

Clearance of 2% Benzyl Alcohol from TOYOPEARL® Resins

Introduction

Traditionally, 20% ethanol has been the preservative of choice as a storage solution for chromatography media. However, more stringent safety requirements are driving the demand for an alternative since large quantities of ethanol may be viewed as potentially hazardous. This is becoming more common as process scale chromatography columns increase in size and volume.

Benzyl alcohol is a commonly used preservative in the pharmaceutical industry. It is soluble in water up to approximately 40,000 ppm (4%), though it is typically used at much lower concentrations as a preservative in parenteral therapeutics. Benzyl alcohol solutions are microbiocidal, but are not considered sanitizing agents. Benzyl alcohol is non-flammable, inexpensive, and has fewer regulatory issues concerning its disposal than solutions containing ethanol.

A 2% solution of benzyl alcohol in water has been identified as a suitable alternative to 20% ethanol as a preservative in resin storage solutions. This application note evaluates the clearance of 2% benzyl alcohol from a variety of TOYOPEARL chromatography resins.

TOYOPEARL chromatography media are hydroxylated methacrylic polymer resins and are made commercially in many different pore sizes and particle diameters. TOYOPEARL Butyl-650M, TOYOPEARL SuperQ-650M, and TOYOPEARL AF-rProtein A-650F resins; hydrophobic interaction (HIC), ion exchange and affinity chromatography resins respectively, represent three of the available ligand modes available from Tosoh Bioscience. All three resins share the same polymethacrylic backbone and the same 100 nm pore size.

TOYOPEARL Butyl-650M resin is the second most hydrophobic ligand in the TOYOPEARL HIC product line. TOYOPEARL SuperQ-650M is a strong anion exchange resin containing quaternary ammonium groups and TOYOPEARL AF-rProtein A-650F is an affinity resin with a recombinant protein A ligand. Together, these three products serve as a representative sample of all TOYOPEARL resins that may be stored in 2% benzyl alcohol solution as an alternative to 20% ethanol.

Table 1 shows the general properties of the chromatography resins being evaluated in this study.

Table 1. Properties of TOYOPEARL Resins Used in this Study

	TOYOPEARL Butyl-650M	TOYOPEARL SuperQ-650M	TOYOPEARL rProtein A-650F
Mode	HIC	AIEX	Affinity
Backbone	polymer	polymer	polymer
Ligand	butyl	quaternary ammonium	recombinant protein A

Experimental Conditions/ Results

This study was executed in an effort to determine whether a 2% benzyl alcohol storage solution could be effectively removed from TOYOPEARL resins through repeated washes with DI water.

Samples of the three TOYOPEARL resins to be tested (TOYOPEARL SuperQ-650M, TOYOPEARL Butyl-650M, and TOYOPEARL AF-rProtein A-650F) were prepared by adding 100 mL of aqueous 2% benzyl alcohol to 100 mL of suction filtered resin.

The resin and benzyl alcohol mixture was re-slurried and allowed to settle three times to ensure that a homogeneous mixture of resin and preservative was achieved. This homogeneous slurry was then suction filtered to remove the benzyl alcohol supernatant.

After filtration, a 100 mL aliquot of DI water was added to the filtered resin and stirred to make a slurry. This resin/ DI water slurry was allowed to stand for 5 minutes and was then suction filtered to remove the supernatant. This procedure was repeated 14 more times, for a total of 15 washes (Protein A resin was washed a total of 20 times).

Samples of the filtered supernatant from the TOYOPEARL SuperQ-650M and Butyl-650M were taken after the 5th, 10th, and 15th washes and analyzed for benzyl alcohol concentration (**Figure 1 and Table 2**). Samples of the filtered supernatant from the TOYOPEARL AF-rProtein A-650F were taken after every wash and analyzed for benzyl alcohol concentration (**Figure 2**).

The TOYOPEARL AF-rProtein A-650F was packed in a 1.6 cm ID × 10 cm column and washed with DI water at a flow rate of 800 cm/hr. A sample of the effluent was taken after 5, 10, and 15 column volumes and analyzed for benzyl alcohol concentration (**Figure 3**).

Figure 1. Concentration of Benzyl Alcohol in Resin Supernatant (batch wash)

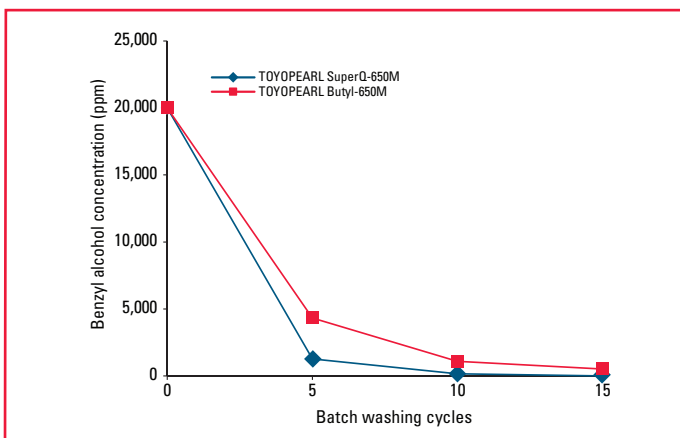


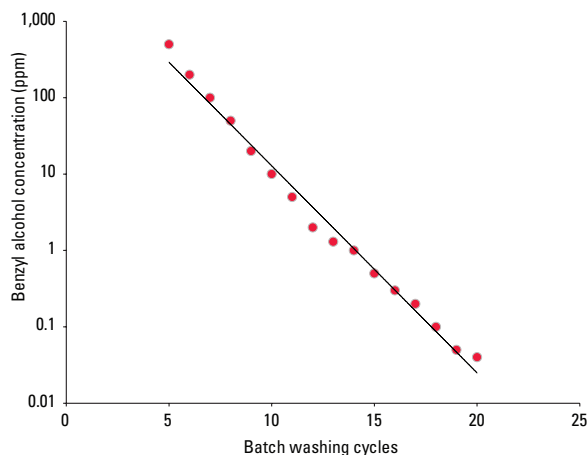
Table 2. Concentration of Benzyl Alcohol in Resin Supernatant (batch wash)

Batch washing cycles	Benzyl Alcohol Concentration (ppm)	
	TOYOPEARL SuperQ-650M	TOYOPEARL Butyl-650M
0	20,000	20,000
5	1,277	4,325
10	155	1,093
15	12	524

Column: TSKgel ODS-80Ts, 5 μ m, 4.6 mm ID \times 15 cm
 Mobile phase: MeOH/H₂O (70/30)
 Flow rate: 1 mL/min
 Detection: UV @ 254 nm
 Temperature: ambient
 Injection vol.: 10 μ L
 Sample: washed resin filtrate

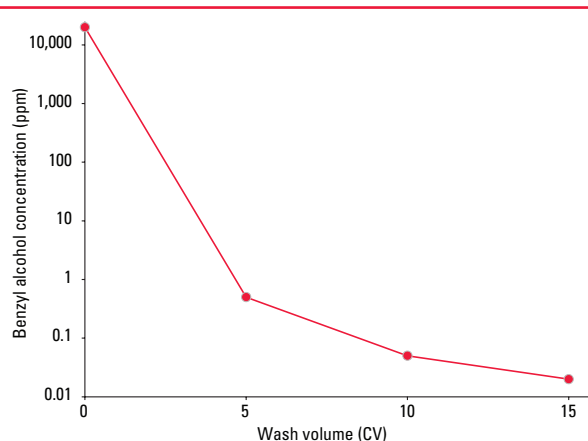
Samples measured against a 3-point calibration curve (benzyl alcohol vs. peak area) at concentrations of 2,200 and 20,000 ppm.

Figure 2. Concentration of Benzyl Alcohol in TOYOPEARL AF-rProtein A-650F Supernatant (batch wash)



Resin: TOYOPEARL AF-rProtein A-650F
 Resin sample: 50% slurry, suction dried (wet cake)
 Mobile phase: DI H₂O
 Wash procedure: A. re-suspend sample in 2 \times resin volume of DI H₂O
 B. let stand for 10 minutes
 C. suction dry (wet cake)
 D. test filtrate for benzyl alcohol concentration

Figure 3. Concentration of Benzyl Alcohol in TOYOPEARL AF-rProtein A-650F Supernatant (packed column)



Resin: TOYOPEARL AF-rProtein A-650F
 Column size: 1.6 cm ID \times 10 cm (20.1 mL)
 Mobile phase: DI H₂O
 Flow rate: 800 cm/hr (26.8 mL/min)
 Residence time: 45 sec

Conclusions

A 2% benzyl alcohol solution can be effectively removed from the TOYOPEARL SuperQ-650M and TOYOPEARL AF-rProtein A-650F resins by thorough washing with DI water. It is less effectively removed from the TOYOPEARL Butyl-650M resin with the same procedure.

As benzyl alcohol is a hydrophobic molecule, it may not be possible to adequately reduce its concentration from hydrophobic interaction chromatography resins due to interactions between the preservative and the ligand.

The use of benzyl alcohol (2%) with chromatography media that are un-functionalized or are functionalized with non-hydrophobic ligands is an acceptable alternative to the recommended 20% ethanol.

Please note: customers wishing to store TOYOPEARL resins in 2% benzyl alcohol should evaluate clearance of the preservative under operating conditions for their individual processes.

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