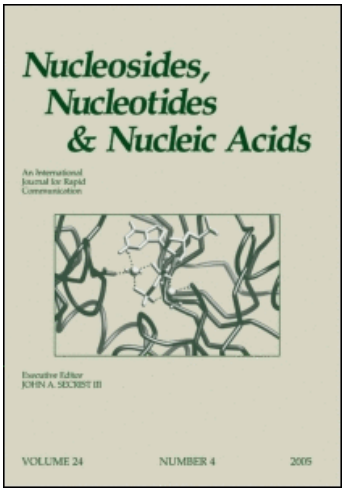

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OLIGOPREP PVA SUPPORT FOR OLIGONUCLEOTIDE SYNTHESIS IN COLUMNS ON A SCALE UP TO 10 μMOL

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□ *OligoPrep is a macroporous polyvinylacetate (PVA) biodegradable support that has been designed for cost-effective automated synthesis of oligonucleotides using standard phosphoramidite chemistry. Originally developed for large-scale oligonucleotide synthesis in beds and reactors, we present here its utility for medium-scale work of 1–10 μmol in column syntheses on standard DNA synthesizers. We show how an increase in scale, and, therefore, yield, can be achieved without significant increase in reagent quantity. Additional deblock and oxidation cycles can provide high coupling yields, and the use of concentrated ammonia in aqueous methylamine (AMA) for oligonucleotide cleavage and deprotection results in excellent recovery.*

Keywords OligoPrep; biodegradable; polyvinylacetate; medium-scale DNA synthesis

INTRODUCTION

The need for manufacture of synthetic oligonucleotides on a large scale using solid-supported phosphoramidite chemistry has significantly increased over the last decade due to their proven and potential use in therapeutics. The commercial success of such technology will, of course, depend on cost-effective manufacture. In particular, the selection of a low-cost, optimized solid support is fundamental to successful therapeutic oligonucleotide synthesis.

Ideally a solid support should have the following properties: (1) its size and shape should facilitate easy manipulation and throughput of liquids; (2) it must swell appropriately in process solvents; (3) it must be compatible and inert to all process reagents and solvents and remain mechanically robust; (4) it should offer suitable chemical functionality for attachment of the first monomer and have inter-chain spacing conducive to synthesis of oligonucleotides of the desired length; and (5) it should be of low-cost and

OligoPrep is a trademark of Merck KGaA, Germany.

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disposable without serious damage to the environment. OligoPrep was developed to meet these criteria.[1,2]

The native PVA support bears a secondary hydroxyl chain attachment terminus that is used for functionalization up to 350 μmol/g. The support is designed to provide extensive distribution of these functional groups throughout a highly solvated polymeric matrix ideal for assembly of 20mer (primer-length) oligonucleotides, although sequences up to 40 bases in length have also been synthesized in high purity.[3] After nucleoside loading, any residual terminal OH groups are easily capped ensuring efficiency of oligonucleotide synthesis (supports with terminal NH_2 groups can be more difficult to cap leading to ~1–2% loss of product).

The present study extends the utility and scope of OligoPrep to medium-scale work of 1–10 μmol in column syntheses on standard DNA synthesizers.

RESULTS AND DISCUSSION

We initially investigated the use of dT OligoPrep support loaded to 244 μmol/g in columns under standard conditions. When using 1 μmol of OligoPrep (pre-swollen in acetonitrile) with a 1 μmol protocol on a MerMade 6, a >97.8% coupling efficiency (ave. >99%)[4] was obtained. The yield of oligonucleotide produced with CPG is around 70% from a 99% coupling. We quickly established that the 30% yield obtained from OligoPrep could be increased to an average of 75% by using AMA as the cleavage and deprotection solution after oligonucleotide synthesis. This could be further increased by the use of dichloromethane to pre-swell the support prior to synthesis. Using 1 μmol of OligoPrep with a 1 μmol protocol on an ABI 394 gave similar results to the MerMade. The coupling efficiency could be increased to >99% and the yield from 65% to >80% by increasing the number of deblock and oxidation steps to 3 of each.

We then looked into 5x scale-up using 0.2 μmol protocols. When using 1 μmol of OligoPrep (pre-swollen in acetonitrile) on the MerMade, >97% coupling efficiency was obtained. This was improved further by pre-swelling with dichloromethane and/or increasing the number of deblock and oxidation steps. No change in phosphoramidite quantity or concentration was required for yields in excess of 70%. Using 1 μmol of OligoPrep on the ABI, ≥97% coupling efficiency and a quantitative yield of oligonucleotide was produced when the support was pre-swollen with either acetonitrile or dichloromethane. This could again be increased to >99% with additional deblock and/or oxidations.

A stepped change in scale was examined using the 1 μmol protocol on the ABI. A trend of decreasing coupling efficiency with increasing quantity of oligonucleotide was produced (see Figure 1). The yield at the 10 μmol scale had dropped to 37% and although the coupling efficiencies were high

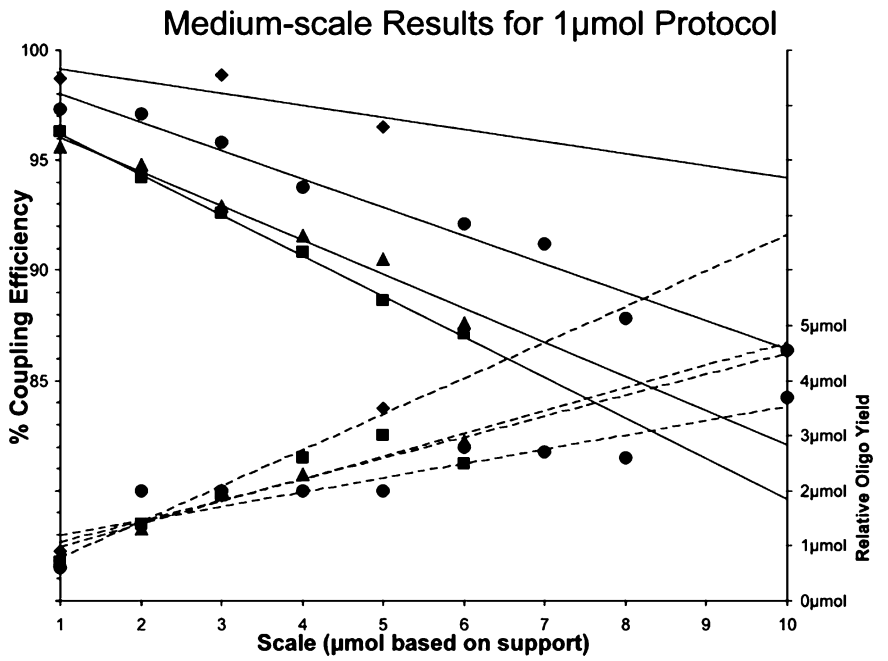

FIGURE 1 Percentage coupling efficiency and relative oligo yields for 1–10 μmol syntheses (based on amount of support) run on 1 μmol protocols on an ABI 394 DNA synthesizer. Key: ●—pre-swollen in MeCN, standard protocol; ▲—pre-swollen in MeCN, standard protocol (repeat); ♦—pre-swollen in MeCN, double deblock and oxidizer; and ■—pre-swollen in DCM, standard protocol. Lines drawn are linear trendlines.

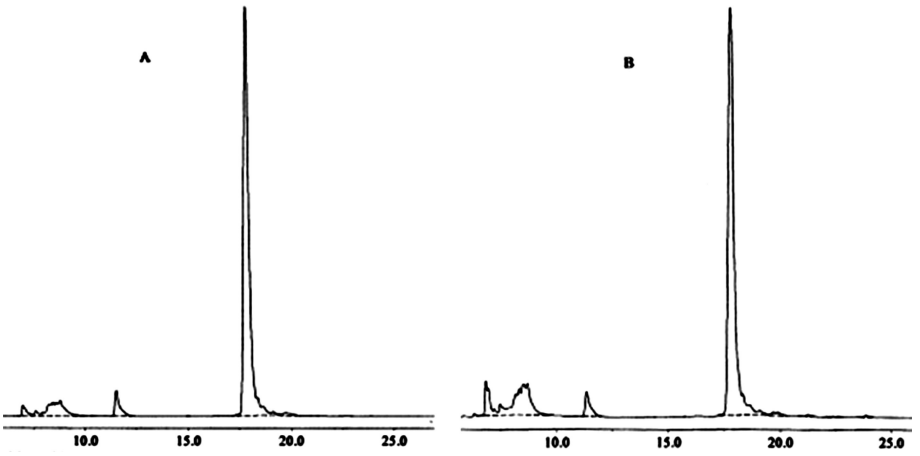

FIGURE 2 HPLC (C18 ODS Hypersil column, 0.1 M TEAA/MeCN) traces of a crude 10mer (TGTAGAGTCT) synthesized on an ABI 394 using (A) 1 μmol support, 1 μmol protocol, and (B) 5 μmol support, 1 μmol protocol.

at the 5 μmol scale, the yield was average. This reflects the decreasing number of equivalents of phosphoramidite. The maximum scale possible on the ABI without changing the phosphoramidite coupling step appeared to be 3μmol with a coupling efficiency of >98.7% and a yield of 70%.

A 10mer (TGTAGAGTCT) was synthesized on the ABI using either the standard protocol or with triple deblock and oxidation steps. In this case, the standard protocol furnished the best results and the yield at the 5 μmol scale was still in excess of 60% with AMA cleavage and deprotection, while the coupling efficiency was 96.1% (see Figure 2).

The flexibility of the MerMade 6 machine with respect to column size, phosphoramidite concentration and volume of delivery opens the possibility of synthesis scales previously unachievable for in-column synthesis. In fact, syntheses of up to 250 μmol on OligoPrep have recently been reported.[5]

CONCLUSIONS

We have shown that OligoPrep is an ideal support for oligonucleotide synthesis in columns up to 10 μmol. We have shown that it is possible to scale the synthesis in columns from 1–5 μmol without increasing the quantity of phosphoramidite. Syntheses have resulted in good quality and high yielding oligonucleotides. With the use of OligoPrep support, and exploiting the versatility of the MerMade 6, we intend to further increase the scale of synthesis.

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