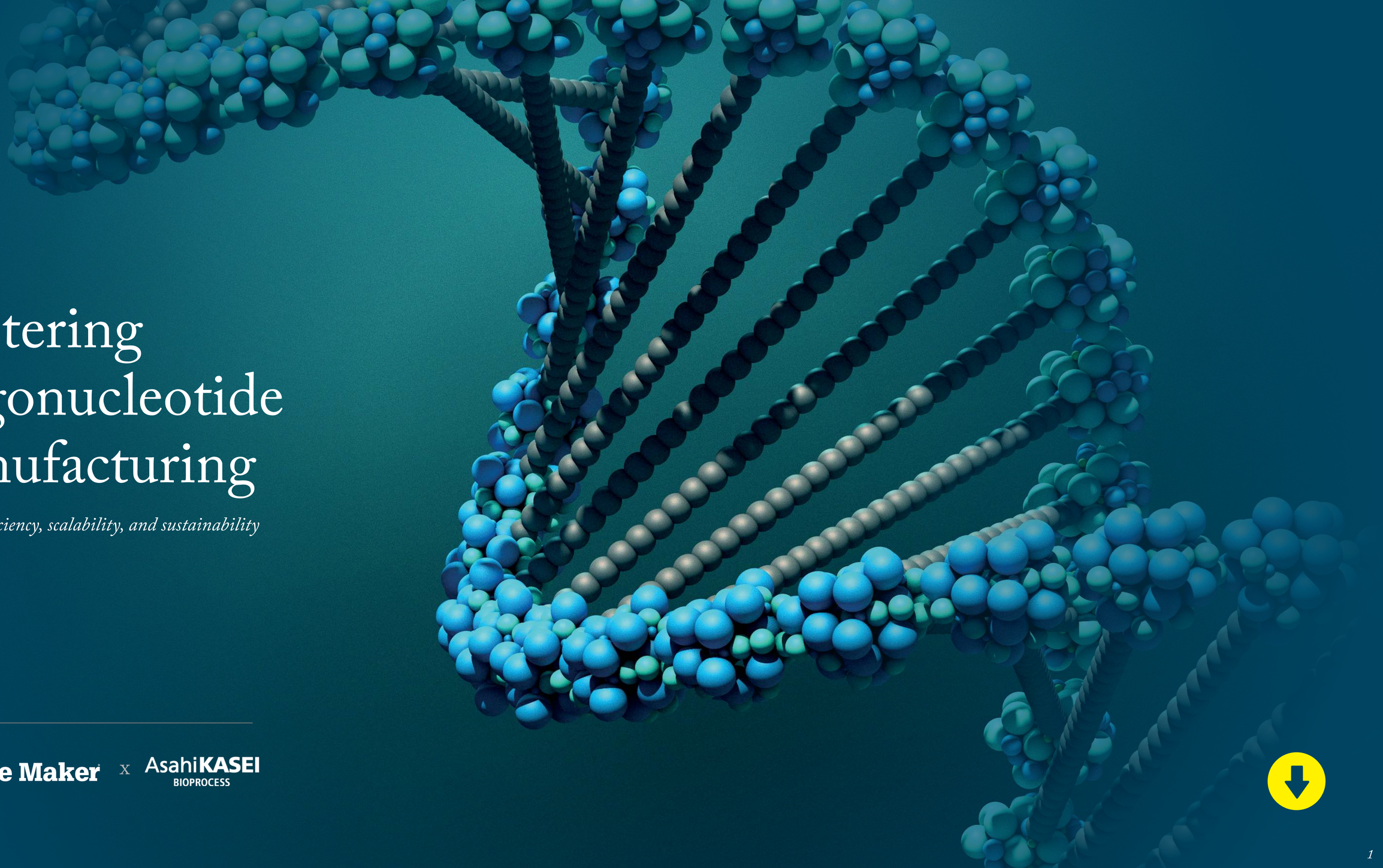
Mastering Oligonucleotide Manufacturing

Unlocking efficiency, scalability, and sustainability



the Medicine Maker x AsahiKASEI BIOPROCESS

Oligonucleotides: Getting Equipment Selection Right

The oligonucleotides market is growing rapidly, with drug developers increasingly attracted to this intriguing drug modality. But manufacturing can be challenging – and choosing the right equipment for the job is essential. Established suppliers – such as Asahi Kasei Bioprocess (AKB) – can not only provide valuable guidance on equipment selection based on your process but also help guide further optimization when producing these complex therapeutics.

Featuring Tom Krebstakies, Sales Manager for Europe and Asia at AKB Deutschland GmbH in Cologne, Germany.

Why are oligonucleotides such a hot topic in drug development?
Oligonucleotide therapeutics are characterized by high efficiency and high specificity. They can directly target the site of action, such as the regulation of gene expression, and have great potential for treating metabolic diseases, genetic diseases, and cancer, as well as preventing infectious diseases.

The science, production, and commercialization of oligonucleotides have all advanced quickly in recent years – and the global market has developed tremendously. The global oligonucleotide synthesis market was valued at around $7 billion in 2023 and is projected to potentially double or triple by 2030. The calculated CAGR is therefore easily >12 percent during the forecast period. Though delivery into the target cell has historically been a challenge, platforms have now emerged, such as LNPs or GalNAc conjugates for siRNAs for liver delivery. Now, the focus is also on developing delivery solutions for very specific tissues and cell types, opening avenues to tackle a broader range of diseases.

As anything grows, scaling up manufacturing processes and capacities becomes a major topic. Priorities include modernizing facilities, optimizing machinery, and implementing more efficient synthetic processes. At the same time, there is also a need to consider sustainability. Oligonucleotide manufacturing processes are connected to a certain amount of waste; for example, 1 kg of product requires 1000 kg of acetonitrile. Finding ways to reduce waste is obviously better for the environment and the planet, but can also connect to improved production efficiency and cost control. The industry is working on optimizing existing technologies and developing new approaches to reduce waste and byproducts. Digitalization, automation, and Pharma 4.0™ will be important for both scalability and environmental care. A better understanding of processes will also help guide optimization and create the ideal synthesis.

What is the manufacturing process for oligos?
The most widely used state-of-the-art process is solid phase oligo synthesis. The synthesizer controls the reoccurring four-step cycle of detritylation, coupling, capping, and oxidation. The reaction

Oligonucleotides: Getting Equipment Selection Right (cont...)

occurs in synthesis columns, while the synthesizer precisely delivers the amidites and all required reagents. The sizing and pairing of the synthesizer with the columns are key for effective coupling and yield.

Every cycle, a phosphonamidite is added to the growing and immobilized oligonucleotide chain. Once finalized, the oligo is then cleaved from its solid support and protecting groups are removed to obtain an active API. This step is recommended to be automated using a cleavage and deprotection system, ensuring optimized conditions for high efficiency.

The downstream phase starts with purification (reversed-phase, anion exchange, or hydrophobic interaction chromatography) to remove undesired shortmers and impurities. Also in this step, the use of dedicated medium pressure liquid chromatography systems with matching dynamic axial compression columns is imperative for efficiency and high-quality yields. Auxiliary components and features are available to increase handling, safety, and process efficiency. After purification and before lyophilization of the end-product, the oligos undergo the process of desalting by buffer exchange and concentration. For this application, AKB recently launched an ultrafiltration/diafiltration (UF/DF) system intended for use in oligonucleotide manufacturing environments that require specific safety directives.

What are the biggest considerations and challenges when setting up a new manufacturing line?

From our perspective as a technology supplier, we recommend considering scalability, as well as the integration into the facility and the overall production approach. Is the production line intended for one product? Or is the line for a CDMO producing multiple products where the highest flexibility is required?

Because of the complexity of scale- up, partnerships and collaboration with organizations that have the right expertise and technological solutions can be beneficial to make the process faster and easier. The number of variables that need to be considered in both upstream and downstream processes require more than a "plug-and-play" approach with large-scale equipment.

How should companies approach the challenge of choosing the right equipment?

After determining the right oligonucleotide synthesis type and considering the desired functionality, target application, and cost, carefully evaluate the characteristics of each required step and then select the equipment that best suits your goals. High quality equipment and components play a crucial role in ensuring the efficiency and reproducibility of oligonucleotide synthesis at scale.

All the steps in the manufacturing process require in depth analysis of the parameters, which should be aligned with your process requirements; however, there are a few general features that I can highlight. I recommend looking for an innovative and thoughtful mechanical design that is complemented by automation. Automation speeds up the process steps while ensuring consistency across multiple batches – a prerequisite for reproducible results. Also, choose a system that will allow for seamless transition from small scale to large scale. AKB's systems and columns are specifically designed with scalability in mind, offering a range of sizes that cater to varying synthesis volumes and throughput demands.

Finally, keep in mind that, though a truly high-performance production line usually requires a higher initial investment, this cost is repaid in long-term impact on operational efficiency and overall cost-effectiveness. Opting for high-quality equipment should result in lower maintenance requirements and less downtime – ultimately leading to higher productivity and cost savings by ensuring synthesis processes run smoothly and efficiently with maximum uptime.

How is AKB innovating oligonucleotide manufacture?

AKB offers state-of-the-art equipment and components from synthesis through concentration, including the THESYS™

Oligonucleotides: Getting Equipment Selection Right (cont...)

Oligosynthesizer, THESYS ACS Column, THESYS SCS Column, THESYS Cleavage & Deprotection System, CURSIV™ MPLC System, CURSIV DAC Ergo LC Column, SLURIPREP™ System and VANTIJ™ Ultrafiltration/ Diafiltration TFF System. All our products are "built for you" to ensure that a customer's individual requirements are met. The ability to use the same equipment to manufacture different products with different chemicals or at different scales eliminates the need for costly new investment or reconfiguration of equipment.

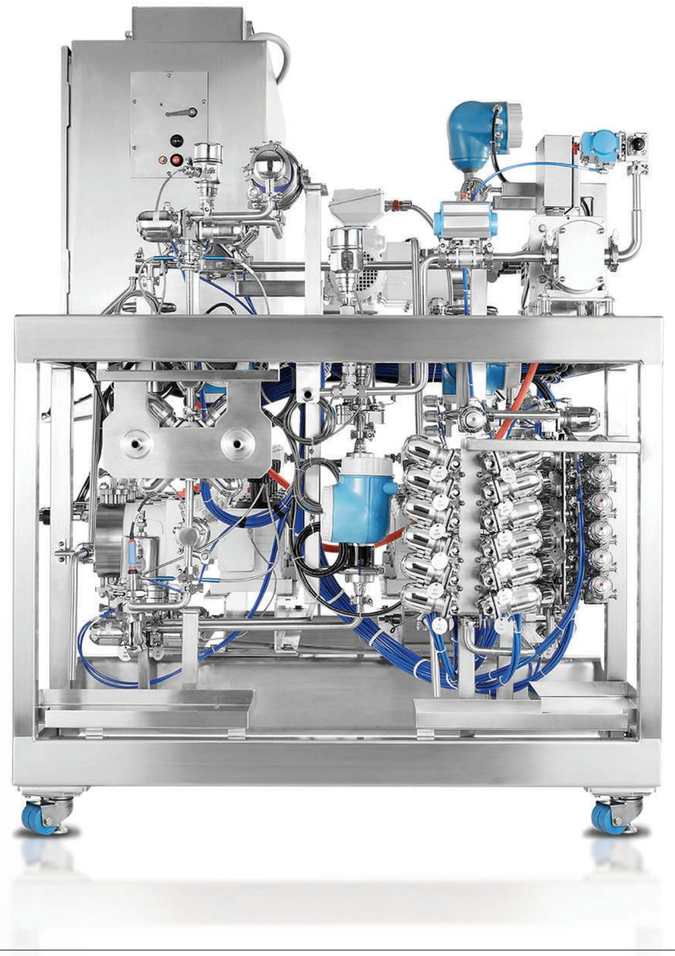
AKB relies on user familiarity with the cross-platform components of all systems. Devices and components are designed to work together seamlessly in a single integrated system, minimizing compatibility issues and ensuring optimal performance and efficiency. This also applies to the OCELOT® system control for all automated system types, which is an intuitive, powerful software platform that can be integrated into plantwide control systems. That said, the operator is certainly not disregarded; rather, the operator is always considered in the ergonomic design of a system, to enable increased efficiency and safety.

With 30 years of experience in various areas of biopharma manufacturing, AKB can apply technical principles precisely to the requirements of different drug modalities – or rethink them to develop innovative products. Our strength is the ability to apply our expertise and experience individually in every project to achieve the best possible results – together with the customer. Close collaboration strengthens relationships and communication channels. As a trusted advisor, we encourage interaction with project managers, engineers, and service during and after successful project completion.

What advice can you offer to manufacturers that are just getting started with new lines and investments in the oligo field?

Make sure you have a clear understanding of the oligonucleotide synthesis process before purchasing equipment or components. And choose a supplier that has a comprehensive range of equipment – and don't forget to consider their customer support. A good supplier should have exceptional customer support that ensures smooth procurement and ongoing help with any issues that arise, including advice on improving efficiency and maintaining compliance with industry standards.

To ensure long-term cost efficiency, evaluating the cost of initial equipment and operations is a given, but you'll see greater benefits if you are willing to collaborate with partners that can help you take performance and efficiency to another level – and in a future-proof way. AKB's experience in oligo scale-up and the comprehensive portfolio of flexible, future-ready technologies – combined with a breadth of technical expertise and end-to-end support – make the company an ideal partner to quickly navigate the complex challenges in this exciting and rapidly growing therapeutic space.



LINKS

Find out more
https://fluidmgmt.ak-bio.com/

Built for You

How AKB is incorporating ergonomics into biopharma equipment to make manufacturing safer, more efficient, and more productive.

What's the secret behind great equipment? High performance and high quality should be a given in the world of biopharma, but we shouldn't forget the overall design and useability of a system, including its ergonomics. AKB designs and manufactures large-scale biopharmaceutical manufacturing equipment that is deployed all over the world – and it prides itself on deep collaboration with customers. Part of the company's ethos is "Built For You" – a philosophy that ensures equipment is truly made to help biopharma companies to develop the best products possible, in a safe and efficient manner.

Here, we speak with Steve Foy, Manager, Products and Brand Strategy, to find out more.

What inspired AKB's "Built for You" philosophy?

Our mission and vision are centered around being a trusted advisor for customers. The only way to achieve that is to partner with customers – and that means developing and fostering relationships. It shouldn't just be about the sale of products and equipment; we have a duty to help our customers perform to the best of their ability.

We're already well-known for custom equipment, but our "Built For You" philosophy is about more than that. It is a broader story that goes beyond custom equipment into a deep understanding of customers and their specific requirements, and our willingness to design solutions on their behalf to help them be more productive.

Why is ergonomic equipment design so important?

Ergonomics have naturally been an important aspect of our equipment design for a long time, but we've never really focused specific messaging around it. Ergonomics is the study of ease and comfort – and a stepping stone to greater efficiency and safety – and plays a key part in "Built For You". An ergonomic chair, for example, increases efficiency and productivity for somebody working at a desk by increasing their safety and comfort. Poorly designed equipment can potentially reduce productivity and increase risks for the operator, the product, and the company in general.

To give an example of how ergonomics affects pharmaceutical manufacturing, consider our commercial-scale CURSIV™ DAC Ergo liquid chromatography column. Large columns can be difficult and tedious for operators to use and maintain. The DAC Ergo works on a hydraulic system rather than on a solitary frame. The telescopic legs are much larger and separated from the column. A single operator can remove the bottom plate assembly in roughly 30 minutes, which makes unpacking and maintenance much easier.

Is ergonomics typically overlooked by customers?

Customers often focus on the technical specifications of equipment. Certainly, equipment needs to have the right specs and deliver high performance. However, if your equipment isn't easily usable – for example, if it is difficult to operate or to learn how to use – then it will be an obstacle to optimized productivity.

In addition to looking at the technical details, we always advise customers to think about ergonomics. For example, look for a human–machine interface screen that is positioned appropriately – not too high or too low; look for a keyboard and a touchscreen input

Built for You (cont...)

option to make it comfortable for the individual operator. Customers should also think about maintenance and accessibility. Are all the parts inside easy to access? The alternative is to take everything apart, retrieve and replace a component, and put it all back together. These are aspects that customers may not consider outright.

As well as ensuring our equipment is high quality, high performing, durable, robust, and reliable, we always look at how we can make things more accessible, more efficient, and simpler. All these factors can have a big impact on day-to-day operations. There is a level of productivity that a customer may not even know they're able to achieve. And so, it is up to us to focus on that and innovate on the customer's behalf. Even if they've become familiar and proficient with older designs, could they still save hours of downtime when it comes to process changeover? That could mean extra batches per week.

One driver for the design for our recently released THESYS™ ACS Ergo column was input from customers. Our typical synthesis columns are very high performing – and popular with customers all over the world. But changing the media after synthesis involves unscrewing a series of threaded bolts, which can be a bit tedious. After listening to feedback, we modified the design to improve usability and productivity.

How does AKB work with customers during the design process?

We start by understanding the space we must work with. Very early in the design phase, we ask the customer for a floor plan of their facility that shows the route the equipment will take from the loading dock, as well as the room and location for installation. We note the maximum dimensions that can be safely wheeled through the hallways and around corners, the sizes of entryways, and areas to avoid placing routine maintenance items.

During our formal internal review, we have several checklist items related to safety and ergonomics. We verify the constraints with the lead engineer and ensure that the designed skid will fit. We look at every single component that will require routine maintenance or access to control panels and consider ease of use, such as the position of pump faces and pump motors, the support of heavy valve blocks and other components, and the accessibility of control panels for sensor calibration.

We locate the HMI and keyboard at commonly accepted heights for ergonomic operation and, during our formal external review with the customer, we suggest they include representatives from their maintenance and metrology departments. We then go through all these points with the customer and come out with alignment or suggestions to better meet their needs. It is not uncommon for us to export a 3D model of the system for the customer to import into their plant plans for further confirmation that all components and ports are located to provide ease of use.

Why should new customers consider working with AKB?

I am deeply proud of our people and their ability to develop great equipment and form great relationships. In addition to providing high quality equipment with excellent performance – which can be table stakes – we focus on ergonomics and usability as key selling points. Another differentiator is the service we offer and how we communicate with the customer – from sales and the proposal process, to design, manufacture, installation, and field service.

A cornerstone of AKB is our collaboration with customers. Our success is derived from our customer's success. Our equipment plays a crucial role in bringing new therapies to patients and we have a responsibility to supply the best solutions that can help customers to be as productive and efficient as possible.

EXAMPLES OF ERGONOMIC AKB TECHNOLOGY

CURSIV™ DAC Ergo – liquid chromatography columns with a patented design to ensure swifter, safter column changeover between batches.

THESYS™ C&D Systems – designed to accommodate both DNA and RNA processes with numerous in-process controls to automated steps.

THESYS™ ACS Ergo – synthesis column designed to simplify time between batches by being built for faster unpacking and cleaning.

MOTIV® Single-Use Inline Buffer Formulation System – streamlines buffer production by removing CIP/SIP between batches.

VANTIJ™ Single-Use Virus Filtration Controller – includes features and design elements for reproducible, reliable filtration.

SLURIPREP™ line of media management products – designed for DAC LC columns to ensure seamless, efficient operation.

The Status Quo of Oligonucleotides Is Not the Future

The science of oligonucleotides is advancing, and advanced manufacturing techniques are needed to optimize their impact.

Historically, oligonucleotide therapies have targeted small patient populations with rare conditions, but with excitement growing in tandem with the potential of the treatments themselves, innovators are looking to target indications with larger numbers of patients.

When one particular company was looking to optimize a manufacturing space for oligonucleotide manufacturing, AKB and CRB were invited to offer expertise in terms of the floor design and process equipment. The focus of the project: improving quality, purity, and output, while minimizing environmental impact. Here, AKB Vice President of Operations Brian Crawford and CRB Process Engineer Doug Chritton tell us more.

What are the big trends in oligonucleotide manufacture?
Doug Chritton: CMOs are building out capacity to support these drugs and capacities are also being boosted on the clinical side with an eye to even larger throughput in the future. There's also interest in advancing production techniques. The traditional way of building a molecule – one nucleotide at a time on solid phase supports – is no longer the best approach; it uses a lot of solvents, isn't sustainable, and isn't designed to produce quantities to suit the demands of a large patient population.

We're now seeing a push for enzymatic synthesis to link molecules and reduce the number of harmful chemicals and flammable liquids, while also increasing yield.

The Status Quo of Oligonucleotides Is Not the Future (cont...)

For example, if a 20 nucleotide long oligo product (20-mer) is 98 percent effective at every linkage, you have around a 67 percent yield, which isn't great but is acceptable for drugs targeting small patient populations. As we look at longer chains and larger populations, however, that yield becomes less feasible. A 100-mer, for an application such as a guide RNA for CRISPR therapies, may only have around a 13 percent yield. These longer chain molecules aren't necessarily targeting large patient populations, but would still benefit from enzymatic production methods and improved yields. There is also room to improve other manufacturing steps. Advanced drying techniques, such as spray drying, are seeing increased attention, as are enhanced purification methods.

The industry is recognizing that the status quo is not a sustainable production approach if we need large quantities of oligonucleotide therapies in the future.

What are the main challenges in optimizing a manufacturing space for oligonucleotides?

Brian Crawford: The oligonucleotides field is booming, but there is a dearth of experienced personnel for this emerging modality. And it is always a challenge to outfit a facility for current state-of-the-art manufacturing methods, while also considering and building flexibility for future manufacturing advances. It is important to keep advanced methods in mind. Whether enzymatic processes, liquid phase synthesis, building the sequences with nucleotide triphosphates rather than phosphoramidites, there are all kinds of advanced manufacturing techniques that could be employed to solve the current challenges being seen in oligo manufacturing.

You are also best finding a partner who is familiar with the challenges. There are many aspects to consider – and many timelines must converge – to get a new manufacturing space qualified and ready to go. You must consider local government permits, inspections timing, associated utility supply and piping, HVAC, qualification, construction of warehouse space and storage, supporting labs for product testing and clearance, and more. Simply ensuring that essential equipment can fit into elevators, through halls and corridors, and into the appropriate room is often overlooked!

Collaborating with an experienced partner brings familiarity and expertise in navigating all challenges. Scale up is not always about making everything bigger. The emphasis should be on increasing throughput, yield, and purity, which can increase the amount of product manufactured without requiring infinitely scalable machines.

How does the complementary expertise of both companies come together in the project in question?

DC: CRB was initially asked to perform a small gap analysis for a client involving an oligonucleotide project, including a review of the facility and potential improvements, and then was hired to design support systems, such as solvent supply, reagent supply, buffer preparation, and waste handling systems. AKB was tasked with providing a large portion of the process equipment. Both companies worked closely together to ensure the equipment fit the facility and supported the needs of the client.

Even before this project, we already had a collaborative relationship where we would often share trends and help each other understand what clients need and want.

BC: AKB and CRB are aligned in solving shared customers' manufacturing challenges in a way that drives the industry forward. On this project, we were selected as the equipment supplier for three of the manufacturing lines. Our history with CRB was advantageous because they already had experience with our equipment, understanding how we can tie it into the plant-wide controls and ancillaries that feed manufacturing processes.

The Status Quo of Oligonucleotides Is Not the Future (cont...)

What were some of the big discussion points in the project?
BC: There were discussions around how the customer wanted to approach the plant-wide controls for data management, including considerations for the future, such as further automation, industry 4.0 initiatives, and space planning. Indeed, a great deal of space planning went into the design of our oligosynthesizer, our UF/DF systems, and chromatography purification systems to make sure that they fit into the vision for the customer.

DC: In oligo facilities, handling corrosive halogenated waste is a huge discussion point. If you put the deblock or detritylation reagent undiluted into a stainless-steel piping system, then you'll have issues with corrosion. It's a very hazardous safety and environmental issue. Halogenated waste is a small portion in comparison, so evaluating the trade off is something that needs to be weighed carefully.

What will define the success of this project?
BC: Thus far, the project has been a success in that we've been able to handle shifting priorities and keep everything on-track or ahead of schedule. The real success will be defined by the customer when their first batches of clinical and process-scale oligonucleotides are complete. From an equipment standpoint, we know we're on track as two full pilot scale trains are installed and qualified. The process scale train is currently in validation testing and everyone is very happy so far! We do recognize, however, that this is just the start. We're now shifting focus to operator training and setting them up for success prior to production. The aim is to contribute as much as we can by ensuring the equipment is set up optimally and ready to run.

What are your top tips for optimizing an oligonucleotide manufacturing space?
DC: For contract manufacturers, flexibility is key. Don't design your facility by honing in on existing processes; 5–10 years down the road, you will need to either add, change, or modify your equipment and facility. Think about how you can accommodate that by planning, planning, and planning some more! Consider not just the process, but the entire operation.

BC: I agree. Knowing what the ideal use case is and planning for change over time is important. Keeping operations in sequence, thinking about critical interfaces between the building, the operator, and the ancillary support systems are also key focal points. At AKB, we focus on developing systems for these challenges that also save down-time, floor space, and drive up the production capacity for customers.

What are your thoughts on the future of oligo drug development?
BC: I'm excited because of what these treatments represent. Rather than palliative or symptomatic treatments, oligos can be curative – whether through gene silencing or RNA interference. There are many developments targeting rare diseases that have no treatment, but it's also exciting to think about using oligos in diseases with potentially hundreds of millions of patients.

We're also seeing more breakthroughs in effective delivery to tissues and we will see more approvals in the future. The status quo will be pushed to one side; the state-of-the-art will be pushed further. It's exciting, on a professional level, to be able to contribute and help drive the field forward.

When it comes to manufacturing, I don't think there will be a single, golden manufacturing approach. It will likely be hybrid – or possibly something new that hasn't been considered yet. But whatever the approach, it seems clear that there will be an emphasis on larger scales, greater efficiency, and improved sustainability.

DC: Oligonucleotides with conjugates is really exciting. New targeting ligands are starting to unlock parts that have been difficult to treat; access to these targets will create new treatment options. By conjugating to an oligonucleotide, we can harness a long duration of action or potential curative action to treat diseases with unmet needs. As we find more conjugates, the oligo targets will diversify to other organs too, resulting in even faster growth of the market segment.

If we draw comparisons to monoclonal antibodies, there were just a handful of approved therapies 25 years ago. Today, there are over 120. We're on the edge of that with oligonucleotide treatments.

LINKS

Find out more
https://fluidmgmt.ak-bio.com/