Increase Column Performance and Lifetime in Peptide and Protein Purification using Aggressive Wash Conditions on the Ultra-Stable Gemini® Sorbents

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Gemini sorbents were specifically developed for chromatographic conditions that demand a wide pH range (pH 1-12). In this application, we demonstrate how the high pH stability of the Gemini product line can be used to extend column lifetime by column regeneration using aggressive alkaline washes (1 M sodium hydroxide in alcohol 1:1 (V/V)). Column regeneration using caustic washes is especially useful in the case of peptide and protein applications where aggregation and impurities tend to accumulate and generate high backpressures. Combining the high pH stability of Gemini particles with the award winning Axia™ pre-packed preparative column will provide longer lifetime and higher performance for the preparative purification of peptide and proteins.

Introduction

In preparative chromatography, bulk media sorbents or pre-packed columns are typically replaced when washing procedures are no longer able to adequately restore column performance or when backpressure remains high. There are many causes for performance degradation and backpressure build-up including incomplete removal of precipitated compounds, strongly retained sample components and dissolution or other degradation of the sorbent. Typical reversed phase silica-based sorbents are usually limited to standard wash conditions of acetic acid, methanol and low concentrations of sodium hydroxide. The use of more aggressive wash conditions offers significant improvements in the cleaning / washing effectiveness but is limited to the chemical pH stability of the reversed phase chromatographic media.

Polymeric sorbents are able to withstand higher concentrations of sodium hydroxide but they do not possess the needed selectivity or efficiency to make them competitive with silica-based sorbents. However, newer silica sorbents like Gemini offer increased selectivity, efficiency and extended pH stability. Gemini C18, Gemini C6-Phenyl and Gemini NX-C18 media are all compatible with a very aggressive wash condition such as sodium hydroxide solution (1 M NaOH in alcohol 1:1 (V/V)). During the final stage of Gemini TWIN™ Technology manufacturing, a unique silica-organic layer is grafted to create a completely new composite particle. Since the internal base silica is unaltered by this manufacturing process, the particle retains its mechanical strength and rigidity along with excellent efficiency for increased resolution, while the silica-organic shell protects the particle from chemical attack. The Gemini material thus allows the use of more effective wash conditions without compromising the integrity of the media.

By nature, peptides and proteins tend to aggregate and in some cases it becomes almost impossible to regenerate media under standard wash conditions as described above. Proteins such as insulin are prepared by recombinant techniques and contain poorly soluble materials that need to be washed and eliminated periodically from the purification media to maintain column performance. Alternatively, peptides/proteins produced by chemical synthesis contain multiple disulfide bridges. Quite often during folding, polymerization and scrambling may occur which can lead to the formation of impurities. These impurities can aggregate during purification under reversed phase conditions and clog the column inlet frit or bind to the media itself. One of the most common industry column regeneration approaches is to treat the media with strong basic conditions which removes performance damaging impurities from the column frit and/or packed media bed. With this washing technique the lifetime of columns packed with Gemini media can be greatly extended due to the sorbents outstanding chemical stability at of high concentrations of sodium hydroxide.

Materials and Methods

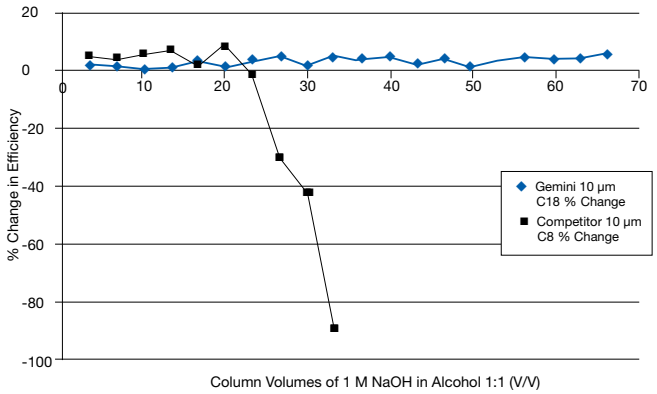
All chemicals and proteins used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents were purchased from EMD (San Diego, CA, USA). Gemini 10 µm C18 250 x 4.6 mm column was obtained from Phenomenex (Torrance, CA, USA) and 10 µm C8 250 x 4.6 mm column was purchased from competitor vendor.

All analyses and purifications were performed on an Agilent® 1100 HPLC system from Agilent Technologies (Santa Clara, CA, USA) equipped with quaternary pump, degasser, variable wavelength UV detector and autosampler. For stability testing experiments (**Figure 1**), the hydrophobic probe was naphthalene, the flow rate was set at 1 mL/min, temperature was ambient and UV detection was 254 nm. The mobile phase was a solution of 20 mM acetic acid in alcohol 1:1 (V/V). The caustic washes were performed under the same conditions of flow rate and temperature using 3.3 column volumes of 1 M NaOH in alcohol 1:1 (V/V). After the caustic washes were performed, the column was neutralized by passing 4 column volumes of 20 mM acetic acid in alcohol 1:1 (V/V). For the protein purification experiments (**Figure 2**), the protein was dissolved at 10 mg/mL, the injection volume was 5 mL, the flow rate was set at 1 mL/min, temperature was ambient and UV detection was set at 260 nm. The protein was purified using a 15-25 % alcohol in water gradient over 25 min. After three preparative protein purifications, the columns were washed and neutralized according to the procedure described previously for **Figure 1** experiments.

Results and Discussion

In the first series of media performance experiments under caustic wash conditions, we tested the effect of repeated wash cycles with a sodium hydroxide solution. The results for Gemini® C18 and a typical reversed phase silica-based sorbent are shown in **Figure 1**. The column efficiency as determined by the test probe was monitored after each washing cycle. As shown in **Figure 1**, the Gemini C18 media maintained a stable performance for >2x more caustic wash exposure than a typical reversed phase silica-based sorbent. Under strongly basic washing conditions, the typical silica-based sorbent started to dissolve creating voids in the column that resulted in significantly lower performance.

Figure 1.
Change in peak efficiency on Gemini C18 and a typical reversed phase silica-based sorbent during chemical stability testing with sodium hydroxide in alcohol.



In a second experiment, the chemical stability performance of Gemini C18 was also demonstrated with a small protein purification (MW < 10000 Da). A protocol similar to the one described in the first experiment was followed for this purification. In this case, we performed three preparative protein purifications followed by a column wash with 3.3 column volumes of sodium hydroxide solution. As represented in **Figure 2**, the Gemini C18 maintained constant fraction purity for >2x more purifications and caustic washes than the typical reversed phase silica-based sorbent. Purifications on the typical silica-based column were stopped due to excessive system backpressure during the washing steps. Once again, the high pH wash conditions caused the dissolution of the typical silica-based sorbent resulting in the formation of media fines which lowered purification performance and increased column/system backpressure.

Figure 2.
Purity of recovered protein with repeated purification and wash cycles using Gemini C18 and a typical reversed phase silica-based column. Purifications on the competitor column were stopped due to excessive backpressure during wash step (see **Figure 3**).

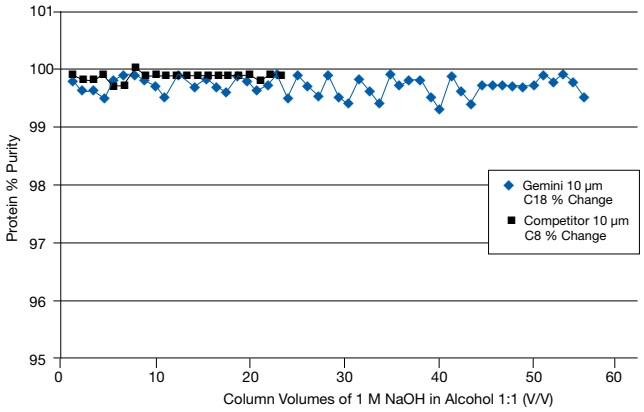
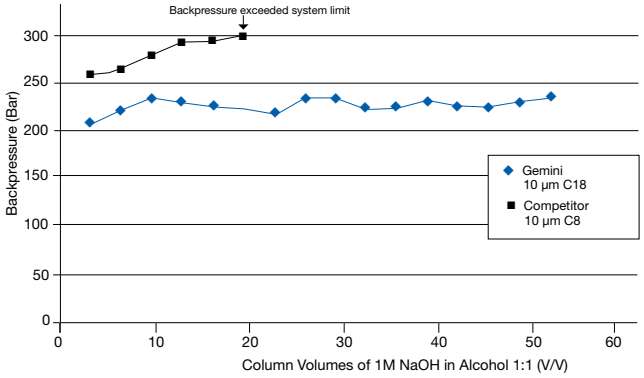


Figure 3 shows the backpressure comparison of the two columns. Gemini C18 shows consistent chromatographic performance without backpressure increases under aggressive wash conditions. After 20 column volumes of caustic wash, the competitor column exceeded system pressure limits while the Gemini C18 backpressure remained stable even after more than 50 column volumes of aggressive caustic wash.

Figure 3.
Maximum column backpressure during sodium hydroxide washes between protein purification cycles



Conclusion

In this technical note, we report that Gemini sorbents are compatible with aggressive caustic wash conditions typically reserved for polymeric sorbents. The superior chemical stability of the Gemini product line in aggressive wash conditions translates into an increased number of purification runs before column replacement is needed. Combining Gemini media with Axia™ packed preparative HPLC columns will provide longer column lifetime and higher performance preparative purifications of peptides and proteins.

APPLICATIONS

Ordering Information

	Axia™ Column	SecurityGuard™ Cartridge
Phases	**Part No.**	**Part No.**
50 x 21.2 mm		
Gemini® 5 µm C18	00B-4435-P0-AX	AJ0-7846
Gemini 10 µm C18	00B-4436-P0-AX	AJ0-7846
Gemini 5 µm C6-Phenyl	00B-4444-P0-AX	AJ0-9157
Gemini 5 µm NX-C18	00B-4454-P0-AX	AJ0-8370
Gemini 10 µm NX-C18	00B-4455-P0-AX	AJ0-8370
100 x 21.2 mm		
Gemini 5 µm C18	00D-4435-P0-AX	AJ0-7846
Gemini 10 µm C18	00D-4436-P0-AX	AJ0-7846
Gemini 5 µm C6-Phenyl	00D-4444-P0-AX	AJ0-9157
Gemini 5 µm NX-C18	00D-4454-P0-AX	AJ0-8370
Gemini 10 µm NX-C18	00D-4455-P0-AX	AJ0-8370
250 x 21.2 mm		
Gemini 5 µm C18	00G-4435-P0-AX	AJ0-7846
Gemini 10 µm C18	00G-4436-P0-AX	AJ0-7846
Gemini 5 µm C6-Phenyl	00G-4444-P0-AX	AJ0-9157
Gemini 5 µm NX-C18	00G-4454-P0-AX	AJ0-8370
Gemini 10 µm NX-C18	00G-4455-P0-AX	AJ0-8370
50 x 30 mm		
Gemini 5 µm C18	00B-4435-U0-AX	AJ0-8308
Gemini 10 µm C18	00B-4436-U0-AX	AJ0-8308
Gemini 5 µm C6-Phenyl	00B-4444-U0-AX	AJ0-9158
Gemini 5 µm NX-C18	00B-4454-U0-AX	AJ0-8371
Gemini 10 µm NX-C18	00B-4455-U0-AX	AJ0-8371
100 x 30 mm		
Gemini 5 µm C18	00D-4435-U0-AX	AJ0-8308
Gemini 10 µm C18	00D-4436-U0-AX	AJ0-8308
Gemini 5 µm C6-Phenyl	00D-4444-U0-AX	AJ0-9158
Gemini 5 µm NX-C18	00D-4454-U0-AX	AJ0-8371
Gemini 10 µm NX-C18	00D-4455-U0-AX	AJ0-8371
250 x 30 mm		
Gemini 5 µm C18	00G-4435-U0-AX	AJ0-8308
Gemini 10 µm C18	00G-4436-U0-AX	AJ0-8308
Gemini 5 µm NX-C18	00G-4454-U0-AX	AJ0-8371
Gemini 10 µm NX-C18	00G-4455-U0-AX	AJ0-8371
50 x 50 mm		
Gemini 5 µm C18	00B-4435-V0-AX	—
Gemini 10 µm C18	00B-4436-V0-AX	—
Gemini 10 µm NX-C18	00B-4455-V0-AX	—
100 x 50 mm		
Gemini 10 µm C18	00D-4436-V0-AX	—
Gemini 5 µm C6-Phenyl	00D-4444-V0-AX	—
Gemini 5 µm NX-C18	00D-4454-V0-AX	—
Gemini 10 µm NX-C18	00D-4455-V0-AX	—
250 x 50 mm		
Gemini 10 µm C18	00G-4436-V0-AX	—
Gemini 5 µm NX-C18	00G-4454-V0-AX	—
Gemini 10 µm NX-C18	00G-4455-V0-AX	—

* SecurityGuard cartridges require SecurityGuard PREP Holder
For columns with Internal Diameter (ID) 18.0- 29.0 mm, use holder: AJ0-8223
For columns with Internal Diameter (ID) 30.0- 49.0 mm, use holder: AJ0-8277

Bulk Media

Phases	100 g	1 kg
Gemini 10 µm C18	04G-4436	04K-4436

guarantee

If Axia™ packed columns do not provide at least an equivalent separation as compared to a competing preparative column of the same particle size, same phase, and dimensions, return the column with comparative data within 45 days for a FULL REFUND. Only applies to 21.2mm ID columns.

APPLICATIONS

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