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WHITE PAPER

Solid-Phase Oligonucleotide Synthesis (SPOS) and Liquid-Phase Oligonucleotide Synthesis (LPOS): Complementary or Competitive Processes?

Yong Xu, Consultant II, Pharmaceutical Development

Oligonucleotide synthesis has made significant progress, driven by the growing popularity of oligonucleotide-based therapeutics in drug discovery, as well as the increasing number of approved oligonucleotide drugs. Unlike traditional small molecule drugs that primarily act on proteins, oligonucleotide therapeutics function by binding to specific mRNAs, enabling the regulation of targets that were considered undruggable by conventional small molecules. This unique mechanism makes oligonucleotide therapeutics theoretically applicable to virtually any therapeutic targets. Since the approval of the first therapeutic oligonucleotide, Fomivirsen (Vitravene), by the U.S. FDA in 1998, oligonucleotide-based therapies have emerged as powerful therapeutics for the treatment of human diseases. Recent innovations and breakthroughs in oligonucleotide delivery technologies have paved the way to various oligonucleotide modalities for tackling previously considered undruggable targets and unmet medical needs.

Currently, a total of 20 oligonucleotide drug products has been commercially approved by the FDA and EMA to date.

Hundreds of oligonucleotides are currently in active clinical trials, which, has created a high demand for various oligonucleotide modalities, including antisense oligonucleotide (ASO), small interfering RNA (siRNA), aptamer, gapmer, microRNA, small activating RNA (saRNA), etc.[1] Additionally, as CRISPR-Cas gene-editing technologies progress to clinical trials and commercialization across multiple companies, the need for chemically synthesized guide RNA (gRNA) is also driving advancements in oligonucleotide development.

gRNA, a single-stranded oligonucleotide typically 80-100 nucleotides long, guides the CRISPR-Cas system to a specific DNA target for gene editing. The synthesis of highly pure gRNA is crucial to ensuring the precision, safety, and efficiency of CRISPR gene editing.

Traditionally, oligonucleotides (ONs) are synthesized with high yield and purity using an efficient, automated DNA/RNA synthesizer based on phosphonamidite chemistry. This process follows a four-step coupling cycle, detritylation, coupling, oxidation/thiolation, and capping, on a solid support resin. Today, scaling up oligonucleotide synthesis to kilogram quantities has become a routine and well-established practice, primarily using the Solid-Phase Oligonucleotide Synthesis (SPOS) approach.[2]

Solid-Phase Oligonucleotide Synthesis (SPOS):

With over 40 years of development, Solid-Phase Oligonucleotide Synthesis (SPOS) has become the mainstream protocol for oligonucleotide production, offering several advantages, including high efficiency, high purity, scalability, and automation. Through decades of optimization, solid-phase synthesis has achieved nearly 100% coupling efficiency, improved process control, and efficient byproduct separation using a resin support, primarily controlled pore glass (CPG).[2]

As a result, SPOS typically delivers approximately 50% yield and 90% purity for oligonucleotides ranging from 20- to 40-mer. It is scalable, making it ideal for producing large quantities of oligonucleotides at kilogram scales. SPOS is a highly efficient and labor-saving process, thanks to automation and programmability, which minimizes human error while enhancing efficiency and reproducibility. Even for kilogram-scale synthesis, the production of 20- to 40-mer oligonucleotides can be typically completed in approximately two days.

However, SPOS also has several limitations, primarily due to the large excess of reagents required and the substantial solvent consumption, particularly acetonitrile used for washing at each synthetic step. Because of its heterogeneous nature, SPOS necessitates excessive reagent use to ensure reaction completeness, significantly increasing production costs. This is especially true for the expensive oligonucleoside phosphonamidites used in each cycle. For example, each coupling step typically requires two equivalents of amidite and five equivalents of coupling activator.

From a green chemistry and Process Mass Intensity (PMI) perspective, SPOS is not an ideal process and has considerable room for optimization. PMI is a key metric for assessing the sustainability of an API manufacturing process, measuring the total mass of materials used per unit of product. The PMI for typical 20-mer therapeutic oligonucleotides ranges from 3,035 to 7,023, with an average of 4,299, whereas PMIs for small molecules generally range from 168 to 308.[3] A significant contributor to the high PMI in SPOS is acetonitrile consumption, which can reach up to 1,000 kg per kg of oligonucleotide, making its environmental footprint considerably larger than that of small-molecule synthesis.

In addition, SPOS is generally limited to scales of less than 10 kilograms due to nonlinear flow rates caused by increasing column sizes and reduced coupling efficiency associated with large-scale heterogeneous reactions. The high cost of large-scale anion exchange (AEX) and high-performance liquid chromatography (HPLC) purification further restricts the scalability of SPOS. Moreover, SPOS requires specialized equipment, including automated synthesizers and high-pressure chromatography systems, which demand a minimum investment in the tens of millions of dollars and require significant capital expenditure for a kilogram scale production line.

Liquid-Phase Oligonucleotide Synthesis (LPOS):

The development of Liquid-Phase Oligonucleotide Synthesis (LPOS) has gained increasing research interest in recent years. LPOS is designed to overcome the limitations of SPOS, particularly in scalability, cost reduction, Process Mass Intensity (PMI), and reducing the reliance on chromatography-dependent purification.[4]

Unlike SPOS, where synthesis occurs on a solid resin support, LPOS is performed entirely in solution, like conventional homogeneous organic synthesis. The growing oligonucleotide chain in LPOS remains attached to a soluble tag or anchor, which provides unique properties that facilitate solution-phase chemistry, as well as the workup after each cycle. The purification process typically involves precipitation in an antisolvent using a polar solvent or an alcohol-based antisolvent. If precipitation is not feasible, then the removal of small molecule impurities and by-products can be achieved through nanofiltration technology.

A key requirement for LPOS is the solubility properties of the tag, which must be distinct from those of the reagents and byproducts to enable efficient removal through precipitation, filtration, or extraction. Common soluble tags used in LPOS include small, well-defined molecules and soluble polymers. Among these, the most advanced and scalable options are the AJIPHASE® anchor and PEG-based polymers. These molecules are highly soluble in reaction media such as dichloromethane (DCM) and acetonitrile but insoluble in polar solvents like alcohols, allowing for effective separation of the desired product from byproducts and excess reagents.

Since LPOS is performed in a homogeneous medium and follows linear reaction kinetics without the constraints of resin saturation, it eliminates the need for excess reagents at each step. Additionally, the solvent consumption in LPOS is several orders of magnitude lower than in SPOS. Furthermore, LPOS can be carried out in conventional batch or flow reactors, eliminating the need for expensive automated synthesizers required for SPOS. As a result, LPOS enables large-scale production, potentially yielding materials in ton-scale quantities with lower PMI and reduced costs. Furthermore, LPOS enables real-time, continuous in-process control (IPC) without the need for destructive sample analysis. In contrast, SPOS often requires cleavage from the solid support for analysis, leading to delayed IPC results, as the cleavage process takes at least 15 minutes at elevated temperatures or longer.

More recently, Ajinomoto Bio-Pharma Services utilized their AJIPHASE® technology and platform, which employs soluble anchor molecules composed of a phenyl core functionalized with long alkyl chains (>C10). Originally developed for liquid-phase peptide synthesis, this technology has been successfully adapted for oligonucleotide synthesis at scales of up to 200 kilograms.

In this process, detritylation, coupling, oxidation or sulfurization, and capping steps are performed sequentially in a one-pot system, eliminating the need for intermediate separations. The growing oligonucleotide chain is precipitated in an antisolvent only once per nucleotide extension cycle, significantly reducing solvent consumption and enhancing scalability.

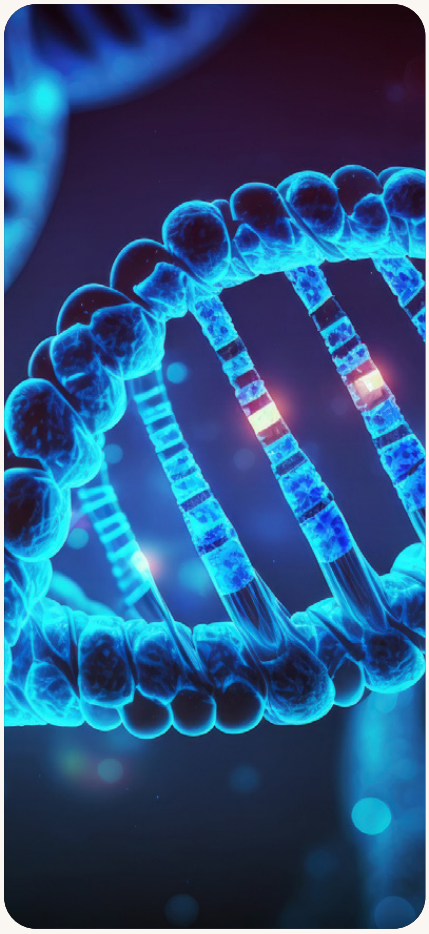
It has been reported that this method enabled the synthesis of a 21-mer oligonucleotide with a 60% yield and a high purity of more than 90% following chromatographic purification.[5] In addition to regular oligonucleotides and peptides, AJIPHASE® technology has also been applied to the synthesis of phosphonodiamidite morpholino oligomers (PMOs) in 10-kg batches to support clinical trials. Most notably, it was recently reported that the FDA has approved the production of a commercial oligonucleotide, whose structure remains undisclosed, using AJIPHASE® technology.[5]

Comparison Summary[3]:

Feature	Solid-Phase Synthesis (SPOS)	Liquid-Phase Synthesis (LPOS)
Efficiency	High	Comparably high
Scalability	~ 10 KG	~ 200 KG
Purity	High	Comparably high
Cost	Expensive reagents and solvents	Lower reagent and solvent costs
Waste Generation	Significant waste due to resin and solvents	Less waste
Automation	High level of automation, faster synthesis	Less automated, relatively slower synthesis
Equipment	Costly and special synthesizer	No additional special equipment
PMI	Higher, 3,035 to 7,023	Lower, up to 90% less compared to SPOS

Conclusion

Despite their differences and some competitive aspects, SPOS and LPOS are best considered complementary rather than purely competitive methods, as each excels in different areas of oligonucleotide synthesis. SPOS is optimal for high-throughput, efficient production at mg to kg scales, while LPOS offers a more cost-effective, environmentally friendly solution for larger-scale manufacturing and enables specific modifications that may be challenging to achieve with SPOS. The choice between the two depends on various factors such as oligonucleotide chain length, chemical modifications, production scale, budget, and timeline.



The complementary methods of oligonucleotide synthesis featured in this review highlight their differences as well as common principles. As an emerging and rapidly growing field, oligonucleotide synthesis and manufacturing are experiencing increasing demand in both academia and industry. We are optimistic that continued scientific and technical advancements in oligonucleotide synthesis will further enhance the sustainability of Solid-Phase Oligonucleotide Synthesis (SPOS) and Liquid-Phase Oligonucleotide Synthesis (LPOS), leading to greener, more cost-effective, and scalable processes to meet the growing challenges of therapeutic oligonucleotide manufacturing. Liquid-Phase Oligonucleotide Synthesis (LPOS) is a relatively new approach and is still in the early stages of development but has significant potential for further optimization, advancement, and breakthroughs.

In summary, SPOS is a well-established approach for oligonucleotide synthesis but is inherently limited by its heterogeneous reaction nature. In contrast, LPOS, as a homogeneous synthesis process, offers a promising pathway toward a more economical, scalable, and environmentally sustainable alternative with continuing technological development.

ABOUT

Syner-G

At Syner-G, we understand that successful oligonucleotide manufacturing requires both mastery of established methodologies such as SPOS and practical expertise in emerging approaches like LPOS. Our team provides end-to-end support in process development, scale-up, analytical characterization, and regulatory guidance to help partners optimize these complementary technologies. Whether the need is rapid, high-throughput synthesis or scalable, sustainable manufacturing solutions, Syner-G is committed to delivering scientifically rigorous, cost-effective strategies tailored to each program's unique requirements.

To explore how our expertise can accelerate your oligonucleotide development, we invite you to connect with us today.

REFERENCES

1. B. P. Vinjamuri, J. Pan, P. Peng, A Review on commercial oligonucleotide drug products. Journal of Pharmaceutical Sciences 2024, 113, 1749–1768.
2. R. Obexer, M. Nassir, E. R. Moody, P. S. Baran, S. L. Lovelock, Modern approaches to therapeutic oligonucleotide manufacturing. Science 2024, 384, eadl4015.
3. B. I. Andrews, F. D. Antia, S. B. Brueggemeier, L. J. Diorazio, S. G. Koenig, M. E. Kopach, H. Lee, M. Olbrich, A. L. Watson, Sustainability Challenges and Opportunities in Oligonucleotide Manufacturing. J. Org. Chem. 2021, 86, 49–61.
4. P. Rosenqvist, V. Saari, M. Ora, A. G. Molina, A. Horvath, P. Virta, Tuning the Solubility of Soluble Support Constructs in Liquid Phase Oligonucleotide Synthesis. J. Org. Chem. 2024, 89, 13005–13015.
5. Ajinomoto company websitehttps://www.ajinomoto.com/innovation/our_innovation/ajiphase

Author: Yong Xu, Synger-G

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