USER BULLETIN

Model 380A, 380B, 381A, 391, 392, 394
DNA Synthesizers

Number 61
May 1991

40-nanomole Polystyrene: New Highly Efficient DNA Synthesis Columns

New, smaller scale DNA synthesis columns are now available from Applied Biosystems. The 40-nanomole columns feature a novel polystyrene support that provides efficient and economical synthesis for oligonucleotides of all lengths. Phosphoramidites can be used with these columns at a double dilution of 0.05 M, saving about 25% of the cycle cost. In addition, the LOW cycle of the Model 391 and the primer cycle of the 392 and 394 can be used with the 40-nanomole columns while maintaining excellent synthesis performance. Finally, these columns extend the life of phosphoramidites in acetonitrile on the instrument to three weeks from the previous standard of two weeks.

The 40-nanomole columns can be used on all Applied Biosystems' DNA synthesizers, and no new cycles are needed. Oligonucleotides of any length can be synthesized on the new support. Currently, 40-nanomole columns are available only with standard protecting groups, and therefore are not compatible with FOD™ (fast oligonucleotide deprotection) reagents, except for those sequences that have a 3' T.

A New Support Material

A form of polystyrene has been developed as the solid support for these columns. Manufactured as rigid beads that do not swell, this solid support has the attractive features of mechanical stability, rapid reaction kinetics, and the ability to be washed quickly and efficiently with organic solvents. Parameters such as pore size, particle size, and both polymerization formula and conditions have been optimized.[1]

Phosphoramidite DNA synthesis using controlled pore glass (CPG) supports can attain 98-99% repetitive trityl yields. However, as oligonucleotide synthesis scale and phosphoramidite excess are both reduced to achieve more economical cycles, the deficiencies of CPG become more apparent. Although the silanol matrix of CPG is exhaustively capped prior to synthesis, a small number of trimethylsilyl capping groups are removed during detritylation with trichloroacetic acid, allowing the phosphoramidite to react with the nucleophilic silanols.[2] Extraneous oligonucleotides can be initiated and propagated during each synthesis cycle, resulting in 3' phosphate terminus failure sequences. These failures become more problematic at smaller scales.[3] With an inert polystyrene support, side reactions during the preparation of the support and during oligonucleotide synthesis are minimized by the lack of reactive functionality.

A 21-mer oligonucleotide primer was synthesized on a Model 380B DNA Synthesizer equipped with both 40-nanomole polystyrene and 0.2-μmole CPG columns. Four milligrams of phosphoramidite were delivered each cycle to test the supports stringently, using the fast cycle and 0.05 M phosphoramidites. Both the crude oligonucleotides and those purified by the oligonucleotide purification cartridge (OPC™)[4] were analyzed on the Model 270A by MICRO-GEL™100 Capillary Electrophoresis (CE)[5] using a new gel-filled matrix optimized for oligonucleotide analysis. The trityl yield for polystyrene was excellent (99.5%), but the CPG trityl yield was only fair (97.1%). The trityl data was corroborated by the quantitation of product on CE. Examination of the CE electropherograms suggests that undesired growth off the support may contribute to the comparatively high (n-1 mer) contamination seen in the CPG samples. Trityl-selective OPC purification and subsequent CE analysis demonstrate that these contaminants are trityl bearing.

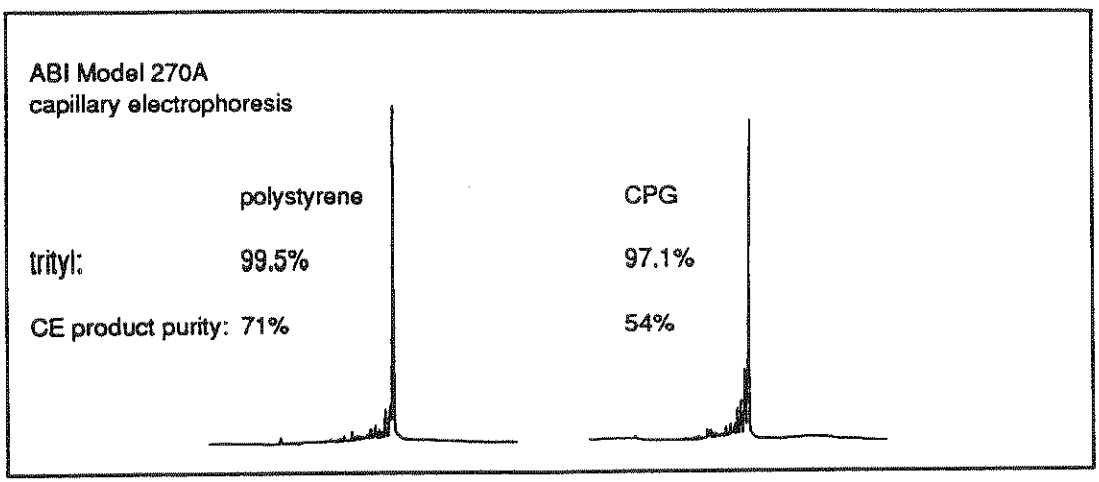

Figure 1. CEs of crude 21-mers.

Electropherograms of crude and OPC-purified samples also demonstrate the superior quality of oligonucleotides synthesized on polystyrene supports. Syntheses conducted with aged, partially degraded reagents also give better results when polystyrene supports are used. In addition to decreased extraneous propagation off the support, polystyrene supports offer coupling efficiency superior to that attainable with CPG. The advantage is especially evident under impaired or more stringent synthesis conditions, such as low phosphoramidite excess. The difference may reflect a more anhydrous environment of the hydrophobic polystyrene.

Recommendations for Use

Dilution of Phosphoramidites

The 40-nanomole polystyrene is especially intended for use with 0.05 M

phosphoramidites to reduce cycle costs and still provide high-quality oligonucleotides. Both the 250- and 500-mg phosphoramidite bottles can be diluted with twice the normal volume of acetonitrile to achieve a concentration of 0.05 M, as shown below:

Table 1. 0.05 M Phosphoramidite Dilution Volumes

Phosphoramidite	Bottle Size	Acetonitrile Volume
A^{bz}	500 mg	11.2 mL
A^{bz}	250	5.6
G^{ib}	500	11.6
G^{ib}	250	5.8
C^{bz}	500	11.8
C^{bz}	250	5.9
T	500	13.2
T	250	6.6

The auto dilute feature of the Model 392 and the 394 can provide 0.05 M phosphoramidites by using 500-mg bottles with the 1.0-g dilution option, or 250-mg bottles with the 0.5-g dilution option.

Increased Phosphoramidite Lifetime

The 40-nanomole polystyrene columns contribute to efficient synthesis even with 0.05 M phosphoramidites that have been on the instrument for 21 days. A parallel synthesis using a 0.2-μmole (small-scale) CPG column demonstrated inferior quality after 14 days. The 40-nanomole polystyrene columns require fewer equivalents of phosphoramidite for efficient synthesis.

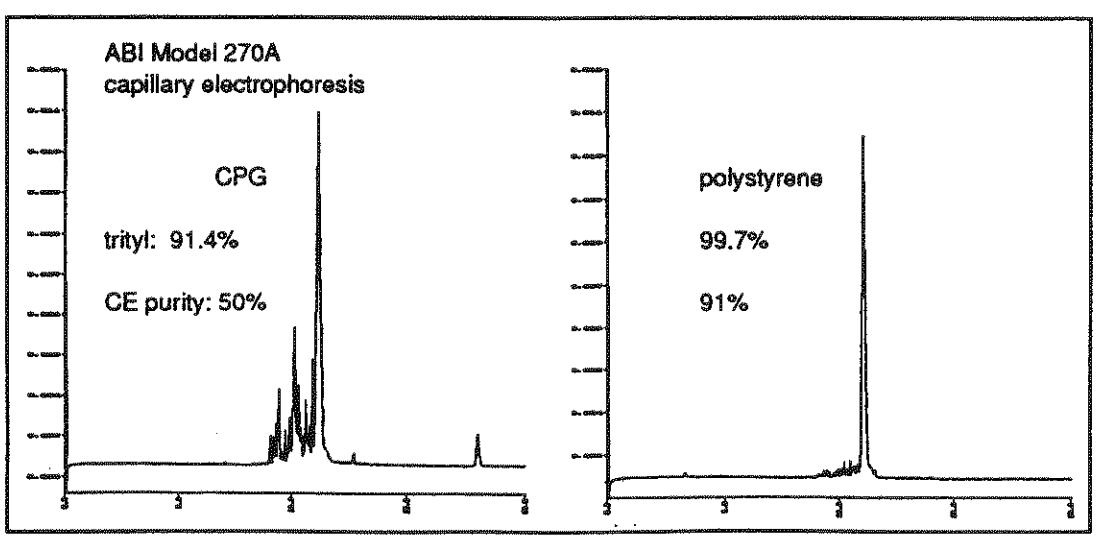

Figure 2. 21-day-old, 0.05 M phosphoramidites, concurrent syntheses of an 18-mer.

Cycle Recommendations

These new columns may be used on all Applied Biosystems' DNA synthesizers. No new cycles are necessary.

Table 2. Cycle Recommendations for 40-nanomole Synthesis

Synthesizer	Cycle
380A, 380B	small-scale cycle (e.g., ssceaf 3, Version 2.01, 380B)
381	.2 UM cycle
391	LOW or .2 UM cycle
392-394	PRIMER or .2 UM CE

- The 40-nanomole columns will support synthesis of oligonucleotides to 150 bases on the above cycles with 0.05 M phosphoramidites.
- The LOW cycle on the Model 391 is not recommended for synthesis of oligonucleotides greater than 50 bases with 0.05 M phosphoramidites and the 40-nanomole columns.

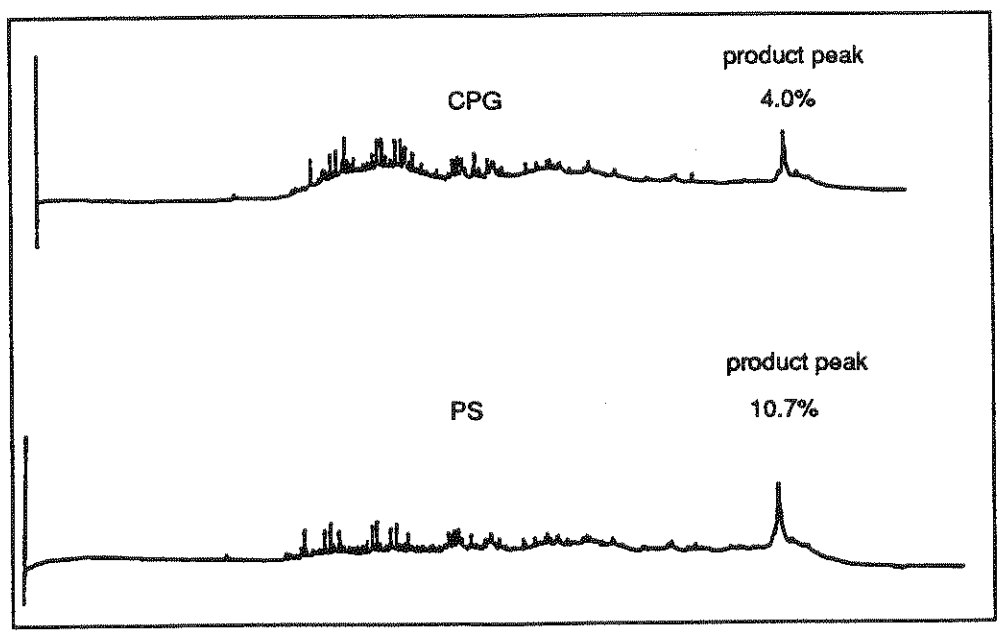

Figure 3. Comparison of crude 120-mers on CE. Syntheses conducted on a 391, with .2 UM cycle, and 0.05 M phosphoramidites.

Trityl Yields

Repetitive trityl yields are consistently at least 98-99% when used as described above. Because of the small amount of trityl released each cycle, an accurate reading of the trityls produced is difficult. Users who wish to read trityls may find it more convenient to dilute trityl solutions to 5.0 mL rather than 10.0 mL. The trityl concentrations will be more appropriate, and less 0.1 M p-toluenesulfonic acid in acetonitrile will be consumed.

Monitoring the trityl cation can be important, but the results must be interpreted with caution. The trityl assay is only an indirect measure of synthesis efficiency. Certainly, trityl cation yields of 98-99% must be present for a good synthesis. However, high trityl yields can be present even when a poor synthesis occurs because this chemistry, although highly refined, is not perfect. Unwanted side reactions do occur, some of which contribute to the trityl cation released each cycle. Sites other than the 5' hydroxyl group can participate in coupling (e.g., branched sites on the nucleic bases).

Low trityl yields (≤ 97%) always predict a less than optimal synthesis. In practical terms, trityl yields of less than 95% do not allow successful synthesis of even short oligonucleotides. For the above reasons, the trityl assay must be viewed only as a preliminary yet convenient monitor of the synthesizer's performance. The assay is useful for the early detection and diagnosis of instrument-related problems. Many laboratories monitor their trityl fractions only by visual inspection. With experience, a failed synthesis is detected this way. Evaluation of the oligonucleotide by PAGE, HPLC or CE is much more informative than the trityl assay. Synthesizer or reagent problems can be adequately diagnosed only by these methods, which are *direct* analyses of the product.

Yield

Most users will find that the 40-nanomole scale provides sufficient quantities of oligonucleotide for their experiments. This scale is compatible with a single OPC purification. With 40-nanomole polystyrene, a typical primer gives 5-10 crude ODU (150-300 µg) and 1-2 OPC-purified ODU (30-60 µg).

Protecting Groups

The current 40-nanomole columns (PN 401072-401075) have the standard protecting groups A^{bz}, G^{ib}, and C^{bz}. Because T has no protecting group, T columns are also compatible with FOD phosphoramidites.

Cleavage and Deprotection

Covering the ammonia collection vials that are used in 40-nanomole syntheses with Teflon-lined caps is especially important. Ammonia will leach a contaminant from Wheaton rubber-lined caps (catalogue no. 225002, for 4-mL vials) into the DNA solution. This contaminant absorbs at 260 nm and 310 nm and will be seen as a fast moving band in a UV shadow gel of the product. The ratio of contaminant to DNA is such that it might not be seen in larger scale syntheses but is readily apparent at the 40-nanomole scale. Teflon-lined caps may be obtained from Baxter (catalogue no. B7508-1). For more details, see User Bulletin No. 32 (July 1986).

A very small amount of pyridine can be retained in the column from the oxidation step, only to be washed into the collection vial along with the DNA during cleavage. This is not noticeable at larger synthesis scales, but at the 40-nanomole scale it may be detected in a UV spectrum of the diluted ammonium hydroxide by the spikes of the pyridine absorbance superimposed on the normal DNA profile. The pyridine should be of no concern because it is easily removable either by evaporation or by OPC. Users who are still concerned may incorporate a 60-second acetonitrile wash at the beginning of the end procedure (before the 60-second reverse flush) to remove the pyridine before cleavage starts. The deprotection time in ammonia for oligonucleotides made on 40-nanomole polystyrene columns with standard protecting groups remains at 8-24 hours at 55° C.

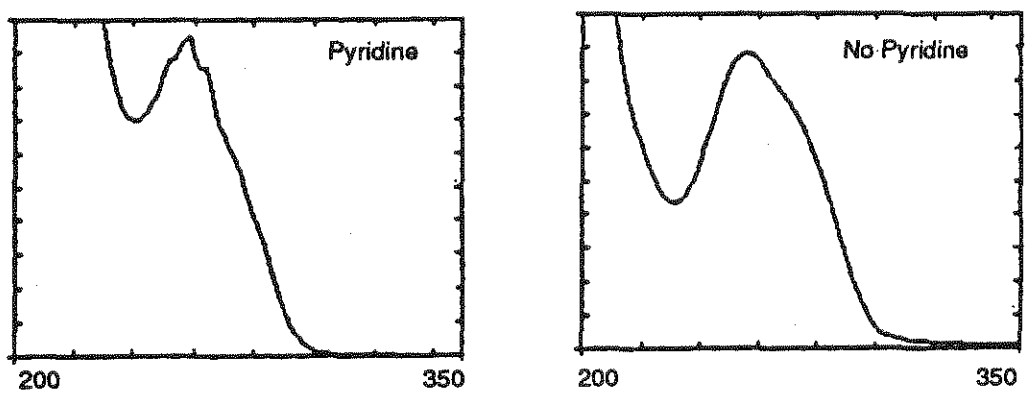

Figure 4. DNA spectra with and without pyridine

Manual Cleavage

Manual cleavage should be performed in the column with a syringe, rather than by emptying the support into a vial containing concentrated ammonium hydroxide. A quick acetonitrile, methanol or ethanol wash and subsequent 60-second reverse flush is recommended before manual or auto cleavage of columns that have dried out since synthesis was performed.

References

1. Andrus, A. and McCollum, C. "A New Support for Automated Oligonucleotide Synthesis," presented at the Symposium on Solid Phase Synthesis, Oxford, U.K., August 1989.
2. Pon, R.T., Usman, N. and Ogilvie, K.K. (1988) "Derivatization of Controlled Pore Glass Beads for Solid Phase Oligonucleotide Synthesis," *Biotechniques* 6:768-775.
3. Andrus, A. and McCollum, C. "A New Support for Automated Oligonucleotide Synthesis," presented at the Ninth International Round Table: Nucleosides, Nucleotides and Their Biological Applications, Uppsala, Sweden, July 1990.
4. McBride, L.J., McCollum, C., Davidson, S., Efcavitch, J.W., Andrus, A. and Lombardi, S. (1988) "Oligonucleotide Purification Cartridge," *Biotechniques* 6:362-367.
5. Moring, S.E., Colburn, J.C., Grossman, P.D. and Lauer, H.H. (1990) "Analytical Aspects of an Automated Capillary Electrophoresis System," *LC•GC* 8:34.