Recycling of Controlled Pore Glass (CPG) Solid Supports for Oligonucleotide Synthesis

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Introduction

As the likelihood of commercially successful oligonucleotide-based therapeutics continues to increase, the need to reduce manufacturing costs becomes more significant. Although critical raw material costs have been reduced, solid synthesis supports need to be further reduced so that oligo manufacture can compete with other classes of APIs. The in-situ regeneration of nucleosidic supports has been proposed (1). However, the general need for in-process quality control testing presents a major complication. A more practical strategy would be to recycle spent supports as re-manufactured products. This approach becomes economically feasible at the metric ton level, where the costs of derivatization chemistry becomes less significant than the cost of underivitized CPG. This paper describes a pyrolysis-based recycling protocol that allows CPG solid supports to be regenerated in a non-destructive manner, resulting in a major cost reduction.

The attributes of CPG synthesis supports are: high specific surface area, dimensional stability in organic solvents, large and stable pore spaces and excellent thermal stability. The structure of CPG manufactured to the initial nucleoside (succinate) base is shown in Figure 1. This covalent linkage is very stable towards the synthesis chemistry, yet allows easy cleavage of the oligo product by a base, such as concentrated aqueous ammonia. Post-cleavage, the spent support is typically disposed of. However, a significant savings could be realized if the base material could be recycled. When attempting to recycle spent synthesis supports, contaminants such as 10-15% of the oligo and fragments of the support linker must first be removed as these would not only impede reactive sites on the CPG, but could also cleave along with the next generation synthesis product, lowering its purity. The main challenge of recycling is to remove these impurities without modifying the original support matrix. A common way of cleaning organic contaminants, such as adsorbed proteins from underivitized, or “native” CPG involves heating in air at temperatures from 600-700°C (2). In this work, a CPG regeneration protocol, based on the pyrolysis of post-cleavage contaminants, developing and evaluated for recycling of spent CPG synthesis supports. The number of regeneration cycles that CPG can endure before replacement with new material is the key parameter in determining the possibility of degradation. Possible degradation markers that could alter CPG include: leaching of pores by ammonia cleavage protocols, breakdown of CPG particles by agitation during washing steps or thermal stresses during pyrolysis, and residual trace organics left after pyrolysis. This study, CPG was examined for these problems after each of a series of regeneration cycles. A cost reduction model, based on projected derivitization chemistry costs at multi-ton nucleoside CPG production, is shown in Figure 2.

Objective & Background

Methods

Price Reduction (%) vs # of Recycles

Figure 1: Nucleoside succinate base covalently coupled to CPG through an intermediate linker, showing base cleavage point for final oligo.

Table 1: Physical and chemical data obtained for samples at the end of each regenerative cycle, along with the corresponding specification ranges.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pore Size (Å)</th>
<th>Bulk Density (g/cc)</th>
<th>Porosity (％/cc)</th>
<th>Fineness Produced (% by wt)</th>
<th>Amino Loading (mmole/g)</th>
<th>Nucleosidic Loading (mmole/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>524</td>
<td>0.30</td>
<td>1.4</td>
<td>189</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>Cycle #1</td>
<td>521</td>
<td>0.31</td>
<td>1.2</td>
<td>2</td>
<td>188</td>
<td>84</td>
</tr>
<tr>
<td>Cycle #2</td>
<td>542</td>
<td>0.29</td>
<td>1.5</td>
<td>5</td>
<td>105</td>
<td>80</td>
</tr>
<tr>
<td>Cycle #3</td>
<td>542</td>
<td>0.29</td>
<td>1.5</td>
<td>5</td>
<td>105</td>
<td>80</td>
</tr>
<tr>
<td>Cycle #4</td>
<td>542</td>
<td>0.29</td>
<td>1.5</td>
<td>5</td>
<td>105</td>
<td>80</td>
</tr>
<tr>
<td>Cycle #5</td>
<td>580</td>
<td>0.25</td>
<td>2.5</td>
<td>3</td>
<td>211</td>
<td>88</td>
</tr>
</tbody>
</table>

Figure 2: Cost Reduction Model

Although the cost savings for nucleoside CPG is 57% after only five regeneration cycles, it begins to drop off quickly with each subsequent cycle. Thus, data was collected for CPG over five recycle periods.

Results

Table 2: Summary of average yield and average purity data after three synthesis runs on CPG initially, and after cycle 3 & 5. This data indicates that for up to 5 cycles, the CPG produced synthesis results that were comparable to those of the original nucleoside support.

<table>
<thead>
<tr>
<th>Recycle #</th>
<th>OD325 (A/mg)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Initial)</td>
<td>108</td>
<td>83</td>
</tr>
<tr>
<td>3</td>
<td>89</td>
<td>91</td>
</tr>
<tr>
<td>5</td>
<td>88</td>
<td>89</td>
</tr>
</tbody>
</table>

Figure 3: Typical recycle/regeneration procedure

This approach becomes economically feasible at the metric ton level, where the costs of derivatization chemistry becomes less significant than the cost of underivitized CPG. This paper describes a pyrolysis-based recycling protocol that allows CPG solid supports to be regenerated in a non-destructive manner, resulting in a major cost reduction.

Discussion

With each cycle, except #2, the data shifted as follows: pore size and pore volume increased, bulk density decreased, and the formation of some fine particles was observed. For cycle #2, the pore size and pore volume decreased slightly, and the bulk density showed a slight increase. These physical data trends are consistent with an expected slight dissolution of silica by the ammonia cleavage solution with each cycle. The exception in cycle #2 is consistent with the formation of precipitated silica in the pores of the support after evaporation of the ammonia. This material would not dissolve in subsequent washes which were insufficiently alkali in pH. This material did, however re-dissolve in the subsequent cycle, which supports the observed larger than average increase in pore size and pore volume after cycle #3. Although a small percentage of fines formed with each cycle, an overall attrition of less than 10% would occur over five cycles due to this effect.

Summary

The overall impact of five regenerative cycles caused insufficient changes to bring the physical parameters of the CPG outside of its specification range. The re-dissolution of amine and nucleoside loadings at each cycle, were good indications that a favorable, clean surface existed after each cycle. The reproducibility of synthesis results further confirmed that the CPG support was suitable for use, even after five regenerative cycles. Thus the CPG supports were shown to be recyclable to a point where almost 60% of their costs could be reduced. With this savings and the economies of scale projected for multi-ton production, nucleoside CPG should sell for $1.50-$2.00 per gram in the future.

Acknowledgements

We would like to thank Albert Rothstein for his help in performing these experiments.

References


Figure 4: Representative IE HPLC chromatograms for the 13-mer test sequence oligonucleotides.

Curves are vertically offset for clarity.

These data indicate that for up to 5 cycles, the CPG produced synthesis results that were comparable to those of the original nucleoside support.

Figure 3: Typical recycle/regeneration procedure

The only exception to this protocol was for cycle 2, where the ammonia solution was evaporated from within the CPG pores prior to washing. This deviation was introduced in an effort to shorten the DI washing time required to bring the pH to neutrality. 13- Mer test sequence oligonucleotides were synthesized on the original support, and cycle # 3 and after cycle # 5 using an AKTA 10 synthesizer (GE Healthcare).

Nucleosides Nucleotides

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