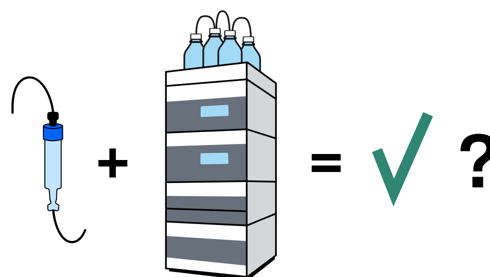
Bio-Works

APPLICATION NOTE

Exploring the compatibility of agarose-based AIEX resin columns with HPLC systems

The increasing demand for sustainable and environmentally friendly purification techniques has led to a shift from traditional rigid media such as reversed-phase chromatography (RPC) and ion-pairing chromatography (IPC), to compressible resin-based methods such as ion exchange chromatography (IEX). While rigid media are compatible with high-pressure liquid chromatography (HPLC) systems, compressible resins, such as agarose-based resins, commonly require low-pressure liquid chromatography (LPLC) systems, like ÄKTA, due to their limited pressure tolerance.

Transitioning from RPC or IPC to IEX would be simplified if agarose-based IEX resin columns were compatible with HPLC systems, enabling use of the same equipment for both techniques. In this study, we demonstrate the compatibility of prepacked agarose-based resin columns with HPLC systems, using a DNA ladder of defined sizes as a proof-of-concept.



Background

Today, reverse phase chromatography (RPC) and ion pair chromatography (IPC) are dominant methods for purifying and analyzing small quantities of so-called TIDES molecules (peptides and oligonucleotides), offering fast and high-resolution techniques. However, they are not sustainable processes, especially not at larger scales, due to the high amounts of organic solvents and high concentrations of toxic ion-pairing reagents used. Ion exchange chromatography (IEX) is an alternative water-based technique that is more sustainable and easy-to-use at all scales. Sustainability considerations have driven the shift from RPC/IPC to IEX. However, system limitations, such as the exclusive availability of HPLC, can hinder this transition. If prepacked IEX resins could be ran on HPLC systems, this shift would be feasible, particularly at small scale.

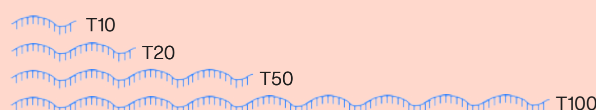
Optimization of running conditions

To evaluate the two system setups, a mix of four DNA sequences of defined lengths were mixed; 10 nucleotides (nts), 20 nts, 50 nts and 100 nts. The sequences only consisted of thymidines to prevent potential inter and intramolecular hydrogen bondings (IDT). Two buffer systems (10 mM NaOH vs. 20 mM tris, pH 8) and elution gradients were evaluated for optimal resolution between the target peaks on the ÄKTA system (ÄKTA Pure 25).

See box below for the running conditions applied after optimisations.

Running conditions applied after optimization

Column:	GoBio Mini Q 1 mL (agarose-based AIEX resin)
Buffers:	20 mM tris-HCl, pH 8 (+ 1.5 M NaCl in elution buffer)
Flow rate:	0.5 mL/min (80 cm/h)
Sample:	Load of 25 µg DNA sequences in a mix per mL resin
Elution:	Linear gradient, 20-50% elution buffer in 20 column volumes (CV)



The optimisations were based on the resolution (Rs) between the peaks. The resolution considers factors, such as selectivity and efficiency (peak width) to determine the separation capacity between peaks. Rs should be as high as possible, and Rs >1.5 means base line separation between peaks. See Table 1 for differences between tris and sodium hydroxide (NaOH) based buffer systems.

Table 1. Resolution values obtained for tris and NaOH buffers

	Resolution (Rs[1])	
Peaks	**Tris**	**NaOH**
T10-T20	3.3	2.8
T20-T50	2.6	2.2
T50-T100	1.1	0.9

Formula to calculate Rs

$$Rs = \frac{t_{R2}-t_{R1}}{0.5(W_2+W_1)}$$

t: retention time
W: peak width

[1] Rs values were obtained by integrating peaks in Unicorn 7.1.

Pressure limitations

The main difference between separations conducted on ÄKTA systems compared to HPLC systems is the system pressure. HPLC is traditionally used for high-resolution separations of small molecules and synthetic molecules (peptides and oligonucleotides) using small particle-sized RPC media generating high pressures, whereas an ÄKTA system operates at low pressures and has mainly been used for more preparative purposes to purify biomolecules.

In theory, the pressure tolerance of the resin is influenced by the pressure over the resin bed independent of the system pressure. If resins are prepacked it is often the hardware that sets the pressure limits. A typical HPLC column can tolerate hundreds of bar, whereas prepacked GoBio Mini columns, designed for ÄKTA systems, can tolerate up to 3 bar. In contrast to the small particles used in RPC separations, GoBio Mini columns are packed with larger more preparative beads (45 µm compared to 2-5 µm in RPC). This difference means that the resins and columns do not contribute to any substantial back-pressures.

The backpressure relevant for the columns is illustrated in Fig. 1C. The system pressure before the column (ΔP_{pre}) does not affect the column itself. Only the sum of ΔP and ΔP_{post} and (representing the pressure over and after the column), is relevant. It is important not to exceed the column hardware pressure limits.

PoC study

The GoBio Mini Q 1 mL column (7x28 mm) was connected to the HPLC system (Shimadzu, 2050-C) using peek tubings (the column was not connected straight into valve but to an adaptor followed by a peek tubing to the outlet valve). Identical running conditions were applied as in previous experiments. At a flow rate of 0.5 mL/min for a 1 mL GoBio Mini column, the pressures were relatively similar between the ÄKTA (with a flow restrictor connected) and HPLC systems, attributable entirely to the system setup. However, when the flow rate was doubled in the ÄKTA system, the back pressure increased only slightly (from 2.5 to 2.7 MPa), whereas the same flow rate increase in the HPLC system resulted in a doubling of backpressure. Note that such a high system pressure obtained in the ÄKTA system is attributed solely to the flow restrictor (otherwise almost no backpressure would be obtained).

Regarding separation, the chromatograms of the DNA sequences looked almost identical with a very similar resolution (Fig 2; 4 major peaks). The smaller peaks prior to the T10 peak are abortive failure sequences presented in the samples (low purity) and they look different due to the difference in delay volumes post mixer in the two systems.

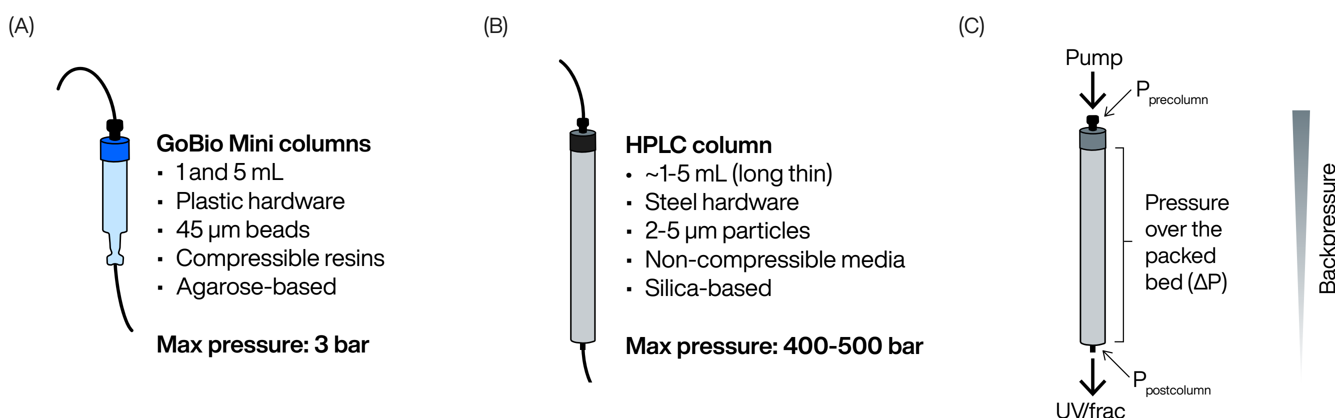


Figure 1. (A) GoBio Mini properties. (B) Properties of a typical RPC/HPLC column. (C) Backpressures and pressure drops in the system and over the column.

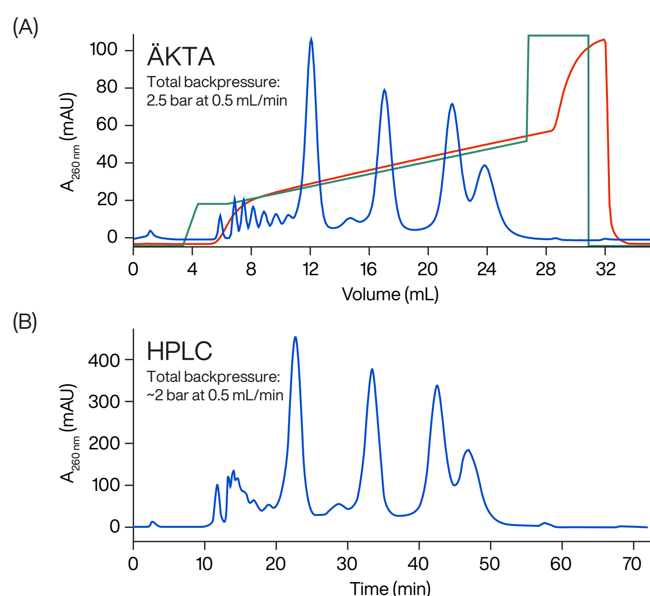


Figure 2. The oligonucleotide mix consisting of four T-sequences separated on either an ÄKTA system or on an HPLC system. Identical running conditions were applied and in the upper chromatogram both the elution gradient (green line) and the conductivity (brown line) is displayed. The delay volumes post mixer is smaller in the HPLC system which makes the actual gradient more consistent to the theoretical gradient

To further validate the GoBio column compatibility with HPLC usage, two additional formats were tested, GoBio Mini 5 mL (13x38 mm, max pressure of 3 bar) and GoBio Screen 7x100 mm (3.8 mL, max pressure of 5 bar). With these larger formats, increased sample volumes were loaded onto the columns, and twice as high flow rate was applied. As shown in Table 2 and in Figure 3 resolution was further improved with these formats under the specified conditions. The total backpressures for GoBio Mini Q 5 mL and GoBio Screen 7x100 40Q were 4 and 5 bar, respectively.

The flow rates must be kept relatively low to ensure hardware pressure limitations are not exceeded.

Table 2. Resolutions for different formats under specified conditions

	Resolution (Rs[1])			
Peaks	**ÄKTA Mini -1 mL**	**HPLC Mini -1 mL**	**HPLC Mini-5 mL**	**HPLC Screen-3.8 mL**
T10-T20	3.3	3.3	5.1	4.6
T20-T50	2.6	2.4	3.4	3.3
T50-T100	1.1	0.9	1.4	1.5

[1] Rs values were obtained by integrating peaks in Unicorn 7.1 or LabSolutions.

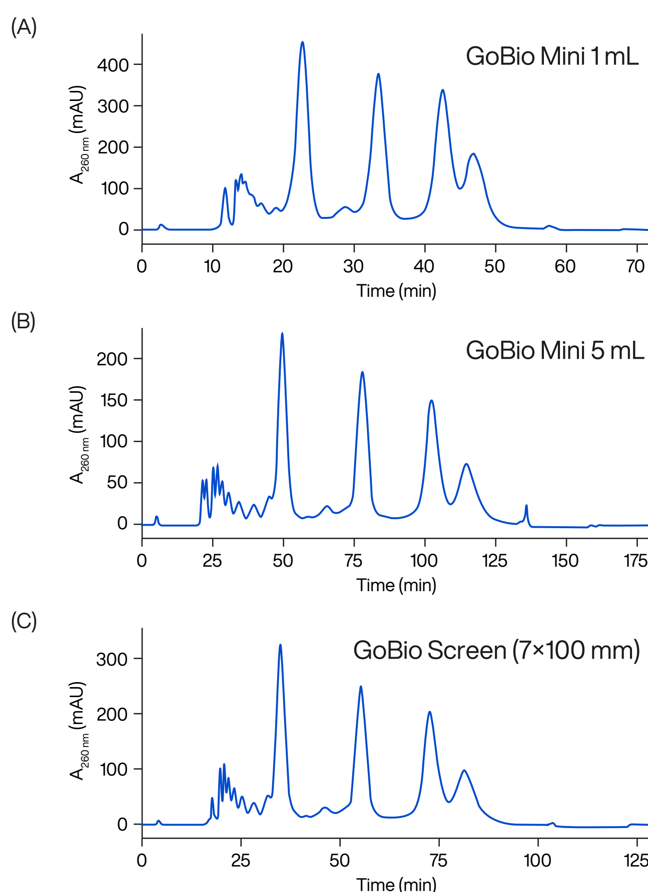


Figure 3. The oligonucleotide mix consisting of four T-sequences separated on different prepacked column formats on an HPLC system. Identical running conditions were applied

These tests demonstrate the ability to separate molecules on a column packed with the agarose-based AIEX resin WorkBeads 40Q (GoBio Mini columns (1 and 5 mL) and GoBio Screen 7x100) using an HPLC system, achieving the same resolution without excessive backpressures.

Conclusion of PoC study

Our results indicate that the selectivity and performance of agarose-based resins in HPLC are comparable to those obtained with traditional ÄKTA systems. This demonstrates the potential for purifying synthetic molecules, oligonucleotides, and peptides using more preparative IEX resins on an HPLC system.

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