

One Size Does Not Fit All: Exploring the Relationship Between Pore Size and Separation Efficiency

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Introduction

Choosing the correct column is fundamental to a successful separation. Column selection is often centered around simple identification of a stationary phase to magnify resolution within certain classes of compounds. While gains in resolution are most affected by selectivity, enhancements can still be made with an intentional selection of the packing material's microstructure. This variable is often overlooked at the expense of potentially significant additional performance gains. Unrestricted mass transfer within the pores of the particle will minimize band broadening associated with hindered partitioning. Increased efficiency, especially when operating at above optimum flow rates, can be gained if the pore size is significantly larger than the solvated analyte. Moreover, reduced access to pores, due to physical hindrance, will limit retention as the majority of surface area and bonded phase exists within the particle.

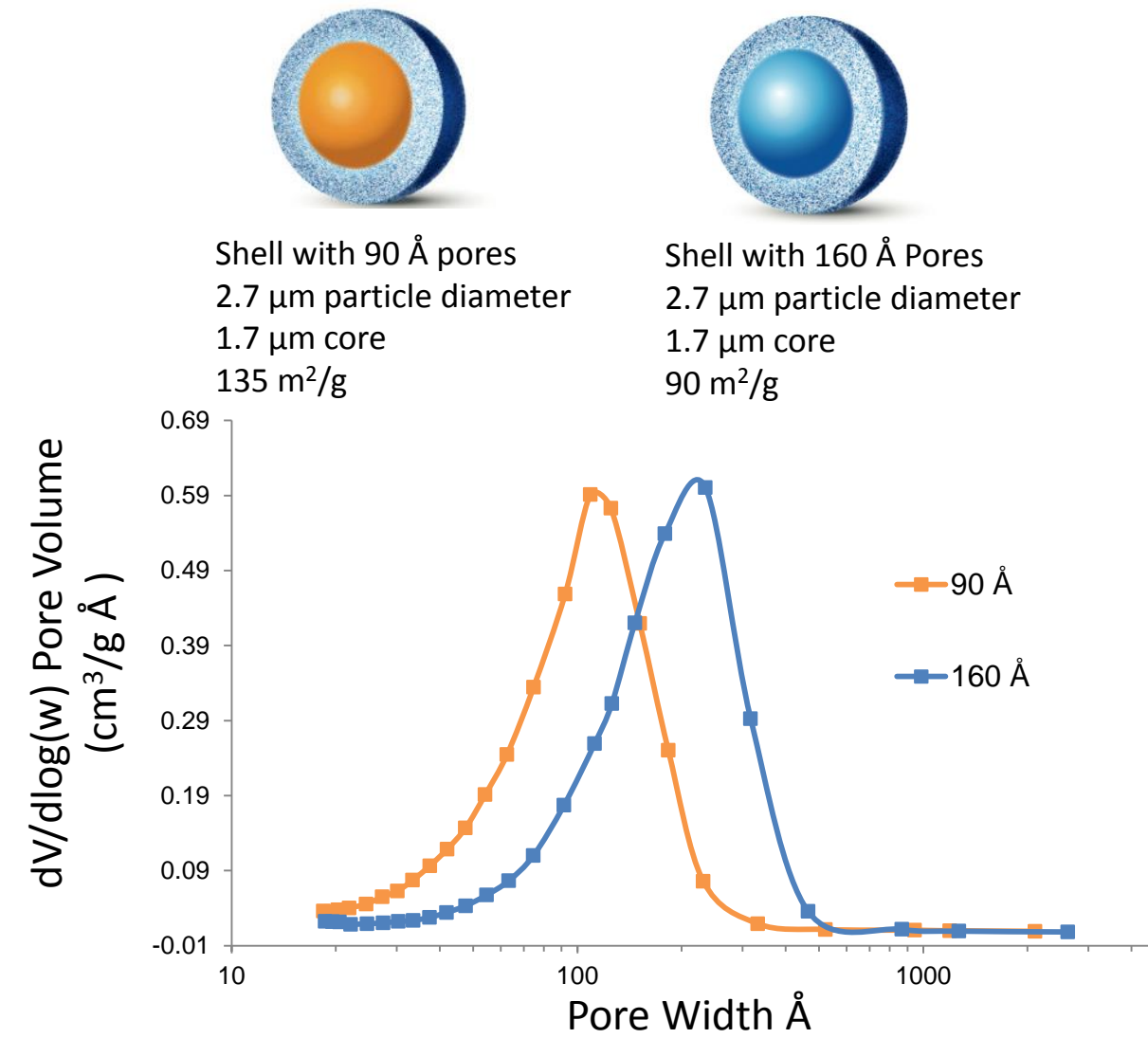
Experimental Conditions and Considerations

Columns: HALO® 90 Å Phenyl-Hexyl, 2.7 µm, 2.1 x 50 mm and HALO® 160 Å Phenyl-Hexyl, 2.7 µm, 2.1 x 50 mm
 Instrument: Nexera X2
 Temperature: 60 °C
 Flow Rate: 0.05 to 1.75 mL/min
 Mobile Phase: Premixed and pumped through a single pump

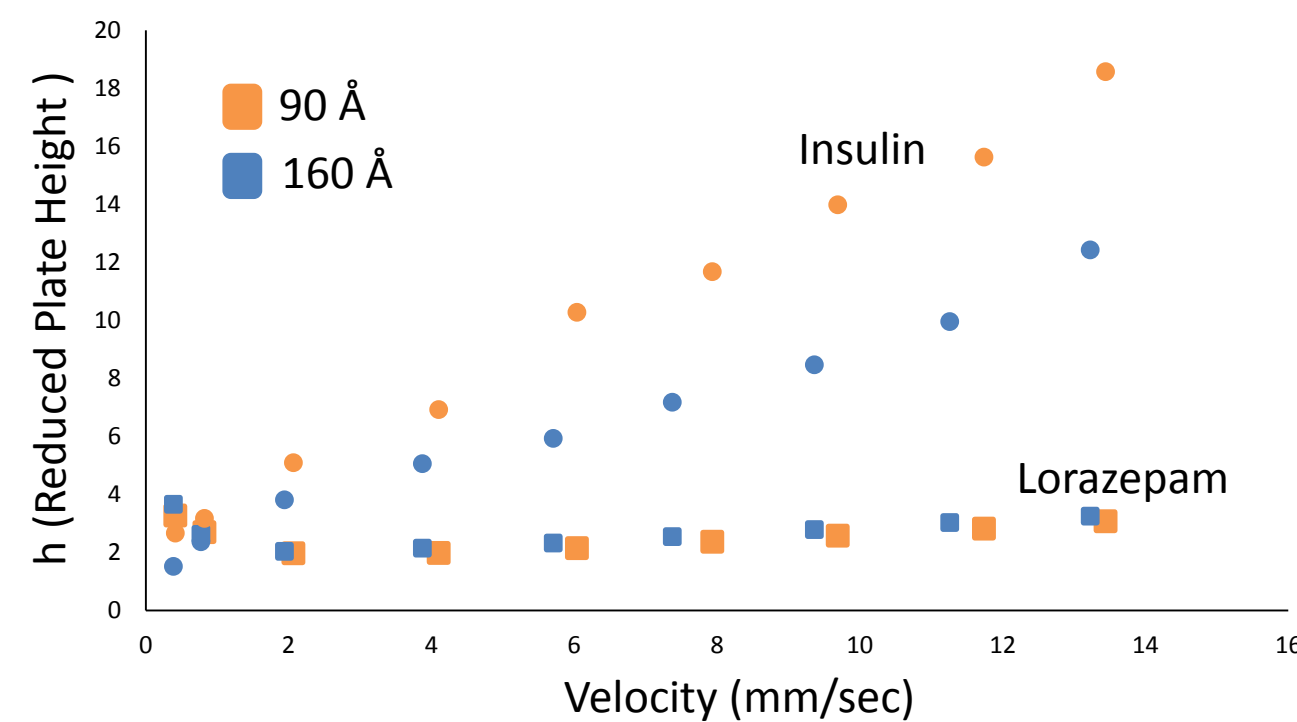
Analyte	MW	Mobile Phase (in water)
Naphthalene	128	50% Acetonitrile
Lorazepam	321	30% Acetonitrile
Angiotensin 1-12	1509	30% Acetonitrile + 0.1% TFA
Bombesin	1619	21% Acetonitrile + 0.1% TFA
Insulin Chain B Oxidized	3496	28% Acetonitrile + 0.1% TFA
Insulin	5777	30% Acetonitrile + 0.1% TFA
Ribonuclease A	13700	22% Acetonitrile + 0.1% TFA

- For direct comparison, columns with identical bonded chemistry were chosen. The bonding chemistry, Phenyl-Hexyl, allows for traditional reverse-phase analysis and minimal loss in pore volume due to stationary phase
- van Deemter analyses were conducted to compare both the effects on retention and efficiency when pore size is changed
- Gradient analysis was conducted to look at separation window across a range of MWs and overall peak width
- Molecules were chosen between 100 and 14000 MW
- As molecular weight (MW) increases the diffusion coefficient of a molecule is reduced significantly. Thus, typical van Deemter fit analysis is limited to probing the C-term region.
- Line of best fit for the C-term region (from the plot of reduced plate height $h = (H(\mu\text{m})/d_p(\mu\text{m}))$) as a function of velocity (mm/sec) is used for comparison
- Velocity is calculated from the column length divided by the elution of an unretained marker

HALO® Superficially Porous Particle Characteristics

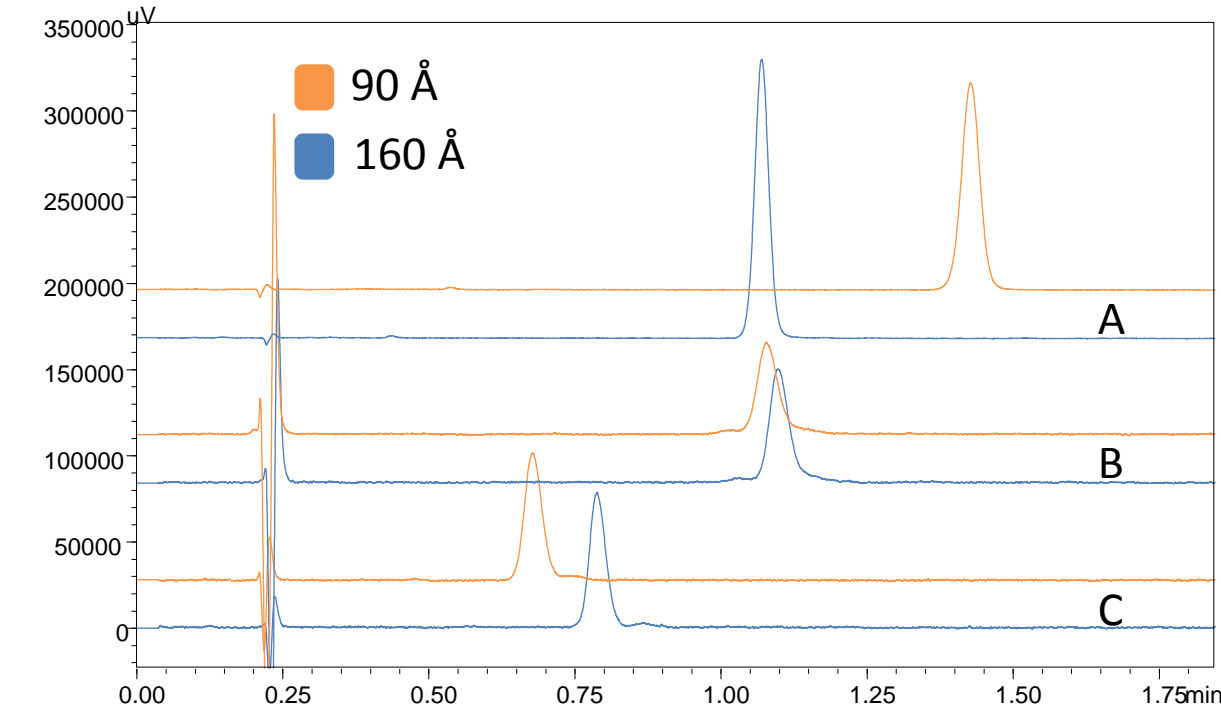


Exemplary Data



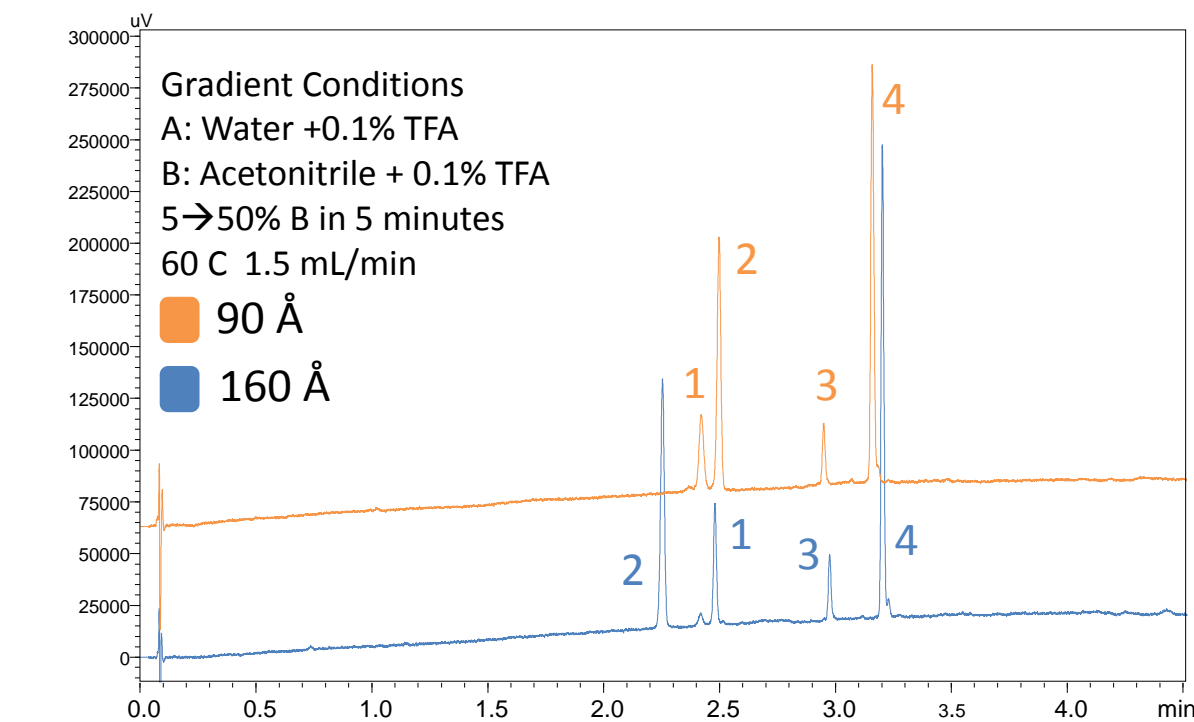
Plot of reduced plate height vs velocity for Insulin and Lorazepam. Nearly identical performance is seen for lorazepam (321 MW) while the 160 Å column shows distinct advantages for the larger molecule Insulin (5777 MW).

Isocratic Comparison



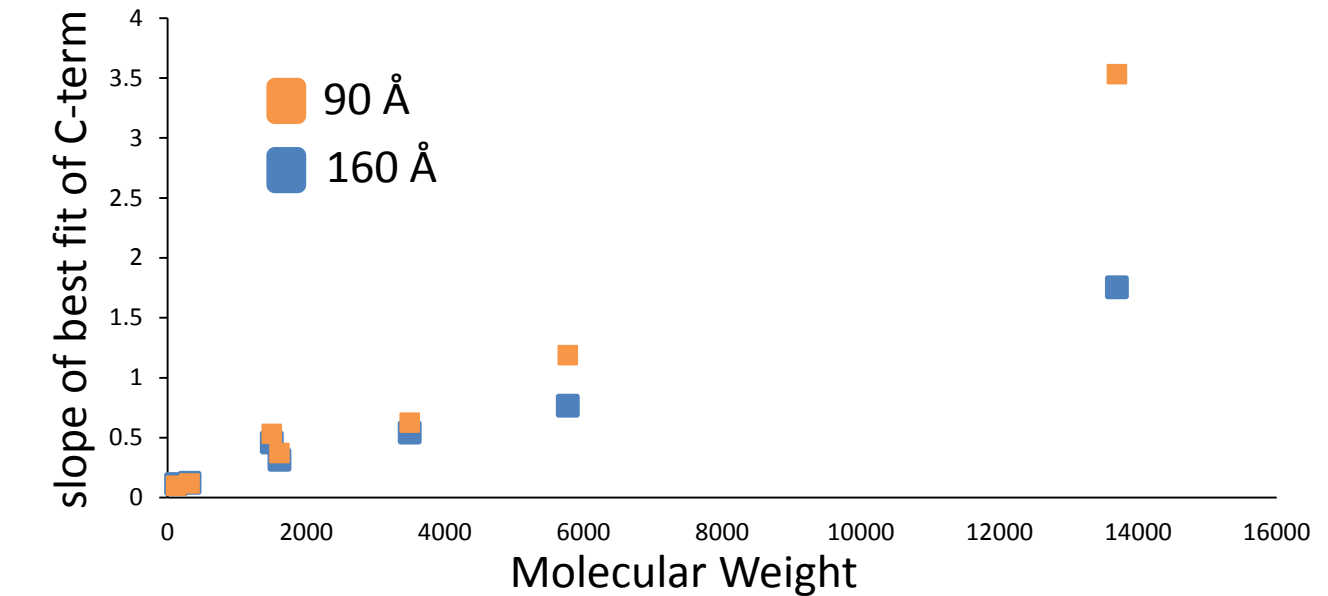
Analytes: (A) Lorazepam (321 MW), (B) insulin chain B oxidized (3496 MW) and (C) Insulin (5777 MW). Conditions: 0.5 mL/min (≈4 mm/sec). Retention order and performance shifts as MW increases and analytes are restricted from stationary phase in smaller pore materials.

Gradient Comparison



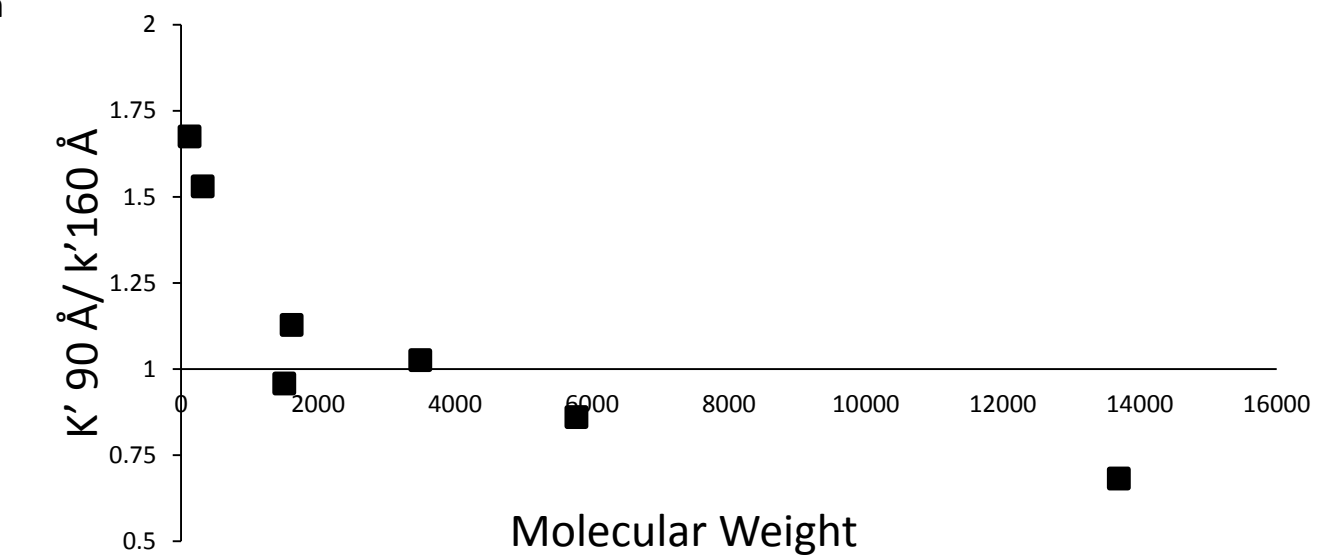
	MW	90 Å			160 Å		
		Peak #	Ret Time	USP Width	Peak #	Ret Time	USP Width
Ribonuclease A	13700	1	2.421	0.038	2	2.480	0.023
Lorazepam	321	2	2.497	0.030	1	2.254	0.030
Insulin Chain B Oxidized	3496	3	2.949	0.022	3	2.976	0.023
Insulin	5777	4	3.160	0.025	4	3.204	0.022
Average Peak Width		0.029			0.025		
Separation Window		0.739			0.950		
Peaks/Window		26			39		

90 Å/160 Å C-Term Region Fit



Plot of the slope of the best fit for the C-term vs MW. Nearly identical performance is seen at very low MW. As MW increases performance differences become exaggerated. Deviation begins to occur at a MW < 4000.

90 Å/160 Å Retention Comparison



Plot of the ratio of k' (retention factor) of analytes on the 90 Å pore column over the 160 Å pore column. Where retention factor is equal the ratio is one. This value indicates where analytes roughly experience an identical amount of stationary phase retention. This region also indicates where a larger pore column should be used.

Conclusion

- Selection of pore size can increase the efficiency of separation
- Clear increase in C-term as analyte size increases, exacerbated when smaller pore materials are used with larger MW analytes
- Larger pore materials mitigate performance loss
- Retention properties transition from surface area dominated to exclusion dominated as MW increases
- The effects of pore size are clear under gradient conditions with samples that widely vary in MW.