BACK TO STIRRED VESSELS AND ON TO METRIC TONS OF OLIGONUCLEOTIDES

Scientists seek to discover new production routes when manufacturing limits arise. Returning to stirred-bed vessels is an innovative way of going forward by returning to the roots while bringing in the necessary chemical and engineering adaptions. The first DNA synthesizers in the 1980s already made use of vessels for stirred bed technology. In the last decades, however, oligonucleotide manufacturers prevailed solid-phase oligonucleotide synthesis (SPOS) in fixed-bed flow through column systems as industry standards. These flow-through oligonucleotide synthesizers allowed better automation, fast chemistry, and low cycle times.

WHY LOOK FOR A NEW SOLUTION FOR OLIGONUCLEOTIDE SYNTHESIS?

Large-scale production of oligonucleotide-based active pharmaceutical ingredients (APIs) is needed, with hundreds of them in advanced clinical trials and a potential metric ton demand. However, the scalability of the manufacturing with flow-through synthesizers is limited by many factors like the sizes of the pumps generating the flow or the thickness of the solid-phase resin bed, in short: the bed height. This thickness is critical because only an optimal bed height for a given flow in flow-through synthesizers ensures that the reaction mixture flows evenly through the resin, allowing efficient coupling reactions. Swelling of the solid phase resins is not exploitable. That's why a scale of over 2 mol of oligonucleotide per batch – corresponding to a double digit-kilogram-scale for an average oligonucleotide – is unprecedented.

In addition, there is another important disadvantage of this technology, a disadvantage for both small and large batches: the concentration gradient over the column, as seen in Figure 1, resulting in batch inhomogeneity. Better batch homogeneity can be achieved by getting away from the flow-through column

principle to agitated-bed systems. This inhomogeneity of conventional packed bed flow SPOS is completely omitted in the SBT-SPOS due to uniform reagent contact time and distribution.

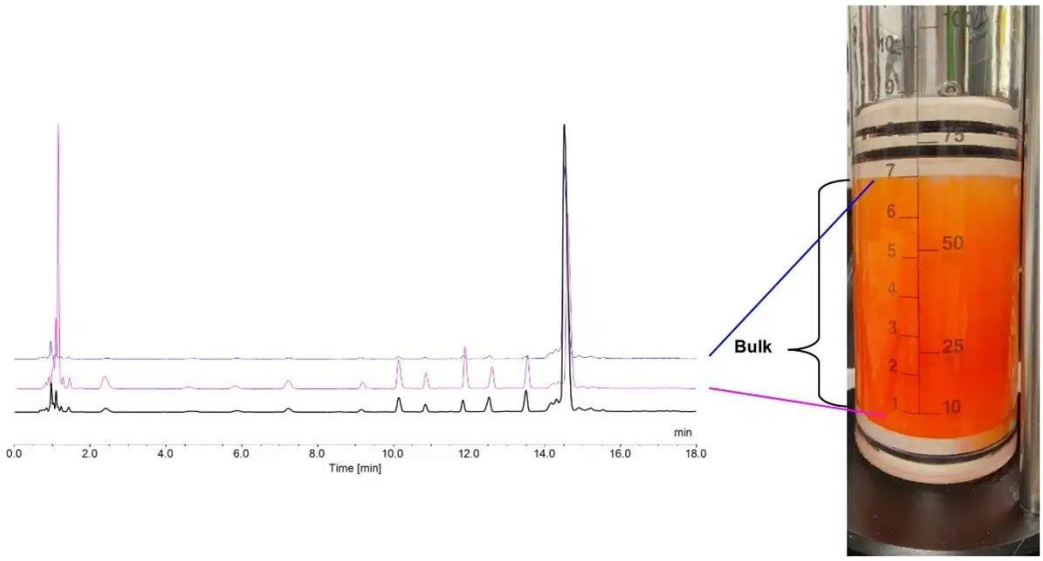

Figure 1 – Flow through SPOS (hover to learn more)

When our scientists started the journey back to stirred vessels, they wanted to demonstrate the feasibility of scale-up to the large scale. They established a toolbox and generic process parameters that apply to most oligonucleotides.

One milestone in this way was solving the problem of depurination. This side reaction, the loss of purine nucleobases in the produced oligonucleotides, as shown in Figure 2, occurs due to the acidic conditions during the so-called detritylation, the removal of the protection groups in SPOS. And this is an unwished effect that decreases yields and purity more prominent in stirred vessels than in flow-through columns.

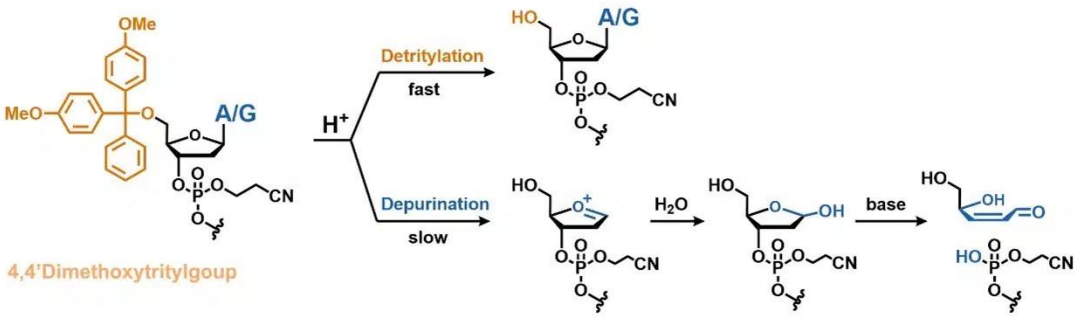

Figure 2 – Depurination is a side reaction during deprotection.

Many experiments tested different reagents, acids, and scavengers to find new deprotection protocols to suppress this side reaction. As a result, the experts moved away from using the common dichloroacetic acid and toluene cocktail during deprotection. Instead, they used an acid with a higher pKA value, a mixture of toluene with trifluoroethanol and special scavengers for the deprotection.

Meanwhile, over 500 lab-scale SPOS applying SBT to make oligonucleotide-based API have been conducted at Bachem. Several case studies were performed at a multi-gram scale with “real” therapeutic oligonucleotide sequences.

STIRRED-BED IN SPOS FOR LARGE-SCALE OLIGONUCLEOTIDE PRODUCTION

As it turned out, SBT is not only suitable for making full-length product sequences. It is also ideal for hybrid approaches: short fragment synthesis with typically less than 10 nucleotides at the hundred-kilogram scale followed by either chemical or enzymatic ligations with no chromatography steps involved. With SBT, the use of greener and more sustainable solvents is possible and the subject of intense investigations at Bachem.

Combining highly efficient synthesis of oligonucleotides with the highly effective purification by multicolumn countercurrent solvent gradient purification (MCSGP) systems[1], which Bachem installed as the first Contract Development and Manufacturing Organization in the field, a further reduction of solvents and improvements of the process mass intensity was achieved. Thus, SBT is a highly efficient process for industrial scale with the benefits:

- Batch and thus product homogeneity
- Swelling of solid support resin acceptable
- Application of high-loading solid-support resin feasible
- Seamless scalability
- On-resin modifications possible
- Potential to save solvents and reduce process mass intensity (PMI)

The PMI is a key performance indicator for solvent consumption. With SBT, a drastic reduction of solvent consumption and the PMI can be achieved by the higher loading of the solid phase resin and the reduction of solvents used for washing.

In a nutshell: The innovative SBT for SPOS represents an economical engineering solution with a simple reactor design and adapted chemistry enabling ton-scale commercial oligonucleotide API manufacture with unmatched process mass intensity.

Read more about manufacturing oligonucleotides with stirred-bed technology in our white paper.

REFERENCES

[1]White paper from Bachem: Continuous Chromatography – Pushing the boundaries of peptide and oligonucleotide production