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Cost Efficient Peptide Purification via ZEOsphere DRP Mixed-Mode Chromatography

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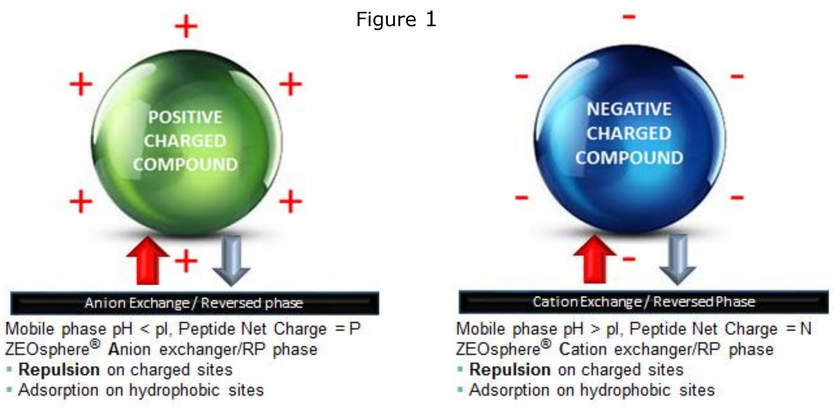
Introduction

ZEOsphere DRP are novel ion exchange / Reversed Phase[1,2,3] Mixed-Mode (HP(LC) stationary phases for the purification of especial peptides, insulins (analogues), oligonucleotides and other charged molecules. Utilizing both ion exchange and reversed phase ligands, a substantial increase in selectivity can be observed. Retention can easily be adjusted by changing organic modifier (changing the dielectric constant) or buffer salt concentrations. As a replacement for standard silica based reversed phase and or ion-exchange materials, this novel type of Mixed-Mode material has shown clear yield increases and substantial decreases in downstream production process costs. ZEOsphere DRP is available for analytical up to process scale applications.

ZEOsphere DRP Interaction Principle

Using ZEOsphere DRP in attractive-repulsing mode is an excellent way to maximize yield while keeping the purity requirements. Two different mechanism interact with the peptide (fig. 1). Hydrophobic effects are attractive (drive adsorption). Electrostatic effects are repulsive (lead to reduction of available interaction area).

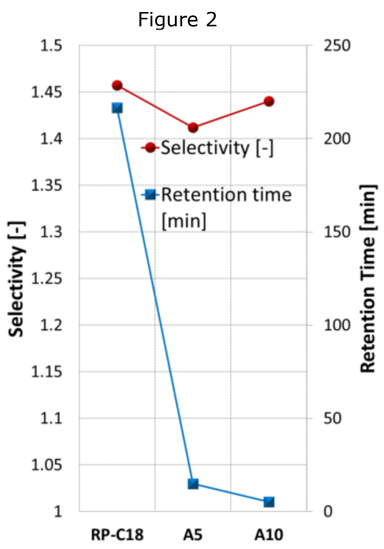
Figure 1



Faster retention, Improved Selectivity

Example: Selectivity between positive charged peptide Goserelin and critical impurity and retention time of main peak in sodium acetate buffer.

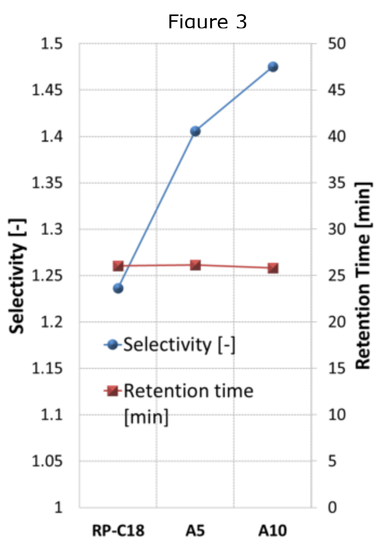
Figure 2



ZEOsphere DRP acts like RP, but….

- Repulsion by SAX or SCX groups.
- Faster retention due to less free surface area, caused by hinderance by repulsive IEX groups.
- Extended selectivity due to ability to work at lower [organic solvent] compared with RP → selectivity decreases along the gradient.

Figure 3



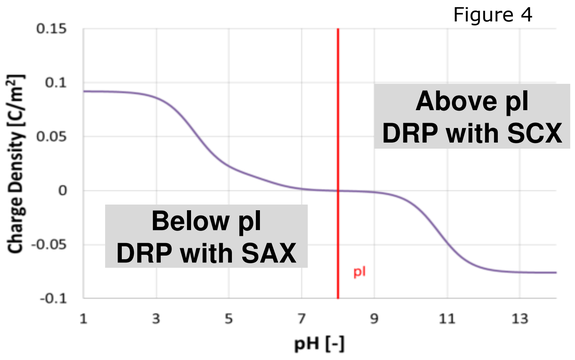
The [CH3CN] and [counterion] are constant for all columns/phases (30mM acetate, 17.6 v% CH3CN)

The retention time is fixed by changing the [CH3CN]. (30mM acetate, 19.8, 15.7, 11.1 v% CH3CN from RP to A10)

E.g. A10 = 10% SAX +90% RP; C10 = 10% SCX +90% RP

Application Ease – Method Development

Figure 4



1. Determination of the isoelectric point (pI) of the peptide of interest
   - pH below pI: compound is positive charged
   - pH above pI: compound is negatively charged
2. ZEOsphere DRP always works in buffered mobile phases. In principle the buffers ionic strength have to be equal.
3. Determination of the pH. Knowing the available pH range allows to understand and select the ionization state of the analyte in the particular mobile phase.
4. Best results in <u>attractive-repulsion</u> mode.
5. The selectivity can be optimized by e.g.: The organic modifier, counter ion and dopant concentration.

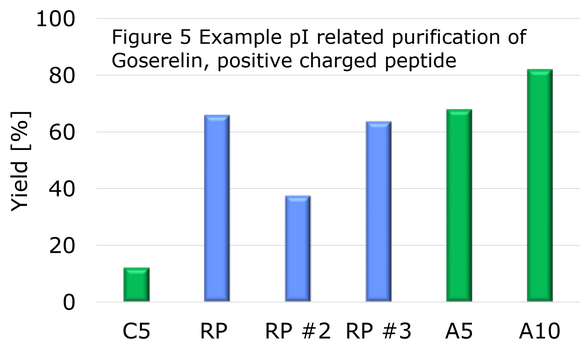


Figure 5 Example pI related purification of Goserelin, positive charged peptide

<u>Chromatographic conditions:</u> Loading: 5g/L of pure positive charged peptide. 300mM acetate buffer, pH 4.8, CH3CN gradient 0.5 vol%/min, T=25°C, required purity: 94%, E.g. A10 = 10% SAX +90% RP; C5 = 5% SCX +95% RP

Enhanced Yield and Efficiency

Example: One Step Purification of Liraglutide by EnzyPep (Preparative column 50x250mm)

EnzyPep Quality by Design.

- ✓ **Final Purity >99%, purification yield ±90%**
- ✓ Used media: ZEOsphere DRP 120 C5 / 10µm
- ✓ After lyophilization the acetate salt of Liraglutide is obtained, no re-salting step needed.
- ✓ The enzymatic reaction mixture was directly loaded on the preparative column.
- ✓ Working conditions equal to analytical → easy scale up.
- ✓ Internet published RP purification: Multiple step, Purity >99%, purification yield <60%

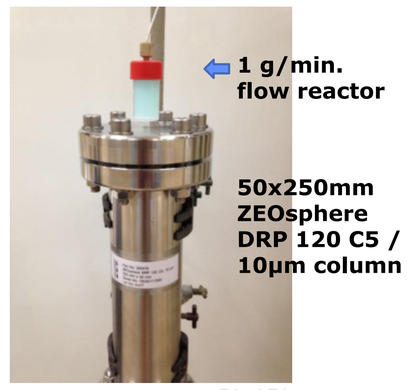


Figure 7 Preparative 50x250mm column with direct connected flow reactor

Costs Efficiency Liraglutide Purification

We can describe the production process costs efficiency as the total outcome between Purity versus Yield versus Loadability. With the ZEOsphere DRP Mixed mode it is possible to efficacious work on all 3 parameters at the same time.

In table 1 you see a summary of a production cost calculation model, developed with industry & university. In this costs calculation model we show the results of 3 real Liraglutide preparative purification solutions (a) based on 2-step C8 phase (competitor), (b) 1-step C18 (competitor) and (c) 1-step ZEOsphere DRP 120 C5 / 10µm (as previous described). In the showed summary the production operational parameters were kept the same. The differences in production costs are therefore only caused by selectivity and retention changes

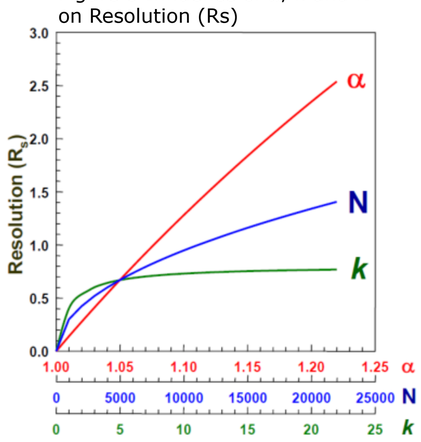
$$Rs = \frac{1}{4}\sqrt{N} \times \frac{\alpha - 1}{\alpha} \times \frac{k}{1+k}$$

Resolution = Efficiency x **Selectivity** x **Retention**

$$N = 16\left(\frac{t_r}{W_b}\right)^2 \qquad \alpha = \frac{k2}{k1} \qquad k = \frac{(t_r - t_0)}{t_0}$$

Selectivity (α) has the greatest impact on improving resolution (Rs).

Figure 8 Influence of α, k and N on Resolution (Rs)



Zhao, J.H. and P.W. Carr. Analytical Chemistry, (1999) 71, 2623-2632

Parameters costs calculation model

- Column: 25x60cm I.D.
- Silica usage: ± 40kgs
- Production cycle time: resp. 132hrs; 108hrs; 96hrs
- Loading: 3 g/L (competitor data)

With crude costs

	2-step C8	1-step C18	1-step DRP
Forecasted Total Campaign Cost (kCHF)	**4317**	**4567**	**2784**
Forecasted Campaign Unit Cost (CHF/g)	**431.7**	**456.7**	**278.4**
Forecasted Campaign Cost (kUSD)	**4317**	**4567**	**2784**
Forecasted Campaign Cost (kUSD/g)	**431.7**	**456.7**	**278.4**
Total Yield (%)	**54.0**	**45.0**	**90.0**

Table 1 Production costs comparison of purification solutions (a) 2-step C8 (competitor), (b) 1-step C18 (competitor) and (c) 1-step ZEOsphere DRP

up to 39 % less costs!

Conclusion

The mostly 1-step ZEOsphere DRP separation shows a substantial better selectivity, leading a to higher yield, lower organic solvent consumption(**greener** separations) and higher throughput. ZEOsphere DRP is an excellent way to lower production costs.

More information Juergen.Machielse@zeochem.com

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[1] R. Khalaf et al, *J. Chromatogr. A* **1397** (2015) 11-18, [2] R. Khalaf et al, *J. Chromatogr. A* **1407** (2015) 169-175, [3] N. Forrer et al WO2013143012 A1 (2013)