Understanding the Oligonucleotide Landscape

Though attracting growing interest in drug development circles, oligonucleotide-based therapeutics present manufacturers with specific processing and characterization challenges

Oligonucleotide-based therapeutics represent the changing face of drug discovery and development. Designed to prevent or modulate the translation of a specific gene, these therapies have the potential to tackle previously undruggable targets and could set the tone for the future of personalized medicine. As of January 2020, the FDA has approved 10 oligonucleotide drugs, but the number of therapeutics moving into and through the development pipeline is blossoming with six of these oligonucleotide drugs approved between 2016 and 2019 (1). Here, Genentech associate scientist Alexandre Goyon outlines the pros and cons of currently available techniques for characterization of oligonucleotides – and considers what the technologies of tomorrow might look like.

What are the main types of oligonucleotides?

When it comes to pharmaceutical clinical development, there are two main categories of oligonucleotides:

- Antisense oligonucleotides (ASOs) – single-stranded, typically 10 to 30 nucleotide units
- Small interfering RNAs (siRNAs) – double-stranded, each strand containing typically 19 to 23 nucleotides.

Each class of oligonucleotide comes with its own advantages and limitations. For example, siRNAs can be more potent than ASOs for some targets as they involve different enzymes for the RNA cleavage mechanism of action, i.e. RNase H1 for ASOs and Ago2 for siRNAs, but siRNAs can lack stability and effective strategies must be employed to ensure their delivery to target sites. Lipid nanoparticles can be used as carriers to transport siRNAs to the target cell's cytoplasm and improve siRNA stability against ribonucleases. siRNAs can also be conjugated to a ligand called GalNAC for transportation to the liver.

What's the regulatory view on oligonucleotides?

Oligonucleotides are considered small molecules since they are manufactured by solid phase synthesis. However, as mentioned by Mohan Sapru of the FDA, there is no ICH and FDA regulations that specifically address the quality expectations of the diverse oligonucleotide products (2). Discussions about legislation are ongoing!

Some of the challenges are addressed in two industry-led white papers produced by a collaborative team from Ionis Pharmaceuticals, AstraZeneca, GlaxoSmithKline, Sanofi-Aventis, Janssen Pharmaceutical, F. Hoffmann-La Roche, Biogen and Novartis in 2017 and 2020 (3,4). In the papers, the authors issue advice on myriad chemistry challenges affecting oligonucleotides and encourage information sharing and chemistry, manufacturing, and control (CMC) harmonization strategies between research groups to reduce the risks that these therapies can potentially pose to human health. They have sparked much discussion about the next steps regulators could take.

What analytical technologies are used for the characterization of oligonucleotides?

There are several, including (5):

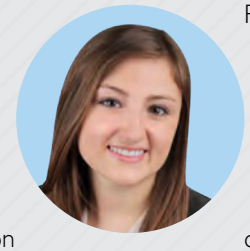
- Ion pairing reversed-phase (IPRP) chromatography – the most common method for the analysis of oligonucleotide impurities. It can separate shortmers, longmers, and phosphodiester (PO) impurities, among others. It can be coupled to mass spectrometry in particular when combining the alkylamine ion-pairing agent with hexafluoroisopropanol (HFIP).
- Anion exchange chromatography – this can also be used to separate impurities, but the large amounts of non-volatile salts involved prevents its direct coupling to mass spectrometry, which is one reason why IPRP is more widely used.
- Hydrophilic interaction chromatography (HILIC) – there have been surprisingly few published reports about the use of HILIC for oligonucleotides. HILIC has the advantage of allowing direct coupling to mass spectrometry without using ion-pairing agents. There is a great diversity of stationary phases, which could provide orthogonal separation to IPRP and thus be complementary.
- Size exclusion chromatography – this method is used with double-stranded oligonucleotides such as siRNAs in order to determine the number of single strands in the double-stranded product using non-denaturing conditions.

How would you like to see analytical technology in this area improve?

The first oligonucleotides were investigated about 30 years ago and it is only in recent years that the industry's interest in them has been renewed in part due to the advances in oligonucleotide chemistry engineering and delivery systems. In my

Oligonucleotides: Perfecting Purification

By Manuela Sevilla, technical expert at Tosoh Bioscience



For the companies pursuing oligonucleotide-based therapies, the relative complexity of their synthesis can pose purification challenges. Oligonucleotides are short DNA or RNA molecules that are "grown" via the addition of nucleotide groups in a series of solid-phase synthesis cycles – a failure in this process can allow impurities to emerge. Some chemical modifications are necessary to improve their pharmacokinetics and stability. These could result in another source of impurities.

Irregularities in the manufacturing process can lead to the formation of the following common impurities:

- Shortmers – oligonucleotides missing one or more nucleotides, or N-1
- Longmers – oligonucleotides that include more than the intended number of nucleotides, or N+1
- Lack of protecting groups (derivatives of existing functional groups that decrease reactivity and increase stability)
- Phosphodiester impurities (PO) in phosphorothioate oligonucleotides

A helping hand

Fortunately, when it comes to removing impurities that occur during oligonucleotide manufacturing, a range of chromatographic techniques ensure that the highest levels of purification can be achieved.

Anion exchange (AIEX) chromatography is the optimal solution for addressing variation in strand lengths – shortmers and longmers. AIEX separates oligonucleotides by the negative charge of the backbone. Because siRNAs are highly polar by nature, Tosoh's high-performance anion-exchange medium, TSKgel SuperQ-5PW (20), can easily separate unwanted synthetic strands from the desired product. Consisting of highly cross-linked hydroxylated methacrylic polymer beads and with a particle size of 20 µm, this resin offers both high resolution and high loading capacity – essential from a productivity point of view. And this resin is available as SkillPak pre-packed columns for faster method development!

Analytically minded

Characterization and quality control of oligonucleotides essentially apply the same modes of chromatography as purification. In AIEX chromatography, TSKgel (U)HPLC columns based on non-porous particles are available with the same surface and ligand chemistries as the TOYOPEARL and TSKgel purification media. TSKgel DNA-STAT or DNA-NPR columns offer fast kinetics and high-resolution separation.

Size exclusion chromatography (SEC) can also be applied in combination with light scattering detection. The TSKgel UP-SW2000 UHPLC column can separate oligonucleotides differing by one base in length. And when coupled with our new LenS3 multi-angle light scattering (MALS) detector, a detailed picture of the oligonucleotide purity can be achieved.

Options aplenty

At Tosoh Bioscience, we've amassed a wealth of experience in the development of chromatographic methods for downstream processes and characterization – and we are happy to help in any process optimization. From small-scale academic environments to big pharma, we can support all your growing needs; we pride ourselves in continually advancing our product offering in line with industry advances.

Oligonucleotides are the next frontier in biopharma – and we are there for you in your pursuit of a healthier future.

opinion, there are three main areas for improvement in the field of analytical chemistry. Firstly, it's important that we limit oligonucleotides' non-specific interactions with metal surfaces and, therefore, the use of truly biocompatible instruments and columns will become crucial. Secondly, the use of superficially porous particles may improve the separation of the larger oligonucleotides being developed today. Thirdly, the use of volatile mobile phase in cation exchange chromatography is an important trend for antibodies. Hopefully, a similar strategy could be applied for the anion exchange analysis of oligonucleotides, but column development would have to focus on reducing the amount of salt needed to elute oligonucleotides.

Developments in the oligonucleotide space are happening at a rapid pace, and it's truly exciting to be a part of this field! Regulators are paying increasing attention to these products and the approval of new therapies will certainly help us push the boundaries of their potential.

References

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