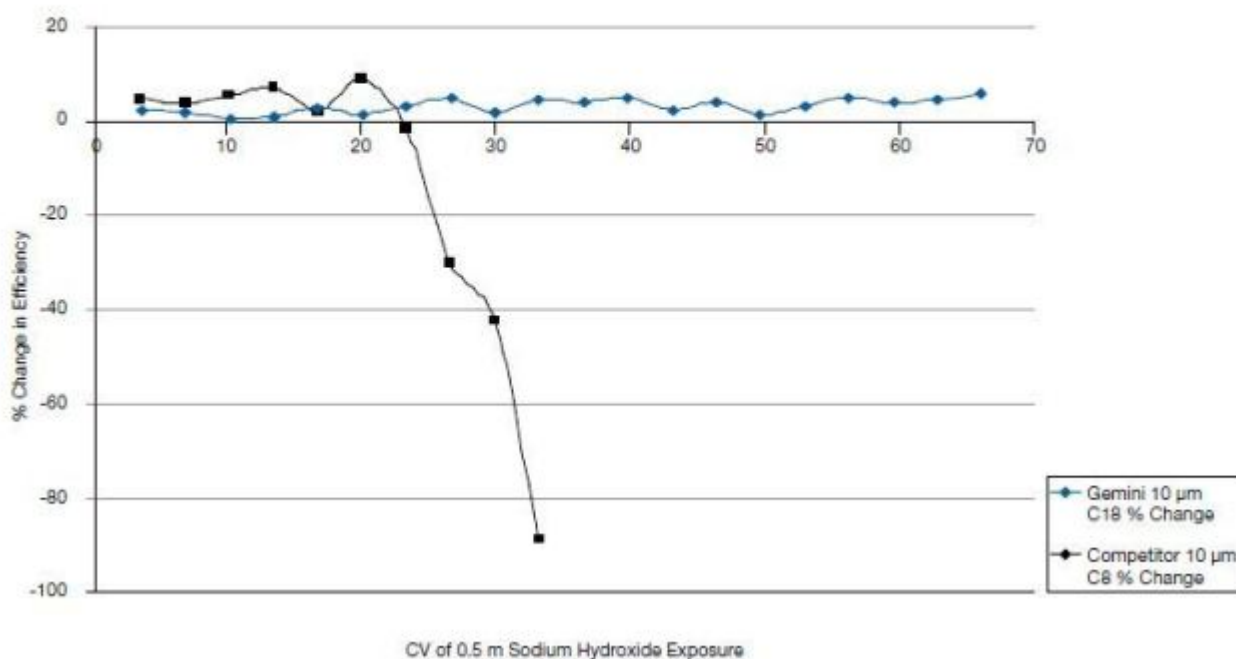


A significant cost in **preparative purification processes** is down time during sorbent replacement. Sorbents are typically replaced when washing procedures no longer restore adequate performance or when backpressure remains high. There are many causes for performance degradation and backpressure buildup including incomplete removal of precipitated or strongly retained sample components and dissolution or other degradation of the sorbent. Current reversed phase, silica-based sorbents are typically limited to 0.1M NaOH for washing.

The use of more aggressive wash conditions can improve the wash effectiveness but the chemical stability of the chromatographic media limits the conditions that can be used. Polymeric sorbents can withstand higher concentrations of NaOH but do not possess the needed selectivity to make them competitive. However, **Gemini® C18** preparative media is compatible with very aggressive wash conditions such as 0.5M NaOH in 50 v/v% alcohol and provides selectivity equivalent to current reversed phase, silica based sorbents. The Gemini material thus allows the use of much more effective wash conditions without compromising the integrity of the media.

One of the largest commercial chromatographic purifications today is used for insulin purification. With the increasing incidence rate of diabetes around the globe the demand for **insulin** is expected to significantly increase in the near future. Increasing sorbent lifetime will allow increased production to meet this need. Therefore, the outstanding compatibility of Gemini C18 with very aggressive wash conditions could yield substantial production economy improvements. The effect of repeated exposure to 0.5M NaOH in alcohol wash cycles on the chromatographic performance of Gemini C18 and a current reversed phase, silica-based sorbent is shown in **Figure 1**.

Figure 1. Change in peak efficiency on Gemini C18 and competitor reversed phase silica-based sorbents during stability testing with 0.5 M Sodium Hydroxide in alcohol.



The stability test was conducted by making four replicate injections of a neutral hydrophobic probe using alcohol/10mM acetic acid mobile phase followed by exposure to 3.3 CV of 0.5M NaOH in alcohol. The efficiency of the test probe was monitored as this cycle of analysis followed by caustic washing was repeated. As shown in **Figure 1**, Gemini C18 maintained stable performance for >2x more caustic wash exposure than a current reversed phase, silica based sorbent.

The outstanding performance stability of **Gemini C18** was also demonstrated with insulin purification. A protocol similar that used above was used for insulin purification except 3 preparative purifications of insulin were performed before the column was washed with 3.3CV 0.5M NaOH in alcohol. As shown in **Figure 2**, Gemini C18 maintained constant fraction purity for >2x more purifications and washes than the competitor column.

Purifications on the competitor column were stopped due to excessive pressure during the wash step. **Figure 3** shows the wash backpressure on Gemini C18 and the competitor column. Gemini shows consistent chromatographic performance without backpressure increases with these very aggressive wash conditions.

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

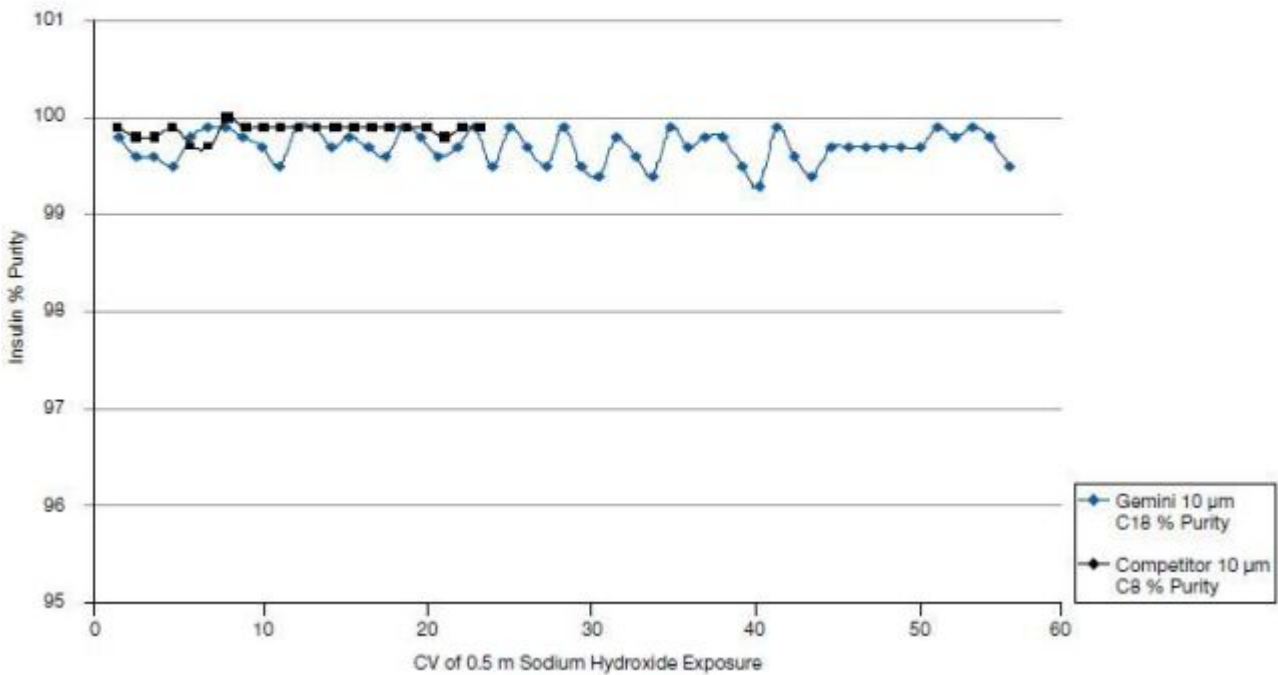
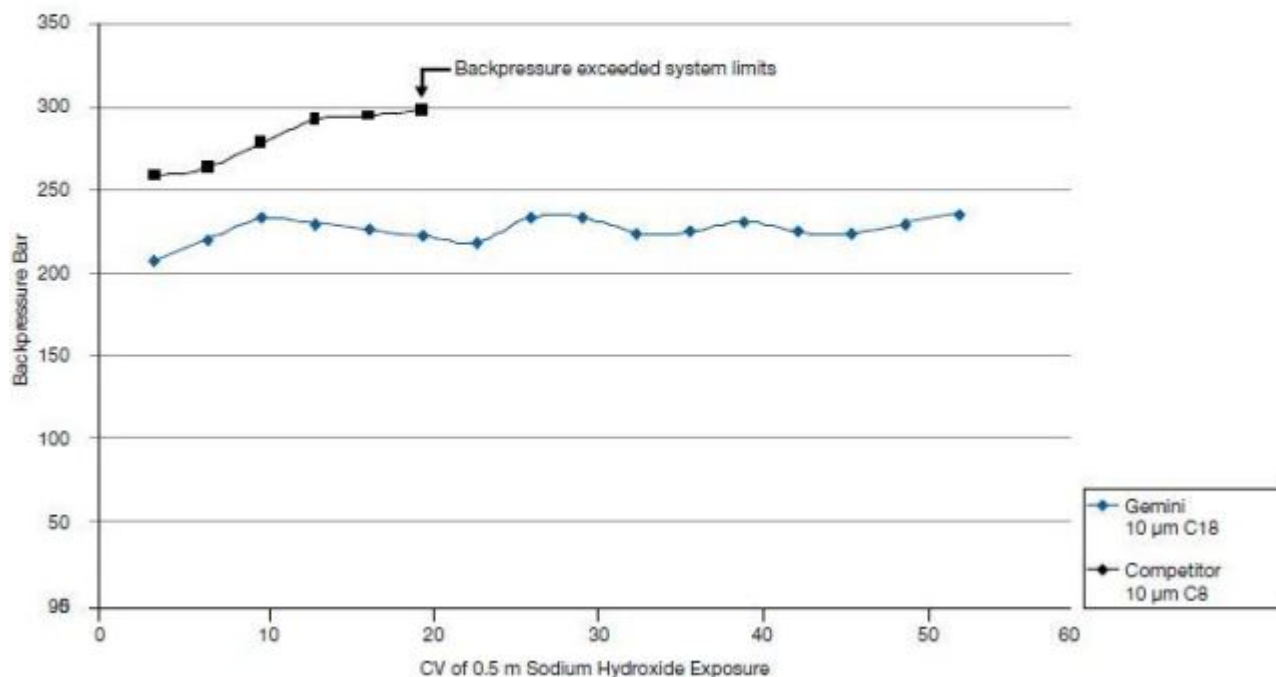


Figure 3. Maximum column backpressure during 0.5 M Sodium Hydroxide washes between insulin purification cycles. After 20 column volumes of caustic, the competitor column exceeded system pressure limits while Gemini backpressure remained stable after 53 column volumes.



Gemini C18 preparative media, therefore, provide the best of both worlds, compatibility with wash conditions typically reserved for polymeric sorbents with selectivity and loadability equivalent to current silica-based, reversed phase sorbents. The superior chromatographic performance of Gemini using very aggressive wash conditions should translate into significant increases in the number of purifications before sorbent replacement is needed and, therefore, greatly improved production economy.

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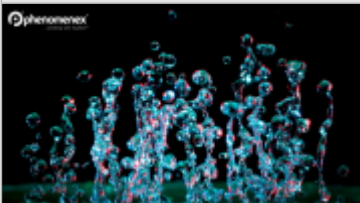
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Summary



Article Name

Increase Production Economy Using Aggressive Wash Conditions

Description

Read about the best methods on how to increase production economy in your lab by using a much more aggressive wash conditions method.