

Efficient peptide-protein purification by flash chromatography based on silica gel—Effect of pore size and hydrophobicity in reversed phase chromatography

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Introduction

Reversed phase silica gel is a potential approach for purification of macromolecules such as proteins and peptides. Based on the molecular weight of these molecules, it is important to select the right pore diameter with the suitable chemistry. A wrong pore size gives a poor chromatography process and not enough resolution using flash chromatography.

The small organic molecules are retained by a distribution and an adsorption-desorption (on-off) mechanisms. On the other hand, peptides and proteins are separated by the latter exclusively. Due to this mechanism, the pore size plays a key role in determination of resolution and loading amount in separation of peptides and proteins.

In this study, the retention mechanism is evaluated on reversed phase conditions with three different ligands: C2, C4 and Cyclohexyl (with 120, 300 and 1000 Å as pore size). The support is a 15 µm particle size spherical silica gel. The importance of the pore size is shown versus the efficiency of the flash cartridges by injecting different masses. Finally, the optimization method for purification of large molecules is discussed in order to get better resolution by flash purification of macromolecules. The efficiency of the column (N) is a good parameter to evaluate the SiliaSep™ cartridges performance.

Experimental

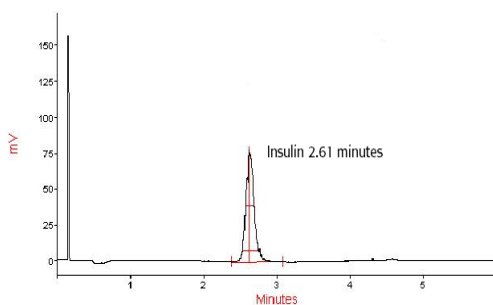
In order to get a good pathway of efficiency for proteins and peptides purification, the following macromolecules are evaluated in this study:

- Insulin from bovine pancreas MW: 5700
- BSA (Albumin from bovine serum) MW:67000
- Apoferritin from equine spleen MW:481000 (marker)

The cartridges used are SiliaSep™ (4g) in each chemistry (120, 300 and 1000 Å). The chromatographic system is a Biotope™ SP4 apparatus model with UV detection (220 nm). The mass injected range for each molecule is: 0.2-1.5 mg.

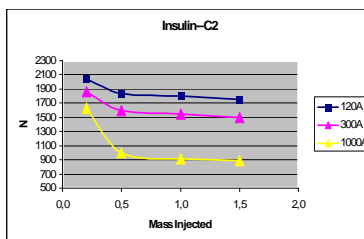
The elution for the protein-peptide is done with a typical mobile phase such as ACN and water that both contains 0.1% TFA. The gradient is 90% ACN (0.1% TFA) to 10% in 5 minutes and one minute isocratic 10% ACN (0.1% TFA). The total run time is 6 minutes at a flow rate of 16 mL/min.

Typical Chromatogram—Insulin (Cyclohexyl 15µm, 120Å)

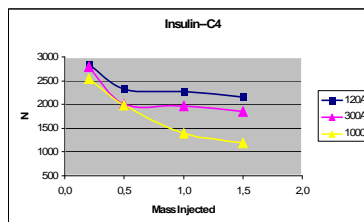


Impact of Pore Size Efficiency—Insulin

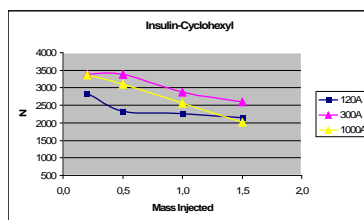
(Efficiency versus Mass Injected for C2)



(Efficiency versus Mass Injected for C4)

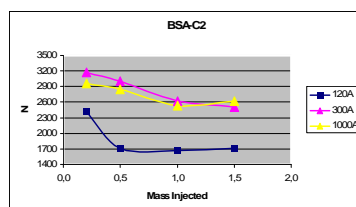


(Efficiency versus Mass Injected for Cyclohexyl)

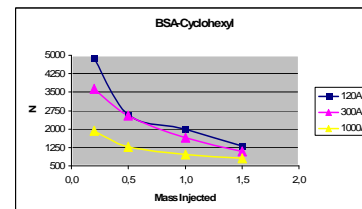


Impact of Pore Size Efficiency---BSA

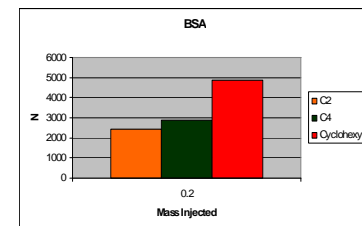
(Efficiency versus Mass Injected for C2)



(Efficiency versus Mass Injected for Cyclohexyl)



Impact of Chemistry with BSA (15µm 120Å)



Conclusion

This work illustrates the use of the flash chromatography technique for the purification of proteins-peptides. The SiliaPrep™ cartridges C2, C4 and Cyclohexyl (120, 300 and 1000 Å, 15 µm) are a powerful tool for analytical chemists in the purification of macromolecules. Based on the results, the smaller MW molecule are better separated with 120Å pore size using a ligand such as C2 and C4. But the best ligand for all loading amounts is the Cyclohexyl phase (N higher than 2000 for all pore sizes).

For higher MW such as BSA, the 120Å is not appropriate in C2 (and also C4). In C2 chemistry, the 300 and 1000Å are the best pore size. However, Cyclohexyl ligand with 120Å pore size gives very nice efficiencies especially for the lower mass injected.

Most chromatographers use C2 and C4 for purifying proteins and peptides. In this paper, we have demonstrated that Cyclohexyl has more advantages for this chromatographic application. The pore size played an important role in the mechanism of interaction with a certain limit. However, the ligand on the gel also played an important role in achieving efficient separation. Appropriate hydrophobicity is critical for good separation.

Finally it is necessary to combine, with the appropriate pore size: hydrophobicity and particle size, to achieve higher chromatographic resolution of peptides and proteins. Large scale purification for macromolecule is now possible with SiliaPrep™ and flash chromatography.

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