DuPont Water Solutions
Life Sciences and Specialties

pH stability of DuPont™ AmberChrom™ Chromatography Resins

Introduction

There are two main types of reverse phase resins – polymeric and silica – and both are used in peptide purification. While both resin families show excellent resolution in peptide separations, polymeric resins have a distinct advantage in pH stability. High pH stability is particularly important in clean-in-place (CIP) procedures where NaOH can be used to degrade aggregated peptides and other molecules. It is also beneficial when feeds are basic and/or the purification requires high pH conditions. Under such conditions, traditional silica can dissolve and leach into the recovered product,[1] and as the silica matrix is hydrolyzed the column separation performance worsens. Over the years, silica manufacturers have introduced modifications to improve the pH stability to raise the typical operational guidance for some silica grades from the traditional pH 2 – 8 to pH 11 or 12. On the other hand, polymerics resins have an inherent chemical stability up to pH 14, which allows the development of sanitization and CIP procedures to help ensure safe, consistent, and reliable separations.[2-3]

A pH stability study was performed on a polymeric resin from the DuPont™ AmberChrom™ chromatography resin family, DuPont™ AmberChrom™ CG161S, and was compared to commercial silica in a cycling experiment that exposed the resin to 1 M NaOH for 100 cycles to illustrate the chemical robustness of the resin and to simulate clean-in-place processes for a resin used over a long lifetime. While this simple study was demonstrated with just a single grade, the other grades in Table 1 have similar compositions and should behave in a comparable fashion.

Table 1. DuPont™ AmberChrom™ Chromatography Resins based on crosslinked DVB chemistry

Grade	Availability as Wet or Dry grade*	Average Particle size (µm)	Average Surface Area (m^2/g)	Nominal Pore Size (Angstroms)	Max P (bar)
XT20	Dry	20	600	300	60
XT30	Dry	30	600	300	60
CG161S	Wet	35	900	150	5
CG161M	Dry, Wet	75	900	150	5
CG161C	Dry, Wet	120	900	150	5
CG300M	Dry, Wet	75	700	300	5
CG300C	Dry, Wet	120	700	300	5

* Bulk resins are sold in mass (g, kg) for dry grades and volume (mL, L) for wet grades. Dry resin is a minimum of 98% solids. Wet grades are sold as a 50% slurry by volume in an ethanol/water mixture (20/80 by volume).

** The mixture contained 5 peptides:
Gly-Tyr, Val-Tyr-Val, Tyr-Gly-Gly-Phe-Met, Tyr-Gly-Gly-Phe-Leu, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe, where Gly = glycine; Tyr = tyrosine; Val = valine; Phe = phenylalanine; Met = methionine; Leu = leucine; Arg = arginine; Ile = isoleucine; His = histidine; Pro = proline

Experimental Details

Materials and methods

The HPLC peptide standard mixture** was from Supelco™ and purchased from MilliporeSigma™ (H2016). It was provided in a dried film with 0.5 mg of each component. Peptide samples were prepared at 2 mg/mL by dissolution in ultrapure water. Acetonitrile (Fisher Scientific™) and formic acid (Thermo Scientific™), were used as purchased to prepare the mobile phases. Mobile phase A consisted of 0.1% formic acid in 5:95 acetonitrile:water and mobile phase B consisted of 0.1% formic acid in 75:25 acetonitrile:water. Samples were held in 2 mL vials with low-volume inserts at 5°C before runs which were performed on an Agilent™ 1260 Infinity II HPLC system equipped with UV detection. Detection was done at 214 nm. Column dimensions were 4.6 x 250 mm for a column volume of 4.15 mL. Resins: DuPont™ AmberChrom™ CG161S chromatography resin and a commercially available C18 silica resin with an average particle size of 16 µm and a specified pore size of 300 Å.

Model for Clean-In-Place

Crude peptide feeds obtained from solid-phase synthesis come with an array of impurities such as shorter or modified sequences resulting from inefficient chemical coupling in chemical synthesis. Peptides made from fermentation processes contain impurities such as host-cell proteins and other organic molecules related to using a living organism. In high-loading scenarios and with repeated use, these impurities can foul chromatography resins and cause a decrease in purification performance, such as a lowering in loading capacity or a shift in retention time that can impact yield and purity. A multitude of cleaning agents, such as organic solvents and alkali solutions, can be used to recover performance. Among these, sodium hydroxide is one of the best solutions as it ensures the degradation of organic substances and is widely used in cleaning resins.

To demonstrate the resilience of the polymeric resins to extreme pH changes, the AmberChrom™ CG161S chromatography resin was exposed to 100 cycles, each consisting of the following:

- 3 CV (Column Volume) equilibration step with 5% B (mobile phase A: 0.1% formic acid in 5:95 acetonitrile:water; mobile phase B: 0.1% formic acid in 75:25 acetonitrile:water)
- 10 CV run with gradient conditions ranging from 5 to 30% mobile phase B (41.5 min)
- 10 CVs in 100% B (41.5 min)
- 10 CVs in 1 M NaOH (41.5 min caustic exposure per cycle).

Injections of a commercial peptide mixture were done every 2-3 cycles to measure consistency of elution behavior. Over 100 cycles, the effective separation of the 5 peptides remained unchanged (Figure 1), with the peptide peak shapes, relative heights, and retention times (Figure 1b) showing no noticeable differences even as the column was exposed to extreme pH conditions in every run.

The same experiment was also performed on the C18 silica resin (Figure 2). Figure 2a illustrates the reason it is not recommended to perform a CIP in a high concentration of caustic on silica. The effects of the base are clear even at the 2nd cycle as the peaks started shifting leftward, all decreasing in retention times. At a certain point, around cycle 11, the resin degraded significantly, causing the fourth and fifth peak to lose the ability to effectively separate the peptide species. This worsening of the separation was accompanied by an increase in the system pressure from an initial 35 bars to 144 bars over cycles 1 to 16, hitting the maximum pressure limit of 300 bars established in the protocol (data not shown). No autopsy was done on the system but presumably, NaOH hydrolyzed the bond between the alkyl chain and the silica, reducing the resin hydrophobicity and shifting the peaks leftward. Moreover, the exposed silica likely dissolved, releasing silica particles that could clog the column and increase pressure. The silica experiment was terminated early, reaching only 16 of the planned 100 cycles.

Conclusions

This simple experiment illustrates that DuPont™ AmberChrom™ chromatography resins based on DVB (divinylbenzene) have excellent resistance to alkaline conditions and can handle wide swings in pH. The chromatography performance was unchanged, even with repeated and extended exposure to 1 M sodium hydroxide for a total of nearly 70 hours over the course of 100 cycles. Such chemical robustness gives end-users flexibility and options for handling difficult manufacturing situations like fouling and sanitization. Although not explored in this paper, the pH resistance may also give end-users a wider window for designing purification processes since pH can be a mobile phase variable to exploit during method development.

References

1. Pettersson, S. W.; Collet, E.; Andersson, U., Chemical stability of reversed phase high performance liquid chromatography silica under sodium hydroxide regeneration conditions. J Chromatogr A 2007, 1142 (1), 93-7.
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3. Grönberg, A.; Hjorth, R. A., Cleaning-in-Place and Sanitization. In Biopharmaceutical Processing - Development, Design, and Implementation of Manufacturing Processes, Elsevier: 2018; pp 675-699.

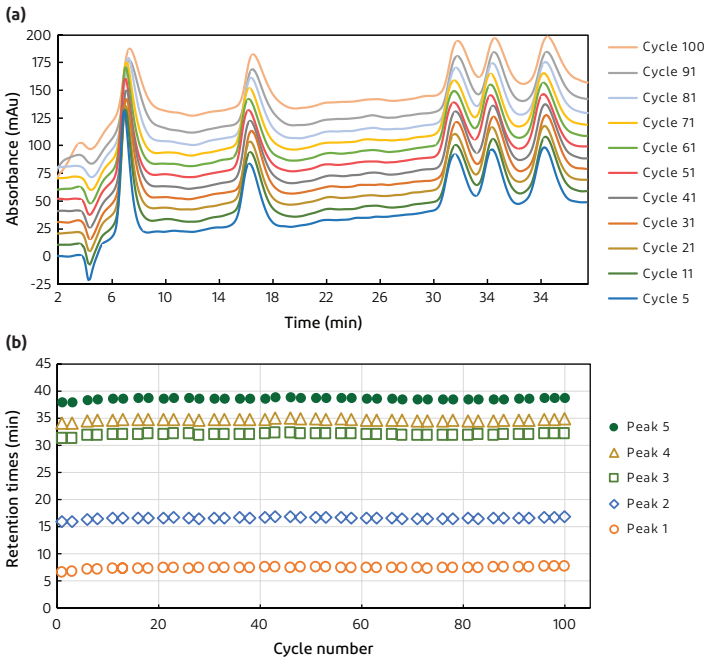

Figure 1. Chromatograms **(a)** and retention times **(b)** of 5 peptides after cycling with CIP on DuPont™ AmberChrom™ CG161S chromatography resin.

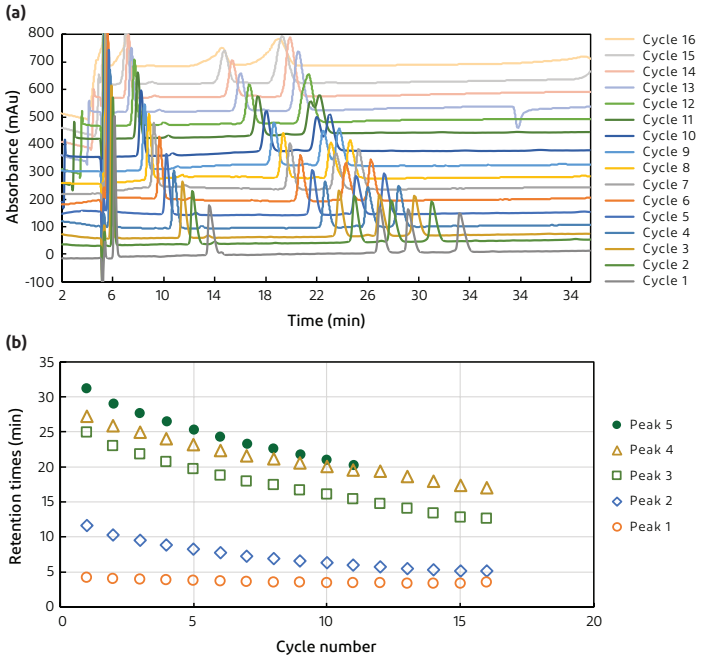

Figure 2. Chromatograms **(a)** and retention times **(b)** of 5 peptides after cycling with CIP on the 16 µm/300 Å C18 silica resin.

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