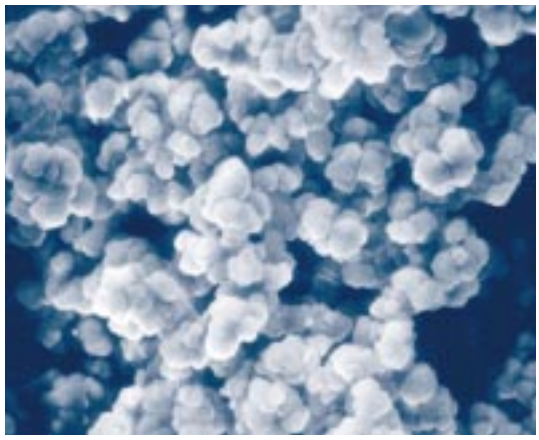
uno

chromatography
BIOCHROMATOGRAPHY COLUMNS

USING AN UNO Q1 COLUMN IN THIS PURIFICATION SCHEME ALLOWED:

- **HIGHER RESOLUTION THAN PERFUSIVE MEDIA**
- **NO LOSS IN RESOLUTION AT HIGHER FLOW RATES**
- **USE WITH VOLATILE, BASIC AND DENATURING BUFFER**

Nucleotide and Oligonucleotide Separations on an UNO™ Q1 Ion Exchange Column



Introduction

The UNO chromatography column is the very first to contain the revolutionary Continuous Bed matrix. Unlike a traditional column, which consists of a bed of packed beads or particles, each UNO column contains an advanced polymer matrix that is completely homogeneous.

This article describes the optimized separation of closely-related synthetic nucleotides on an UNO Q1 column (catalog # 720-0001) using a variety of buffer systems, and a comparison of the separation of RNA 10-mer on an UNO Q1 column and a perfusive beaded anion exchange column.

Major DNA purification methods for solid phase oligonucleotide syntheses include reversed phase (trityl-on and trityl-off), anion exchange chromatography and PAGE electrophoresis.

The drawbacks of using reversed phase chromatography are that the trityl-OH protecting group on the 5'-end is very hydrophobic and requires removal of the group after chromatography; it is often time-consuming; and acetic acid may cause depurination and undesired chain cleavage at depurination sites. Reversed phase purification with trityl-off is straight-forward. However, the hydrophobicity of the main oligonucleotide and the shorter chains are not significantly different, making the separation quite poor. PAGE provides the best oligonucleotide purification, but it is very labor-intensive, time-consuming, difficult to scale-up and the yield is often very low.

Anion exchange chromatography separates oligonucleotides by their chain length, and high resolution separations can be obtained for up to 40-mers. Denaturing conditions can be used to eliminate the effects of the oligonucleotide's secondary structure. The use of traditional anion exchange supports requires long separation times due to their poor mass transfer properties.

The advantages of the UNO anion exchange columns for oligonucleotide purification over conventional media are faster separation, higher resolution for longer oligonucleotides, larger sample loading and the use of a volatile buffer system (TEAA).

30-mer in TEAA Buffer System

A Tris-EDTA-acetate (TEAA) buffer system was used to optimize the separation of a crude 30-mer which contained significant amounts of a 29-mer. By running a 200–500 mM NaCl gradient in 20, 30, 40, 50 and 60 minutes (Figure 1), it was found that the best separation between the 29-mer and 30-mer occurred using the 60 minute gradient. Conditions were further optimized to achieve baseline resolution between the 29-mer and 30-mer by use of a 350–400 mM NaCl gradient in 60 ml (Figure 2). To examine the resolving power of the UNO Q1 column, a sample spiked with a 15, 20, 25, 30, 45 and 60 mer, all sharing the same 3' sequence, was separated using a 60 ml gradient. (Figure 3).

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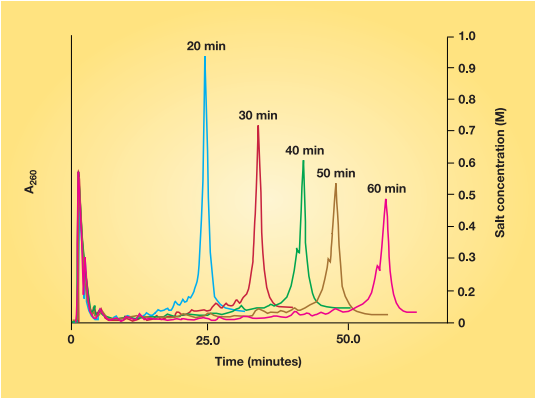

Fig. 1. Separation of a crude 30-mer oligonucleotide on an UNO Q1 column. Sample: 20 µg of TGGTCGTGCAACGGGTCCTCGG ACTTCAAG; Buffer A: 25 mM TEAA pH 7.5; Buffer B: A+1 M NaCl. Flow rate: 1 ml/min; Gradient: 20%–50% B. Gradient times: blue–20 minutes, red–30 minutes, green–40 minutes, brown–50 minutes, magenta–60 minutes. Detection: 260 nm.

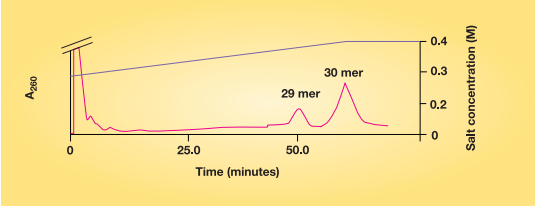

Fig. 2. Baseline separation of 29- and 30-mer oligonucleotide on an UNO Q1 column. Sample: 20 µg of TGGTCGTGCAACGGGT CCTCGGACTTCAAG; Buffer A: 25 mM TEAA pH 7.5; Buffer B: A + 1 M NaCl. Flow rate: 1 ml/min; Gradient: 35–40% B in 60 minutes.

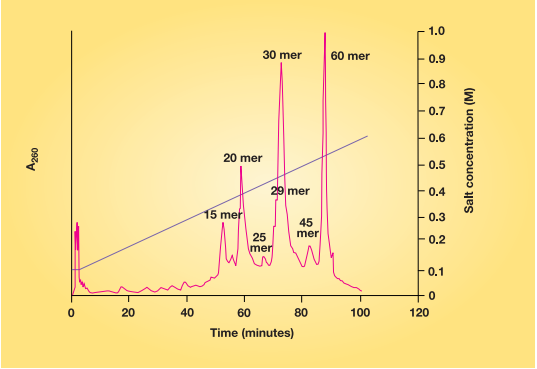

Fig. 3. Separation of a synthetic mixture of a 15, 20, 25, 30, 45 and 60-mer oligonucleotide on an UNO Q1 column. Sample: 60-mer sequence 3'TGGTCGTGCAACGGGTCCTCGGACTTCAA GAGTCTAGGTGACGTCGAACAGTGTCAGTTC5', smaller oligonucleotides share the same 3' end; Buffer A: 25 mM TEAA pH 7.5; Buffer B: A + 1 M NaCl. Flow rate: 1 ml/min; Gradient: 10–60% B in 100 minutes. Detection: 260 nm.

Data courtesy of Lingyan Huang, IDT Inc., Coralville, IA. The results reported here are the copyright property of the following scientists and may not be reproduced in any form without written permission from the owners: Prof. C. H. Lochmüller and Qicai Liu, Department of Chemistry, Duke University, Durham NC 27708 and Lingyan Huang, Integrated DNA Technologies, Coralville, IA 52241.

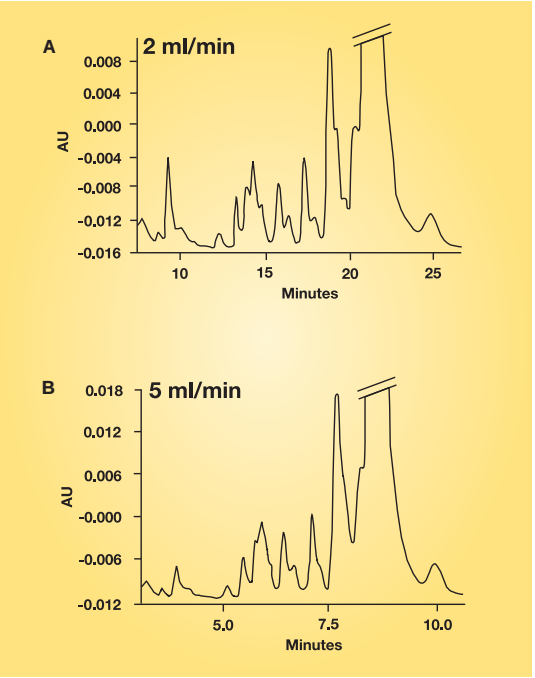

Fig. 4. Effect of flow rate on separation of a 22-mer oligonucleotide separation on an UNO Q1 column. Sample: 66 µg of 22-mer oligonucleotide; Column: UNO Q1 column; Buffer A: 10 mM NaOH; Buffer B: A + 1 M NaCl; Flow rate: A-2 ml/min, B-5 ml/min; Gradient: 0–100% in 60 ml; Detection: 254 nm.

22-mer in 10 mM NaOH Buffer System

The second buffer system used to separate synthesis products of 22-mer was 10 mM NaOH. This buffer is not usually recommended to separate biomolecules on anion exchangers due the long-term instability of the quaternary amine ionic group. However, it is a good buffer to use if the oligonucleotide does not bind to the support using other systems. Separation of the 22-mer from the closely-related nucleotides was accomplished by running a series of increasing gradient volumes from 5 ml to 80 ml (Figure 5). The best resolution was obtained using an 80 ml gradient volume.

For conventional beaded supports, increasing the flow rate decreases the resolution due to the slow binding kinetics of the biomolecule and the mass transfer properties of the support. Increasing the flow rate from 2 to 5 ml/min on the UNO Q1 column did not greatly reduce the resolution for the separation of the 22-mer oligonucleotide (Figure 4).

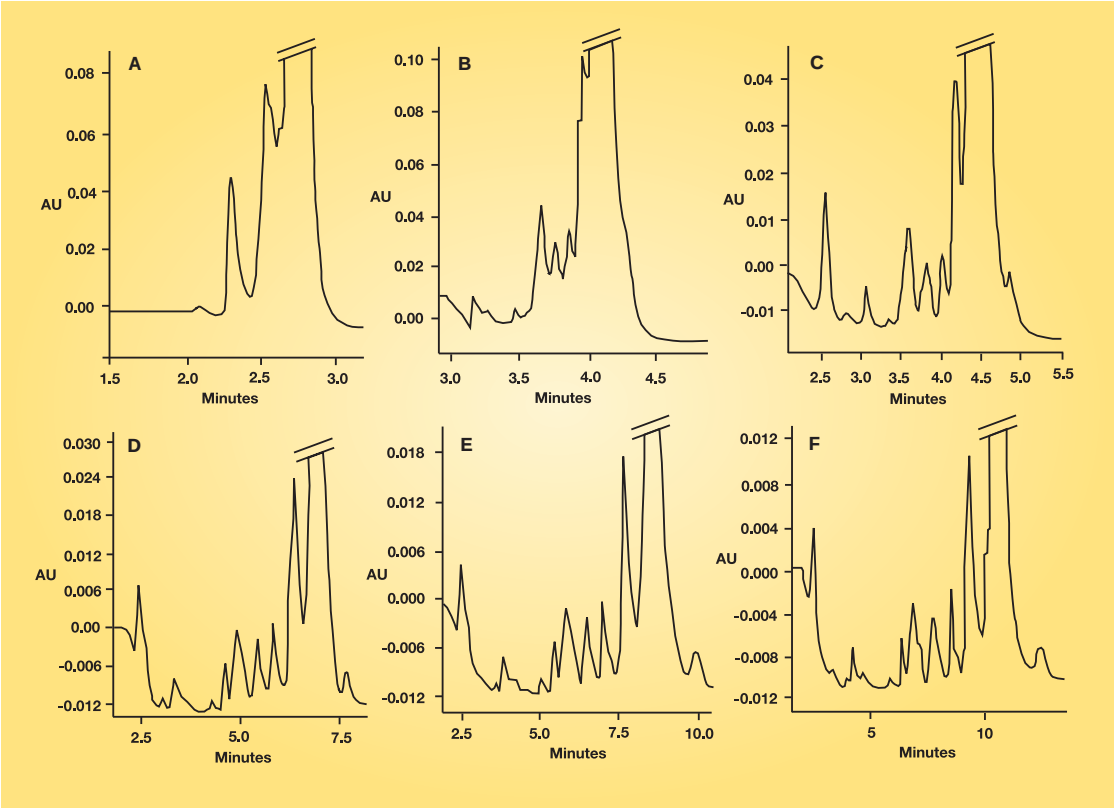

Fig. 5. Optimization of the gradient volume for a 22-mer oligonucleotide on an UNO Q1 column. Sample: 66 µg of 22-mer oligonucleotide; Column: UNO Q1 column; Buffer A: 10 mM NaOH; Buffer B: A + 1 M NaCl; Flow rate: 5 ml/min; Gradient: 0–100% B in A-5 ml; B-10 ml; C-20 ml; D-40 ml; E-60 ml and F-80 ml; Detection: 254 nm.

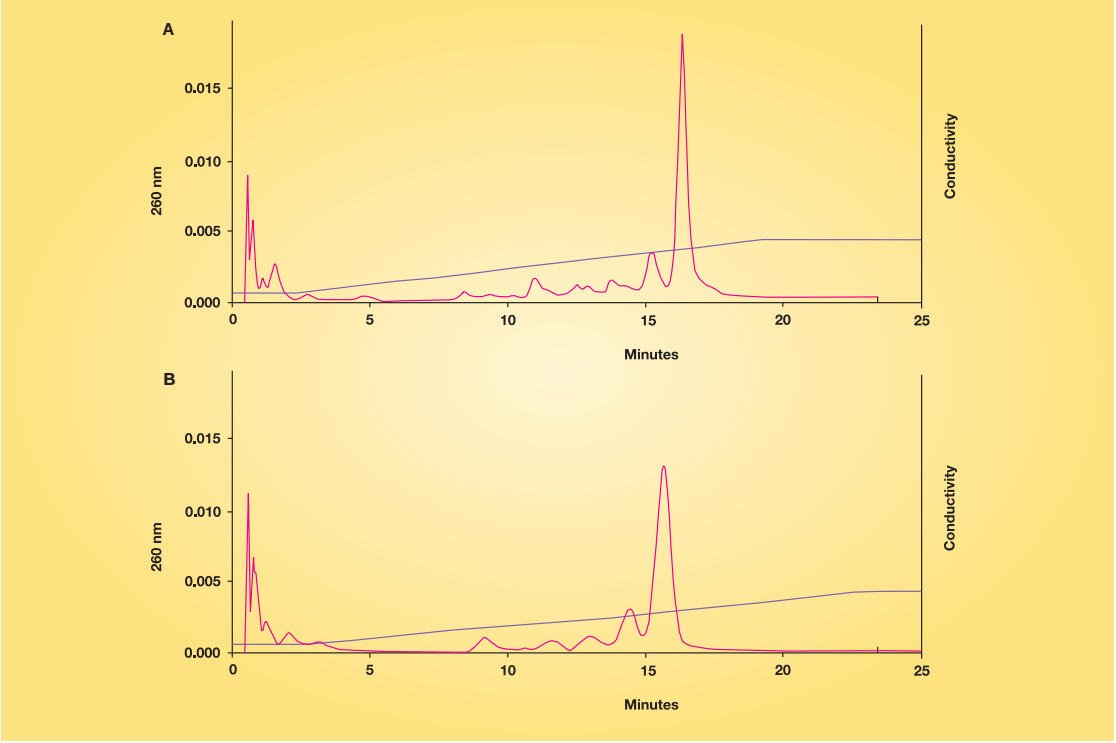

Fig. 6. Separation of 10-mer RNA (5'–GGUCGUAGCA–3') on an UNO Q1 (A) and perfusive anion exchange column (B). Sample: 10 µg (200 µl) of 10-mer RNA; Buffer A: 20 mM Tris-HCl + 50 mM NaCl + 7 M urea, pH 8.0; Buffer B: A + 350 mM NaCl; Flow rate: 2.5 ml/min; Gradient: 0–100% B in 31.6 column volumes; Detection: 260 nm.

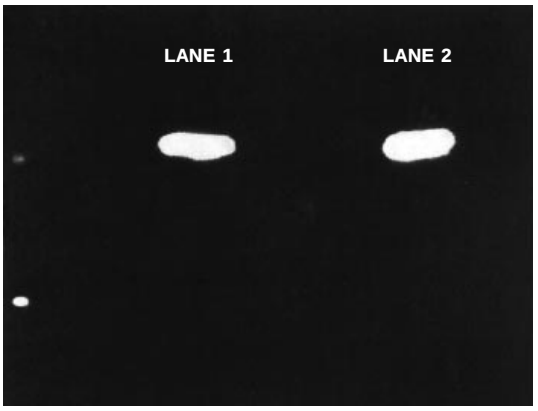

Fig. 7. 20% PAGE Gel (8 M urea) of 10-mer RNA fraction from UNO Q1 column. Lane 1: 5 µg; Lane 2: 10 µg.

Separation of 10-mer RNA in 7 M Urea on UNO Q1 and Perfusive Anion Exchange Column

A 10-mer and its closely-related contaminates were separated in 20 mM Tris-HCl + 7 M urea, pH 7.0, at 2.5 ml/min on the UNO Q1 and a perfusive anion exchange column (Figure 6). Using the UNO Q1 column, the 10-mer was baseline-resolved from the nearest contaminant and had higher peak heights. Pure RNA 10-mer was obtained from the UNO Q1 column as shown in the PAGE gel (Figure 7).

Conclusion

The UNO Q1 column is a powerful tool for the separation of oligonucleotides using a variety of buffer systems including volatile, basic and denaturing buffers. In general, oligonucleotide separations on the UNO Q1 column should be run with a sixty column volume gradient, and the separation is unaffected by changes in the flow rate. The UNO Q1 column demonstrates higher resolving power than a perfusive anion exchange column.

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