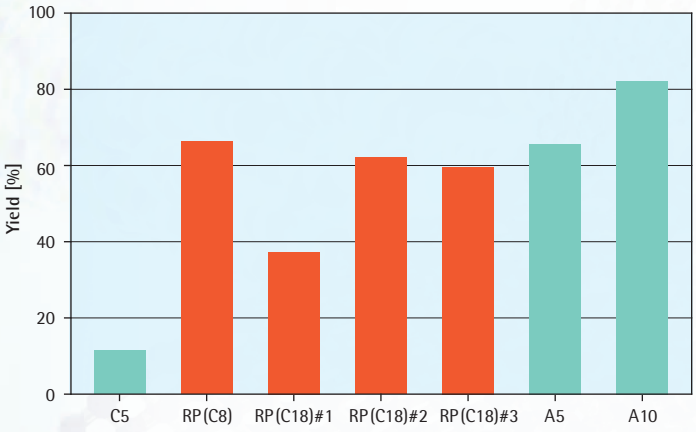
Higher Yield:

Once the required purity of an API has been chosen, the yield of the purification step becomes the most important attribute of the given purification step. Using the ZEOsphere® DRP in attractive-repulsive mode is an excellent way to maximize yield while keeping the purity requirements! In all cases tested (purification of different raw mixtures of synthetic peptides), the ZEOsphere® DRP exhibited superior performance!

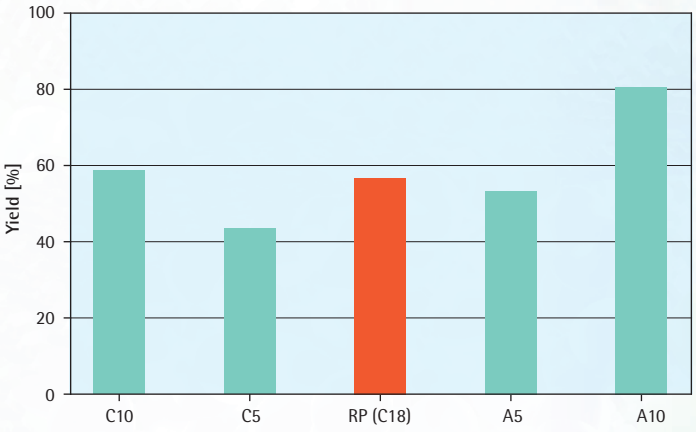


SEM of ZEOsphere® 100 10 µm

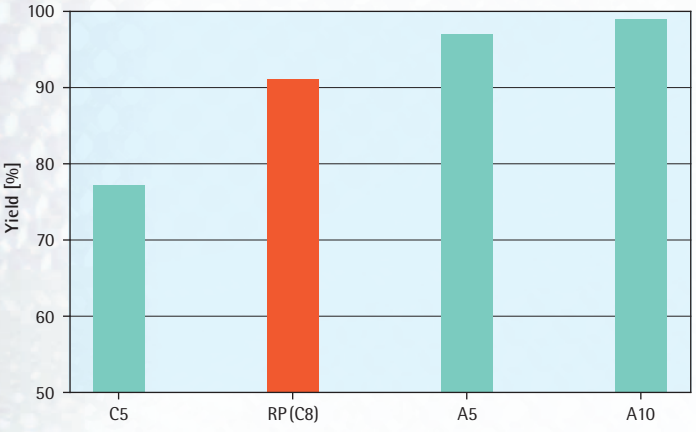
Synthetic Peptide 1: Goserelin (Acetate Buffer)



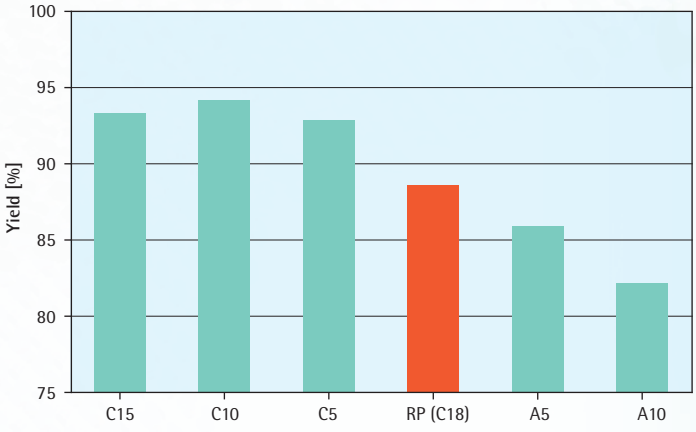
Synthetic Peptide 1: Goserelin (TFA Buffer)



Synthetic Peptide 2



Synthetic Peptide 3

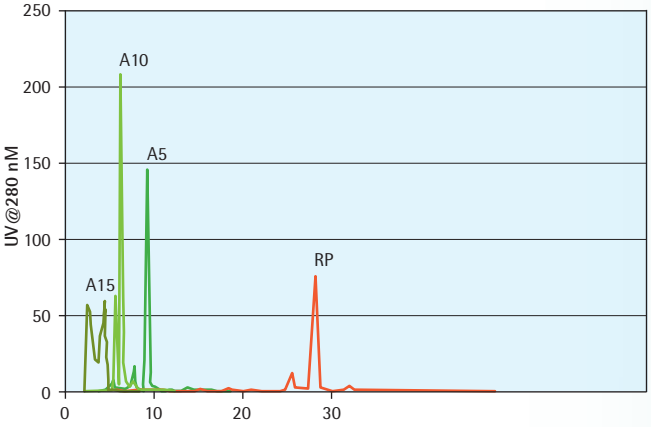


Chromatographic conditions: Loading: 5 g/L of pure peptide
Synthetic peptide 1, Goserelin: 300 mM acetate buffer, pH 4.8, acetonitrile gradient 0.5 vol%/min, T= 25 °C, required purity: 94 % (peptide + charged),
Synthetic peptide 1, Goserelin: 0.1 v% TFA buffer, pH= 4.8, acetonitrile (1% vol TFA) gradient 0.5 vol%/min, T= 25 °C required purity: 95 %, (peptide + charged)
Synthetic peptide 2: 120 mM sodium phosphate buffer pH= 4.8, acetonitrile gradient 0.5 vol%/min. T= 25 °C. Purity= 94 %, (peptide + charged)
Synthetic peptide 3: 100 mM ammonium acetate buffer pH= 6.5, acetonitrile gradient 0.5 vol%/min, T= 25 °C. Purity= 87 %, (peptide - charged)
In all cases, retention time of the peptide of interest was set to 30 minutes by changing the gradient starting point (same slope).
Different starting ACN concentrations to achieve the same tr on all materials.
All RP (C8) and RP (C18) are commercially available and often used reversed phase materials, produced by competitors.

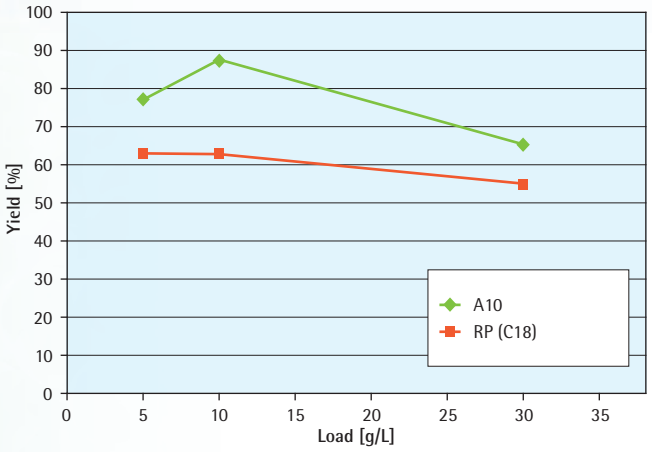
Ease of use

The main advantage of the ZEOsphere® DRP is not only the better performance, but also the easy application of these phases. By using the exact same experimental conditions, the results given by the ZEOsphere® DRP speak for themselves, even at high loaded amounts!

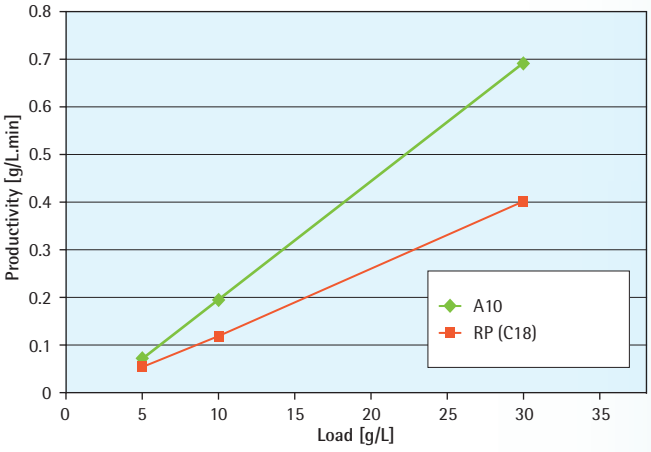
Faster Separation!



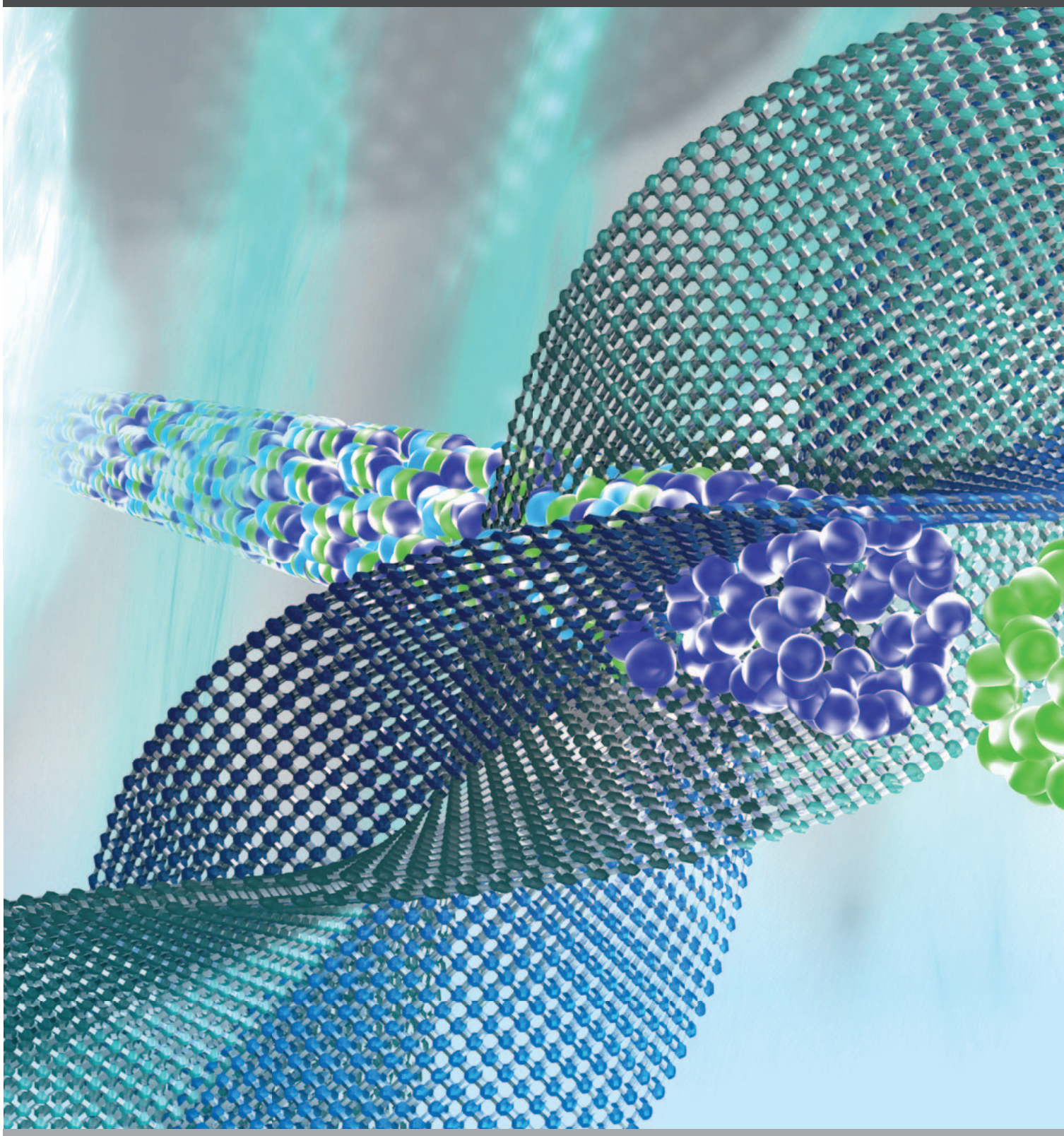
Higher Yields!



More Productive!



Chromatographic conditions:
Peptide: Goserelin, loaded at 5–10–30 g/L of pure peptide
Experimental conditions: 100 mM acetate buffer, pH 4.8 (peptide + charged), required purity: 94 %, gradient slope: 0.42 v% AcN/min.
RP (C18) are commercially available and often used reversed phase materials, produced by competitors.



www.zeochem.com

Zeochem – Innovation & Customization

Process Development on the Zeosphere® DRP can simply be done in the same way as on any traditional reversed phase material! Current processes can even be ported directly! The choice of the ZEOsphere® DRP to use can be done according to simple heuristics:

1. Determination of the isoelectric point (pI) of the peptide of interest.

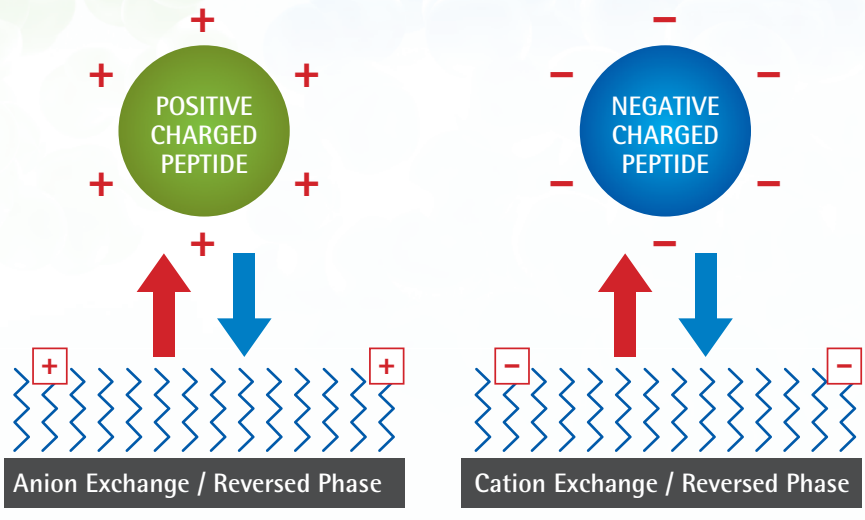
2. Determination of the pH of the mobile phase considered.

3. If the difference pI-pH is positive (pI > pH) , choose a ZEOsphere® Anion exchanger/RP merged phase, or if the difference pI-pH is negative (pI < pH) choose a ZEOsphere® Cation echanger/RP merged phase.

4. If the mobile phase ionic strength is high, choose a highly doped surface, if the mobile phase ionic strength is low, choose a less doped surface.

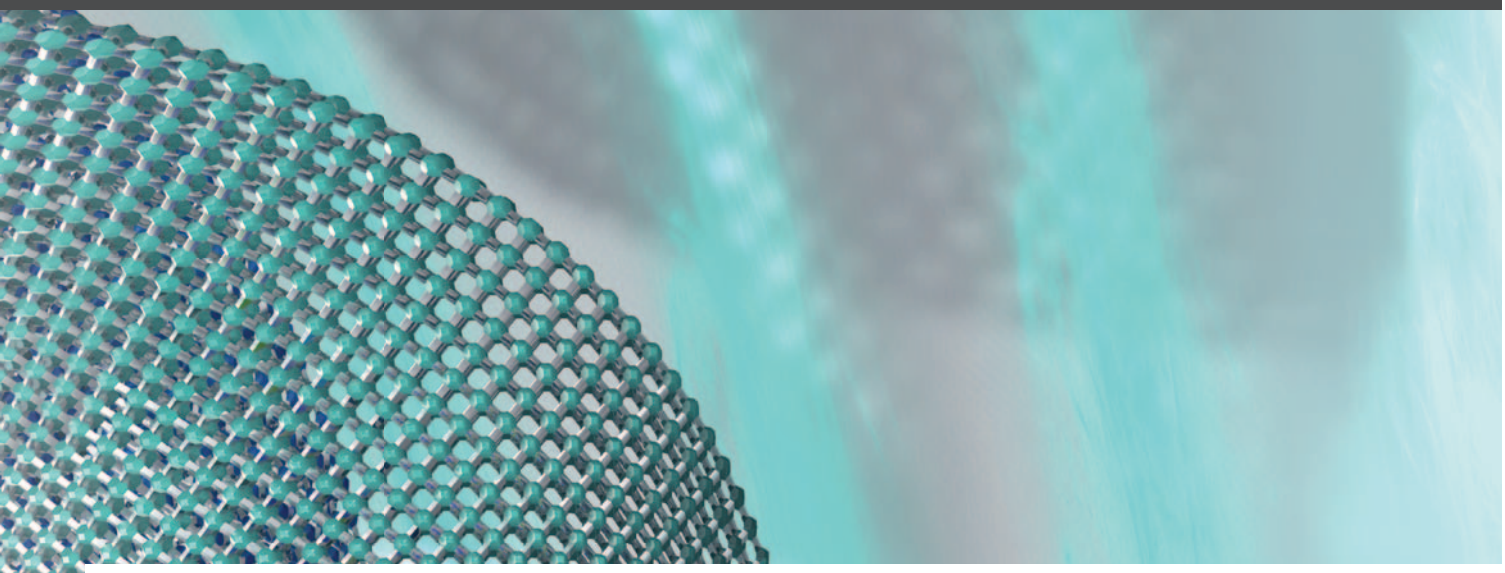
The ZEOCHEM Technology Center can assist you to find the best suitable Doped Reversed Phase. On request we can develop customized Doped Reversed Phases for your applications.

Process Development on the ZEOsphere® DRP



Mobile phase pH < pI, Peptide Net Charge = P
ZEOsphere® **Anion** exchanger / RP phase
- Repulsion on charged sites
- Adsorption on hydrophobic sites

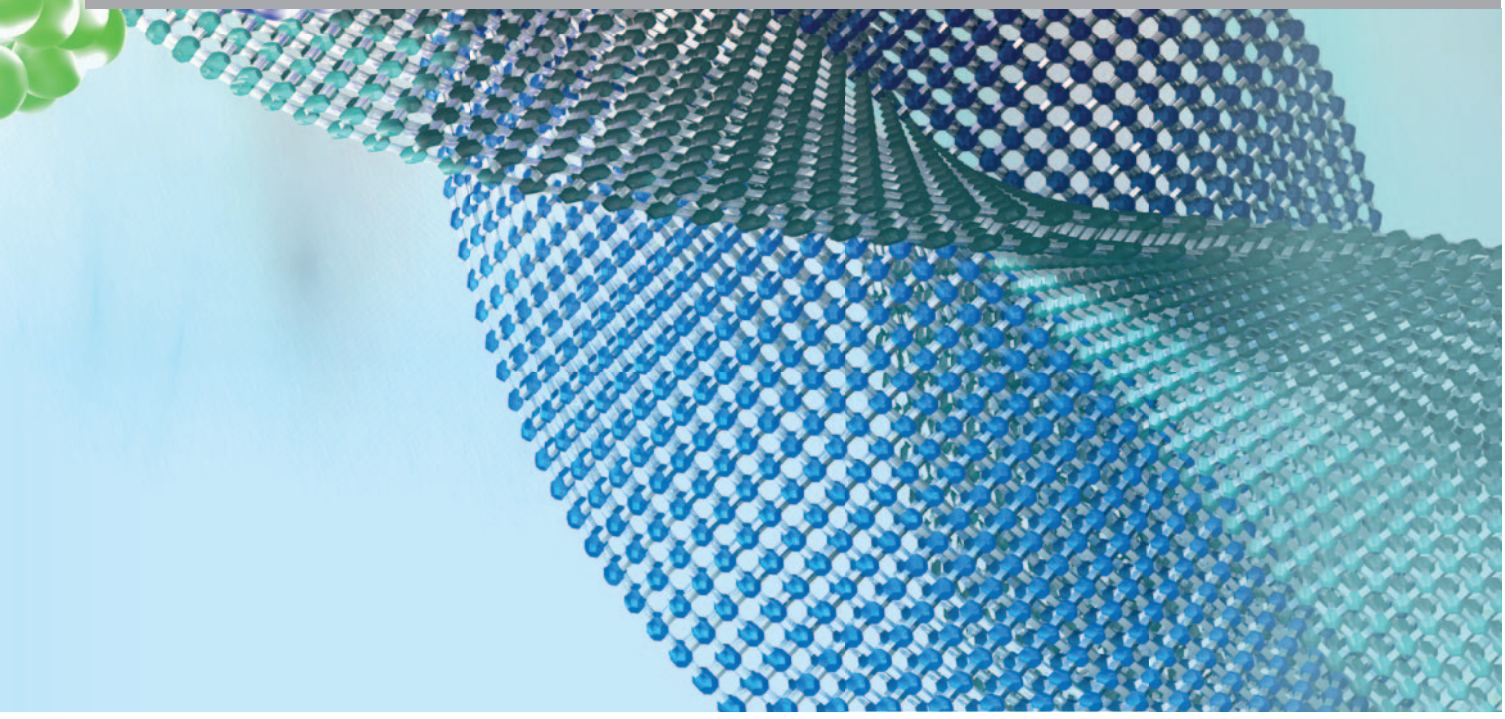
Mobile phase pH < pI, Peptide Net Charge = N
ZEOsphere® **Cation** exchanger / RP phase
- Repulsion on charged sites
- Adsorption on hydrophobic sites

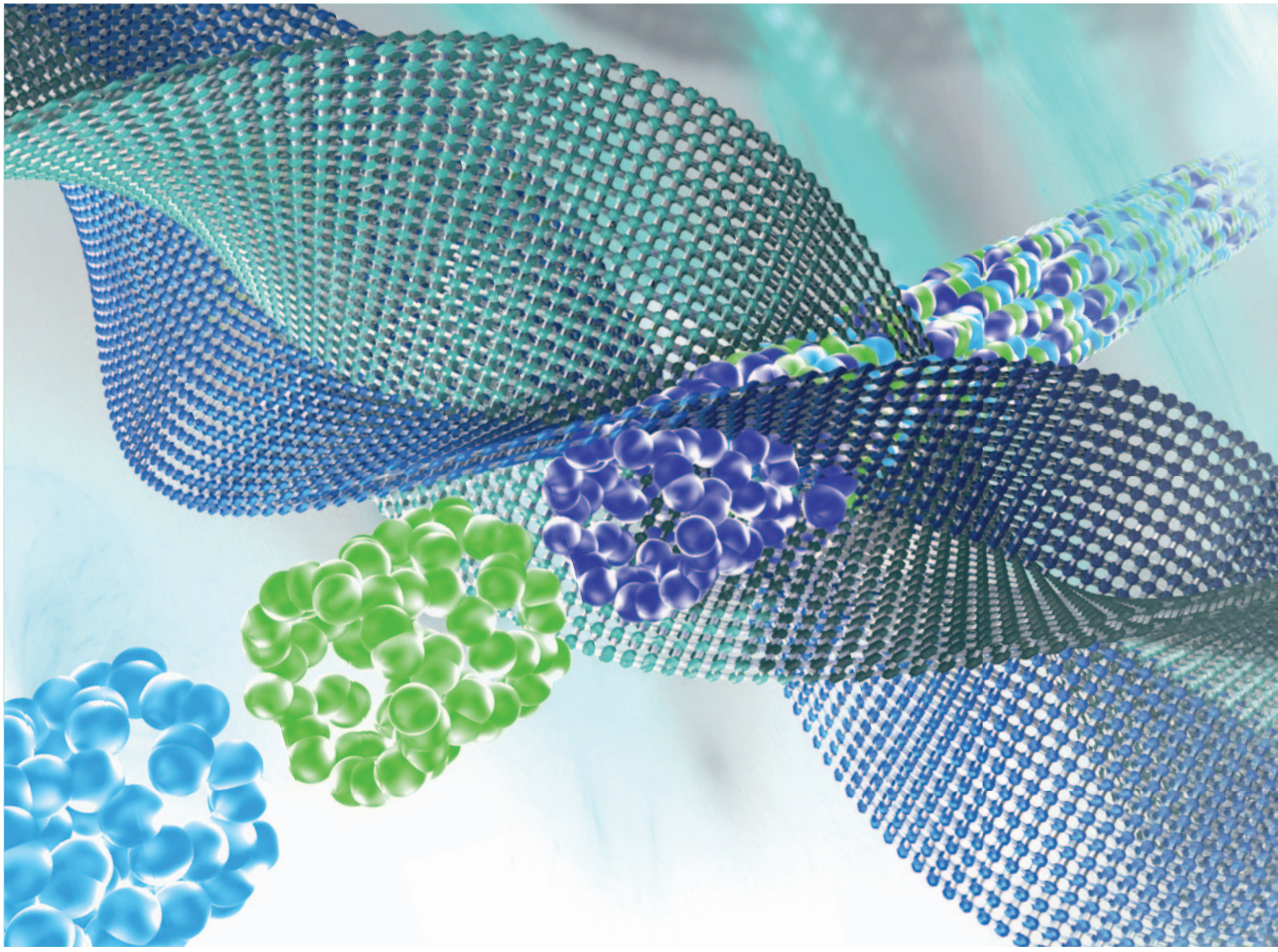


ZEOsphere® silica gels are produced by large batch scale emulsion processes, providing highly reproducible chromatographic separations of excellent quality.

The efficiency of our innovative ZEOsphere® silica gels rely on narrow particle size distributions, very high mechanical and chemical stability and a high specific surface area, to deliver consistent and reliable results.

We are constantly working on improving this process for one main goal:
Absolute Customer Satisfaction!





Zeochem AG
Seestrasse 108 · 8707 Uetikon am See · Switzerland
Phone +41 44 922 93 93 · Fax +41 44 920 20 93
info@zeochem.ch · www.zeochem.com

Zeochem L.L.C.
1600 West Hill Street · Louisville, KY 40210 · USA
Phone +1 502 634 7600 · Fax +1 502 634 8133
info@zeochem.com · www.zeochem.com

For your local agent or distributor please go to www.zeochem.com

ZEOCHEM®

cph **A Company of Chemie + Papier Holding AG**