A New Era for Cell Culture Media

How a better understanding of raw materials has increased the potential for biopharmaceutical companies to understand their process variability.

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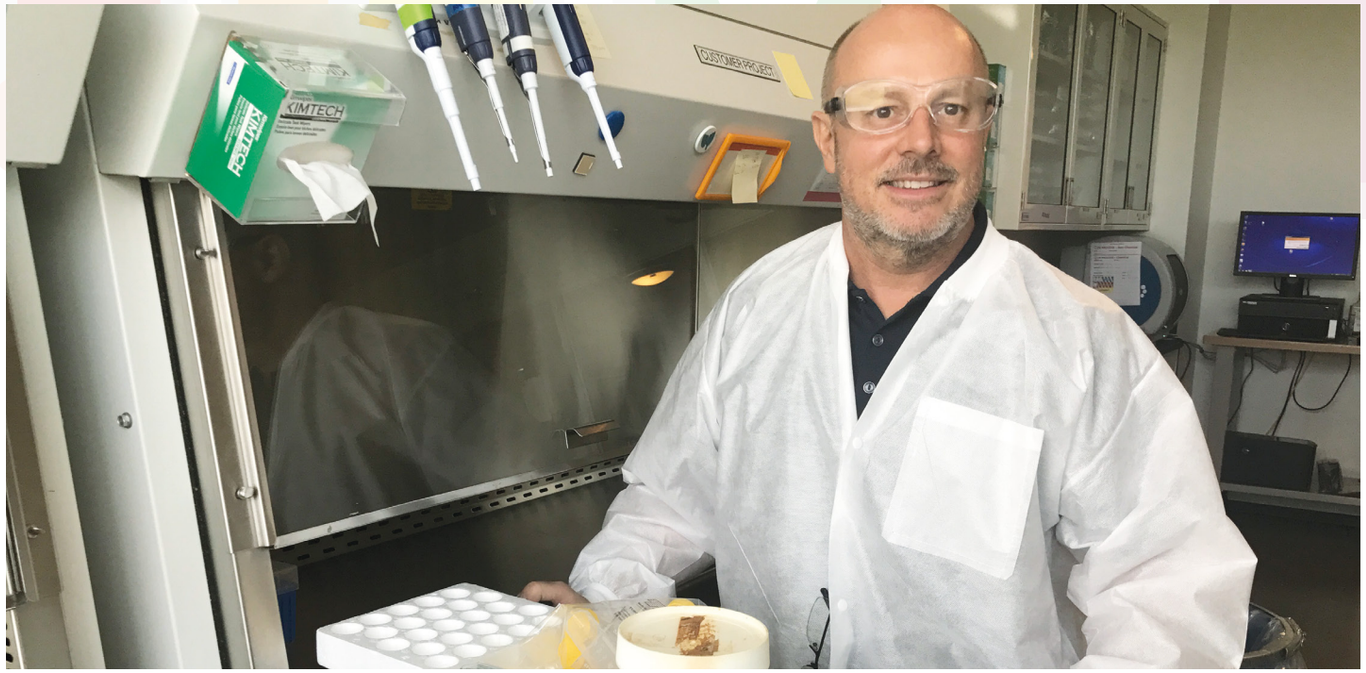

Finding That Special Source

Bioprocesses are highly complex – and we are only now starting to fully understand the impact of cell culture media on the critical quality attributes of bio therapeutics. Here, we trace the supply chain back to raw materials and learn how fundamental research is driving advances in cell culture media.

By Kevin Kayser

I joined Sigma-Aldrich (now Merck) in 2002 as an R&D manager in molecular biology product development, before moving into cell culture media in 2006 and then through several roles to my current position. Looking back, it's amazing to see how far the field has come in just 15 years.

When I entered the cell culture media space, monoclonal antibody titers were around a gram per liter or less; today, we see titers as high as 10–12 g/L range. Redesign of cell lines, cell culture media and advances in bioprocessing technology have both been crucial to this huge boost in productivity. In 2006, the industry was still using serum for many processes. Since then, we've progressed from formulas using non-defined complex components, i.e. full serum, to reduced serum amounts, to a variety of plant-derived hydrolysates, to the use of formulas with much more chemically defined ingredients.

Perhaps the biggest change is our approach to fed-batch processes, both in terms of media and feed design. In the early days, the cell culture media had most of the components needed for cell growth; today, the "basics" are included, and the rest of the components are fed into the process on a regimented basis, providing the nutrients the cells need, and, crucially, when they need them. In essence, we are now able to direct the cells to protein production rather than just increasing cell density – a common goal of the past, when the theory was more cells equal more protein. That's really not the case, and so we've had to learn how to drive specific productivity of individual cells, which is not always an easy task, especially when put into the context of secreting a high amount of a recombinant therapeutic protein that is not a natural part of the cells' architecture! To succeed, we, as an industry, have needed to increase fundamental knowledge of the biochemistry of the cell lines used.

Critical raw material quality

Modern cell culture media are typically made up of anything between 50 to 80 components (although some commercialized therapeutics are grown in cell culture media comprising over 100 components). Each component comes from a particular source – and they also come with a particular "risk". All cell culture media manufacturers must consider those risks, as well as the potential of those risks being passed onto customers. There are two risks which perhaps concern drug producers:

i. Viruses. Is there any chance of inadvertently introducing an adventitious agent into a process? Some may recall the Vesivirus 2117 contamination incident at Genzyme's Allston Massachusetts plant in 2009 – resulting in millions of dollars in lost revenue from delays to Cerezyme and Fabrazyme production and, more importantly, interruption to the supply of life-saving medicines to patients.
ii. Process variability. Individual raw materials originate from diverse sources – mining, complex chemical synthetic routes, and so on – and each one, put simply, must be what we think it is; for example, sodium chloride should consist of sodium chloride and only sodium chloride. Invariably, however, impurities or manufacturing intermediates creep into play. These impurities and/or manufacturing intermediates can have an effect on cell culture and can be a source of process variability.

In the past, the industry never really understood much about the variability of impurities or the biological significance. Over the last four or five years, companies have started to more fully understand the implications of lot-to-lot variability, impurity profiles, and any trace elements that may be present. Some of those trace elements can have a strong impact on CHO-cell enzymes; copper at ppb levels, for example, can activate enzymes that actually alter the critical quality attributes of the resulting therapeutic (1). Consider a pharmaceutical company that is trying to match a certain quality profile filed in its IND material; the company could be hindered by the process variability introduced by trace level copper in a raw material. Likewise, consider a biosimilar developer trying to match an originator profile. In both cases, reducing process variability is essential, which necessitates fully characterized starting materials.

Controlling variability

There is a multiplicity of raw material vendors (including Merck KGaA – although in fact, we are one of our own biggest suppliers when it comes to our cell culture media business). For any cell culture media manufacturer, establishing a robust supply chain is absolutely key. Such supply chains can only be built with time and trust. The credibility of individual suppliers stems from good and effective validation and quality systems, especially in terms of change notification (after all, a seemingly small process change at the start can have big consequences in the final application). A robust supply chain often necessitates multiple suppliers of qualified materials to maintain continuity.

But, importantly, if there is variability, we need to be able to measure it – and understand the potential impact. Going back to my copper example: for one client, trace level concentrations of copper may actually be beneficial by providing the right level of glycosylation. For another client, the glycosylation profile may be affected detrimentally. What does this mean? Firstly, we recognize how essential it is to accurately report copper levels. Secondly, universal specifications are only useful as a starting point. Customers may have some idea of how copper affects their process, but we often have to work side-by-side with them to develop a custom solution.

Room for research

Back in 2009, we put together a research and development team dedicated to raw materials, which focuses on the biological and analytical characterization of all the compounds used in our cell culture media. The team strives to answer some pretty fundamental questions about those compounds. Why does a particular compound need to be there – or, in other words, what is its biological function? In bioprocessing, we're essentially taking cells out of their native environment and forcing them into a new role in therapeutic drug production – so there's a lot of opportunity to remove components that we no longer need. Perhaps selenium is simply there because "we've always done it that way!" (You can actually learn something about the taxonomy of media design simply from where people went to grad school...) The team is also investigating the levels of impurities from lot-to-lot and from vendor-to-vendor – as well as the biological impact of those different levels of impurities.

When it comes to understanding the impact of cell culture media, we shouldn't be passing the responsibility onto our clients – we believe that we, as a supplier, must drive that research. And our R&D team allows us to become raw material masters! Once we started digging, we realized that to answer many of our questions we have to run the full gamut of research – everything from high-level science, such as the fundamental nutritional biochemistry of CHO cells, to more routine work, such as looking at the variability in the manufacturing process for salt, for example.

A dynamic approach

Cell culture media has blossomed from the seemingly simple to the almost infinitely complex. And getting it right – at least for us – demands a multifaceted approach – or, in other words, the involvement of several areas: scientific research and development, quality, supply chain management, operations, and more. If we find something in R&D that may have some sort of impact – copper, for example – we need to set in motion a chain of events that spreads throughout the entire organization. It's not all about cool science – it has to be translated into both the quality and manufacturing organizations to become fully realized.

Our focus on R&D has also allowed us to optimize our own raw materials and additives for use in upstream processes, which gives us a unique advantage; for example, our EMPROVE® product portfolio offers a high level of quality that, in turn, can feed back into our cell culture media business. The result? Improved resource efficiency for both us and our clients. Notably, products bearing the EMPROVE® trademark also come with comprehensive regulatory documentation, which can contribute to quality when clients file registration dossiers.

Right now, we're working in an ever-evolving field, where new scientific knowledge about raw materials and their impact can be dynamically applied to improved products or better process understanding (both of which help our customers reach their own goals, whether that be improved quality or increased yields to drive down costs) – and that's very exciting.

Kevin Kayser is Senior R&D Director at Merck.

Reference

1. *IH Yuk et al., "Effects of copper on CHO cells: cellular requirements and product quality considerations", Biotechnol. Prog., 31, 226-238 (2015).*

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Know Thy Raw Materials

We asked customers what they wanted from their cell culture media suppliers and the reply was, "understand your raw materials". So nine years ago we set out to characterize and evaluate our raw materials – this is what we found...

By Chandana Sharma, PhD

From our perspective as a cell culture media supplier, there are hundreds of possible raw materials which could be in a formula, including amino acids, vitamins, fatty acids, and salts, many with multiple functional groups. Raw materials can come from any number of possible sources, and even the same ingredient from multiple sources. Some raw materials are very well defined and others may lack a complete profile. Understanding raw material differences has become vitally more important to companies like ours. Our customers strive to understand variability and the impact on their biomanufacturing process, they look to improve their cost to manufacture and reduce risk to the patient. So they look to us to know more about our raw materials. This is exactly what our customers told us in late 2008 when we carried out a survey asking what they expected from cell culture media suppliers – the clear message was that a thorough understanding of raw materials and potential variability was crucial. And so Merck KGaA set out on an ambitious program of raw material characterization and evaluation.

At the time, Merck KGaA was focused heavily on developing cell culture medium rather than its building blocks, so we had to take a step back and decide on a strategy. Variability in cell culture raw material is, of course, inevitable, but too much variability can impact the overall performance of the culture medium, bioprocessing parameters and the process output. A cell culture media can consist of 70 to 100 different raw materials and the variability is cumulative, so it needs to be controlled within reason. A good understanding of the raw materials and variability builds a good picture of the medium as a whole and its performance.

A question of variability

The first question for our characterization program was, what should we study? With hundreds of raw materials in our inventory, we had to pick and choose, so we performed a risk assessment to identify "high risk raw materials." From this we created a prioritized list of raw materials. We then undertook an orthogonal approach to characterize those materials, which included chemical and biological assessment. Chemical assessment was focused on understanding the impurity profile, whereas biological characterization was focused on understanding the impact of impurities or variability on cell culture processes.

Using the right tools and techniques for the study was vital to get the best data, which meant investing time and resources into approaches such as mass spectrometry, liquid and gas chromatography, and multivariate data analysis tools. On the biological characterization side, we developed high-throughput biological assays and markers. We took a dose-response approach and studied raw materials in multiple cell lines. There have been some remarkable advances in the sensitivity of analytical instrumentation in recent years, which allowed us to carry out elemental analysis at the parts per billion scale. Overall, we produced a tremendous amount of data and learned what variability was normal and acceptable for our raw materials, and what was not.

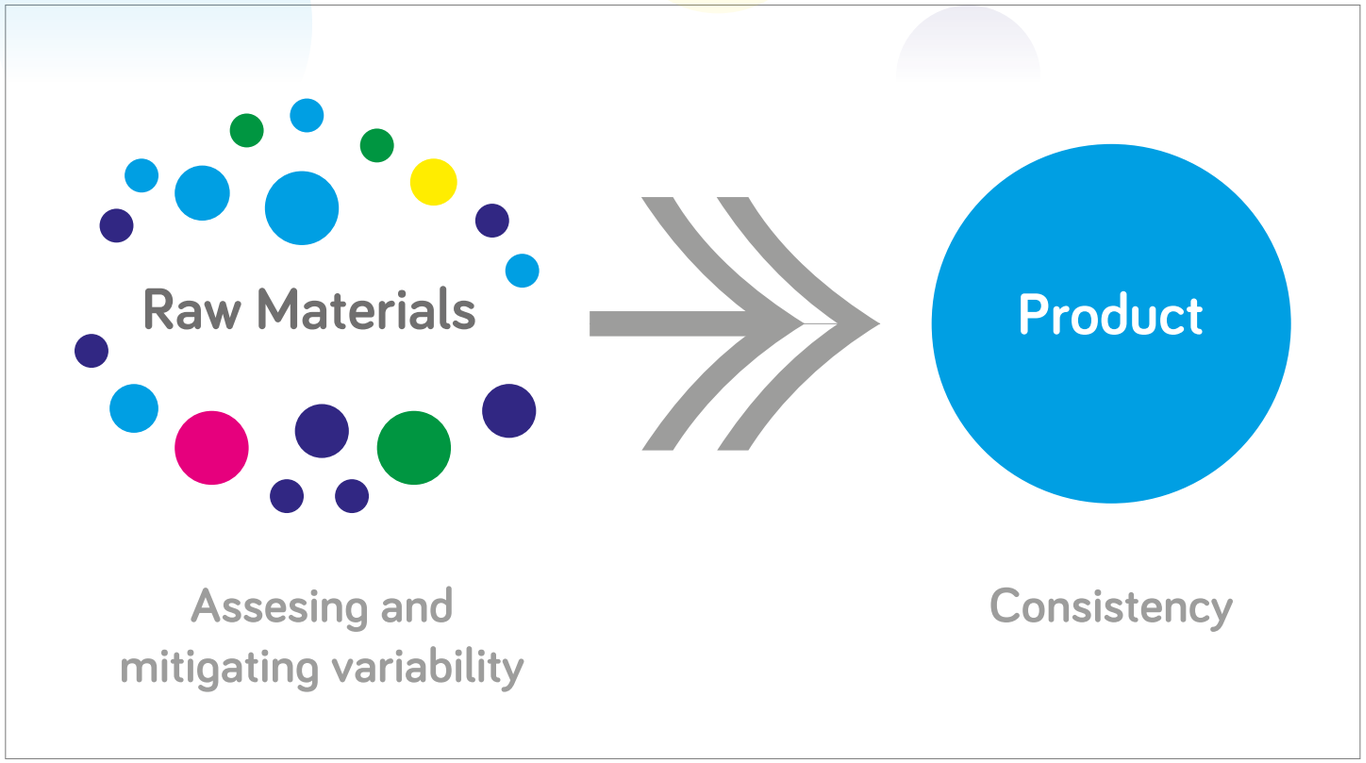
Understanding your partners

One aspect of our raw material characterization program was to study and understand the inter- and intra-lot variability of a given supplier. We had in excess of one hundred different raw materials, but for each of those we also had two to three suppliers (it's always advisable to have some redundancy in the supply chain and not be dependent on one supplier). If, for example, we had L-Lysine coming from supplier A, we had to understand the variability within supplier A, as well as suppliers B and C. Overall, we had to understand how each supply of L-Lysine might differ, and then examine how the variability could be minimized.

We also realized the importance of integrating the characterization program with our quality systems to proactively prevent any variability in raw material from impacting the quality of the final product. Today, we have a two tiered approach of screening changes in the raw material supply. We also have very good relationships with our suppliers and discussed our findings with them. For example, if there was a problem with a certain raw material then we collaborated with the supplier on how we could overcome this.

Knowledge is power

The most important finding was that our raw materials were relatively pure, especially the defined small molecules, which was a relief! When

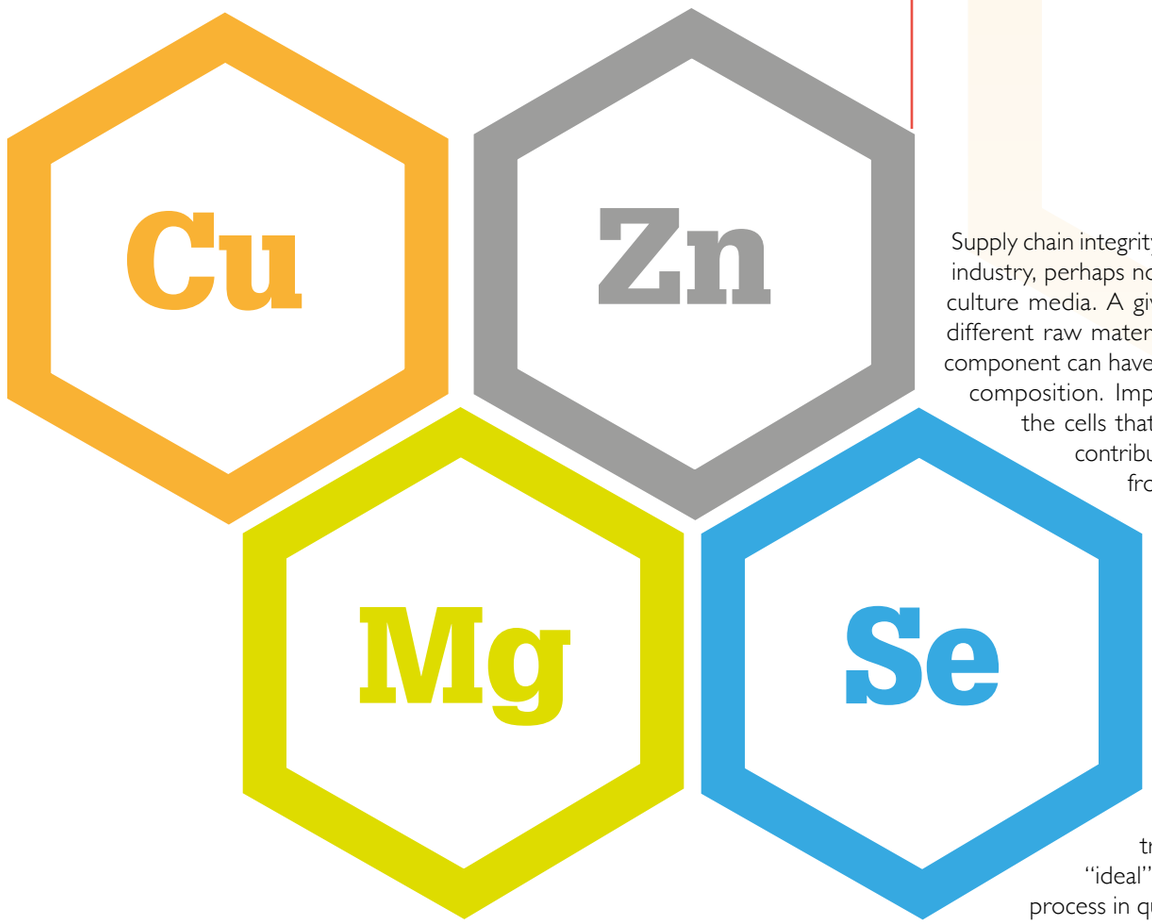


we did see variability, it was coming from trace metal salts or undefined raw materials like hydrolysates. The trace metals finding was surprising because it was not an initial focus of our study. This changed with the results and we ended up diving deeper into the topic of elemental impurities, particularly because it was so important for certain inorganic salts like sodium chloride. In time, we expanded elemental impurity testing to all major raw material groups, including amino acids, vitamins and more.

There will always be some variability in raw materials – since some of our raw materials are byproducts of other processes, and we may not even be the primary industry for it. We have taken steps to ensure that our suppliers are safe, but we need to appreciate that we are one of many customers so we also need to have mitigation and quality control procedures at our end. For example, we learned less than a 1% impurity in poloxamer 188 had a huge impact on cell culture processes, and have developed a quality control cell assay to detect and prevent entry into our supply chain. Thanks to our detailed study, we now have a great deal of information about our raw materials and the impact on cell culture. Information is never a bad thing – you may not choose to act on it, but it allows you to make informed decisions. Many of our customers have used the data we provide about our cell cultures to make changes to their formulations. For example, they may already know there will be a specific amount of manganese or copper as background impurity, so they can tweak their formulations or processes if necessary to account for the variability. Our data can also be used to advise on the most suitable cell culture medium for a given product. A biopharma company may require a cell culture medium with a certain amount of iron, but may have a product that is sensitive to zinc. Now that we have characterized the impurities contained within our raw materials, we can advise our customer that one of our raw materials, ferric citrate for instance, contains zinc. The customer might then ask what other sources of iron are available, to which ferric ammonium citrate might be the answer. We can then supply a cell culture medium that is tailored to the specific needs of the customer. I have seen many success stories where we have collaborated with our customers to overcome problems – all products are different and cell culture systems can be customized. Because of this, deep collaboration with suppliers is crucial. This is true for us with our suppliers, and also our customers.

Overall, the painstaking process of characterizing our raw materials and figuring out how they impact biological systems and our cell cultures has allowed us to be more transparent with our customers. It seemed like quite the task when we first embarked on the project almost a decade ago, but the hard work is now paying off in terms of deepening our relationships with customers and suppliers – securing the supply chain from top to bottom.

Chandana Sharma, PhD, is Head of Cell Culture Raw Materials, Upstream R&D, at Merck.



Chasing Traces

Variability in raw materials can cause product quality issues and lead to regulatory problems. Increasingly, our industry requires specific reassurance on elemental impurities, so how can media suppliers provide confidence in this difficult area?

By Chandana Sharma

Supply chain integrity and reliability is critical for the biopharma industry, perhaps no more so than in the manufacture of cell culture media. A given medium may comprise of 50 to 80 different raw materials, and low levels of impurities in each component can have a cumulative impact on the final medium composition. Impurities can affect multiple pathways of the cells that are grown in cell culture medium, thus contributing to the variability of proteins harvested from those cells. Some trace metals impact certain glycosyltransferases and can alter the protein glycosylation profile. In particular, concentrations of trace elements like copper, manganese, zinc, and selenium, are absolutely key because they have a direct impact on protein quality. Other trace metals are critical nutrient sources in their own right – iron in particular is essential for cell growth. Whether the trace metal is intentional in the media formulation or an impurity, trace components have different effects and "ideal" concentrations may vary according to the process in question. To avoid product quality issues, it is vital that biopharma and biosimilars companies understand the effect of elemental metals on a given bioprocess, and quantify the impurities present in their processes.

Regulatory goals

Recognition of the critical impact of trace metals is reflected in evolving regulatory guidelines. The pharma industry is currently adjusting to new guidelines, such as the FDA's ICH-Q3D "Elemental Impurities" document, concerning acceptable impurity levels in drug products. Broadly, regulators now favor replacement of traditional analytical chemistry methods with more sensitive techniques for trace metal quantification, such as inductively-coupled plasma mass spectrometry (ICP-MS). Industry must familiarize itself with these methods – and media suppliers must adapt to this trend. So how is Merck positioned in this environment?

We now have a state-of-the-art trace metal analysis facility – the result of significant investment and a real development journey. When we first started fully characterizing raw materials, we didn't initially think about trace metals; however, on closer inspection, we saw surprising variability in elemental impurities and realized that we needed to expand our dataset to make sure we understood our raw materials relative to trace metal impurities. As we collected more data, it became increasingly clear that the issue needed serious attention. Unfortunately, the external analytical laboratory that we used at the time wasn't providing us with the data we needed – they worked at a parts per million sensitivity when we needed parts per billion. Eventually, we made the decision to develop an in-house, dedicated facility for the analysis of elemental impurities. We renovated our existing space, procured a high quality ICP-MS instrument, hired some personnel, and started generating our own data. We chose ICP-MS as the workhorse analysis method because it is about as exact and quantitative as you can get, but we do sometimes also use ICP-AES (atomic emission spectroscopy) or ICP-OES (optical emission spectroscopy), depending on the quantity of the element we are studying. In general, however, we rely heavily on ICP-MS.

In short, we quickly went from not even knowing that we should look at trace metals to a purpose-built, in-house facility dedicated to the analysis of elemental impurities.

Measuring what's there – and what it does

Our elemental impurities initiative is a three-tiered approach: i) quantifying trace metals in individual raw materials and in our final product; ii) determining whether our process of mixing and milling these raw materials itself contributes to impurities in the final product; and iii) understanding the impact of individual impurities on final protein quality. The first element was perhaps the most

time-consuming, but the third part – understanding impact on protein quality – was the most important. To study this, we also utilize a CHO model system that produces a specific protein where we can assess the impact of elemental impurities on the ability and quality of the protein. The findings will not apply to all biopharma processes, but given that 70 percent of the biopharma industry uses CHO cells, the data is relevant to most systems.

One example of impurities I'd like to share is our learnings around ferrous sulphate. Cell culture formulations and cells in general, must have a source of iron. Ferrous sulphate is a common choice. Ferrous sulphate is sourced from mining from the earth and as such may have companion ingredients in the form of impurities. We found that our ferrous sulphate had very high levels of manganese, which in parallel the industry was learning had a high impact on protein quality. We changed our ferrous sulphate supplier to one that offered a product with a lower manganese level. To our surprise, however, one of our clients then reported a sudden change in the glycosylation profile of their product. Our subsequent investigations showed that this was caused by lower manganese levels in the medium, which was a consequence of our switch to a more pure ferrous sulphate. Essentially, the client's process relied on manganese impurities for the required product profile. It was easy to fix with a manganese supplement, but the case serves as an interesting example of how a higher quality product can have an unexpected negative impact. (I might add that, for most customers, reducing the manganese impurity level was a positive development!) The whole topic really emphasizes the importance of understanding a product and its processes; it is unwise to rely on impurities for a bioprocess; far better to understand what the process requirements are, and then work with media suppliers to ensure those needs are met.

Another example of the importance of being able to accurately track down and quantify trace metal contamination also involved manganese. We were working with a customer to establish the source of a ten-fold excess of manganese in certain lots of the same cell culture medium. After many dead ends, we isolated the source of the manganese in the most surprising culture component: vitamins. Vitamins are synthesized using processes in which impurities are well-controlled, so this was unexpected. Nevertheless, a specific lot of vitamin B6 had up to 500 ppm of manganese, and was clearly the source of manganese contamination in the final product. Once again, we fixed the issue by working with our vitamin B6 supplier, and again it shows how a sophisticated trace metal analysis initiative can help identify even the most unusual problems.

Control and customization

At Merck, our growing understanding of the effects of elemental impurities, and the expertise we have developed in the quantitative analysis of trace metals, has led us to focus on our raw materials and ensure that each individual material is as pure as possible. By ensuring that each component is high quality, we minimize the cumulative effect of impurities on the final culture medium and, hence, on protein quality. From the data we collect through our three-tiered approach, we continually modify our systems and guide ourselves to do things better, and to make better supply chain decisions.

Looking ahead, we have identified a market need for a customized trace metal analysis service. We are now in the process of developing this as a formal offer to help clients quantify trace metal impurities in their cell culture media. It also matches our philosophy of data visibility. Making data available to customers allows them to manage process variability according to their needs; for example, by mixing different batches of medium to ensure the cells receive optimal levels of given trace metals. Raw materials will never be 100 percent clean, but if you can measure impurity levels, you can manage impurity levels. Without a data-driven approach, it is far more difficult to control the impact of trace metal variability on bioprocesses.

My advice for industry is this: first, understand your particular process; second, communicate with your media supplier to ensure you build robustness into your supply chain; and third, use data to design a process that achieves the desired product profile.

Chandana Sharma, PhD, is Head of Cell Culture Raw Materials, Upstream R&D, at Merck.

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A Media Match Made in Heaven

When it comes to a good cell culture media, biopharma manufacturers desire a chemically defined formula, controlled variance and, increasingly, a specific protein quality profile.

By Bruce Lehr

Cell culture media consistency is crucial when trying to avoid unnecessary introduction of variability in protein production. Over the last five years, customers have been coming to Merck in the search for chemically-defined cell culture media; they want us, as the manufacturer, to not only understand every chemical within our formulas but also what impact they could have on production processes. It's no small task given that cell culture media has evolved from using serum, cell proteins and hydrolysates – all of which have undefined characteristics that can result in variation.

However, cell culture media have advanced significantly in the last two decades and now, despite the challenges, we at Merck are reaching a point where we understand every single chemical included in our formulas and where we can control variability within specific ranges.

In previous articles in this series (1,2), Chandana Sharma summarized how the raw material characterization team at Merck was formed to ensure that our raw materials are as pure and as well understood as possible. From my perspective, I need to know that our cell culture media will never be a factor that causes issues with a customer's process. When our cell culture media cause a shift in protein quality, it should not be accidental – we want to elicit the shift by design.

As well as ensuring that trace elements and impurities are controlled in our media, we need to balance the ratio of raw materials so that a given formula has everything the customer needs to produce their protein. I have been working with cell culture at Sigma Aldrich – now part of Merck KGaA – for 26 years. My current role involves working with customers to develop cell culture media formulas that enable them to produce proteins with the right quality profile.

Traditionally, customers have prioritized high protein productivity, but today we find that greater attention is paid to quality, with customers often seeking a particular N-glycan profile, a particular charge, and so on. Meeting a very specific protein profile is particularly crucial for manufacturers of biosimilars, who must copy the innovator product as closely as possible. Depending on what the innovator molecule is, this task could be easy or extremely difficult. But you can probably guess that, as molecules become more complex, we're often working at the "extremely difficult" end of the spectrum. Sometimes there are also intellectual property considerations to contend with; some innovators patent certain methods to perform specific protein quality manipulations.

Success by design

It is impossible for us to supply an effective cell culture medium, if a customer has not identified the endpoints and critical quality attributes, so we target these during development to ensure that we can deliver what the customer actually wants. There is a seemingly unending list of protein modifications that can be inferred by a customized cell culture medium. Whereas finding the right formula was traditionally performed by trial and error, today we use modeling and chemically defined libraries to perform media

screens. For example, if we were working to find a medium for a fed-batch process, we would perform media screens and feed screens, and then use a mathematical approach – multi-variate analysis – to draw correlations between single components, formulas, and the responses we want to see in terms of protein quality. In this way, we can see what is positively or negatively affecting a system, and then do more design of experiments to closely look at the ranges of those particular components to achieve the desired protein profile.

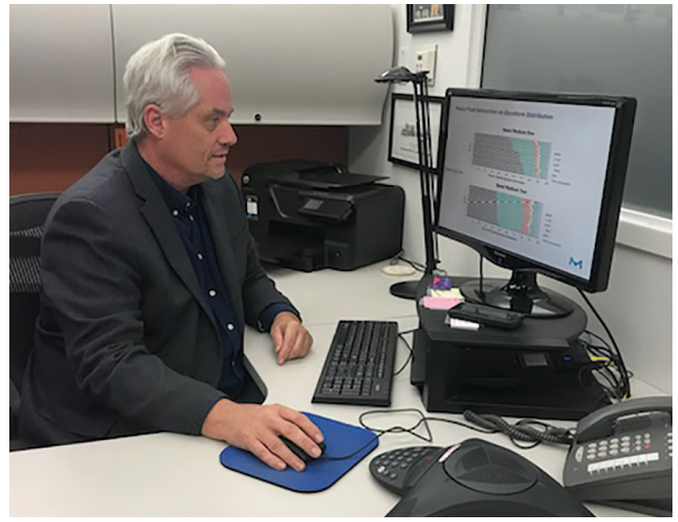
It makes much more sense to use a mathematical approach because, at the outset, you may have 70 components in a formula that would otherwise need to be "tweaked" through trial and error, wasting substantial resources. Screening helps identify the main components – let's say 15 – that need to be interrogated further to ensure they don't give any off-target effects.

How the media is put together depends on whether it's a fed-batch or perfusion process. Perfusion systems are being increasingly adopted, but there are not a lot of good scale-down models, which is a challenge. In fed-batch mode, 96 well plates or TPP tubes can be used to perform experiments under many different conditions. There isn't really a comparable system for perfusion right now, although we are reviewing alternatives, and there are still limitations in the number of conditions that can be run. We are looking at even smaller systems, which will ultimately change our workflows. The end goal is to be faster and as scientifically directed as possible.

Sometimes the actual amount of protein that is produced and the quality attributes move in opposite directions – with quality decreasing as productivity increases. In that case, we discuss the options with the customer – and most choose quality over quantity. If all of the customer's requirements cannot be achieved via a nutritional fix, the problem likely stems from the genetics of the cell line, which may not have the range of responses needed. In the unlikely event that we can't meet a customer's requirements, they may be able to further influence protein quality on the process side, using temperature or pH shifts, or a varied feed schedule. In any case, the customer will always end up with a chemically defined medium with controlled variability that is free of animal components.

Improved matchmaking

Our knowledge of cell culture media has led us to launch a number of commercial products. For example, Ex-Cell Glycosylation Adjust (GAL+) is a supplement that allows users to manipulate N-linked glycosylation by increasing sugar attachment. We are also developing



a GAL-, which, as the name suggests, does the opposite by decreasing sugar attachment. We are constantly assessing other protein quality parameters and will release new commercial products to help customers whenever we can.

We continue to develop and improve our formulas as our knowledge base grows. For example, tyrosines and cysteines are important nutritional elements, particularly for CHO lines, but can suffer from solubility issues, so we've been developing novel forms with higher solubility characteristics and improved liquid-form stability. Other amino acids and vitamins suffer from similar problems.

Finally, I must say that raw materials will always have some level of variability – the key is to ensure that any variability is well understood and controlled as tightly as possible. With an upward trend in quality, as well as calls for more supply chain transparency, cell culture media consistency will become an increasingly important topic.

Bruce Lehr is Director, Upstream R&D, Cell Culture, at Merck.

References

1. *C Sharma, "Chasing Traces," The Medicine Maker, 34 (2017). Available at: http://bit.ly/2hATxvc*
2. *C Sharma, "Know Thy Raw Materials," The Medicine Maker, 33 (2017). Available at: http://bit.ly/2xnRFLi*

The Problem with Poloxamers

Poloxamer 188 has become an essential component of cell culture media, but lot-to-lot variability has been a problem for many biopharma manufacturers. By working with customers directly to understand the science of inconsistency, we're able to supply a product that can be trusted.

By Nina Weis

In previous articles in this series, my colleagues have discussed the importance of well-characterized raw materials, a transparent supply chain and trustworthy suppliers (1-4). As an example of how a well-characterized product leads to benefits for biopharma manufacturers, I would now like to share the story behind Merck's Poloxamer 188, which we launched in March 2017. The product is part of our Emprove® Portfolio, which centers on supporting risk assessment and aiding the development of more robust processes – part of that involves increasing supply chain transparency and offering full GMP documentation.

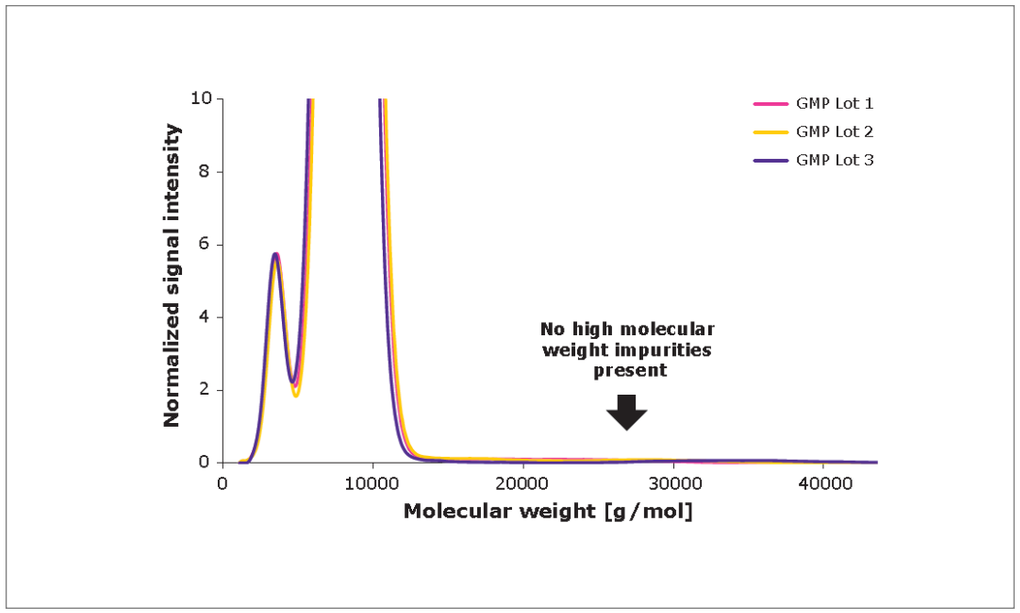
I have been with Merck for almost a decade, working in various functions focused on cell culture media, including product management, strategic marketing and portfolio management. We are always closely watching the market and interacting with customers to understand their needs and devise new technologies and products that will solve biopharma challenges.

Over the years, I have been involved in many projects regarding upstream chemicals, but recently I have been very focused on Poloxamer 188, a surface-active non-ionic amphiphilic triblock copolymer composed of a central hydrophobic chain of propylene oxide flanked by two hydrophilic chains of ethylene oxide. The lengths of the polymer blocks can be altered, allowing different forms of poloxamer to be produced with varying properties. Commonly, poloxamer is used for its surfactant properties – and often employed in drug delivery as a formulation excipient. Poloxamer 188 is a form of poloxamer initially developed for the cosmetic industry to improve or change surface properties for products, such as hand cream or shower gel. As it turns out, it can also play a starring role in cell culture media.

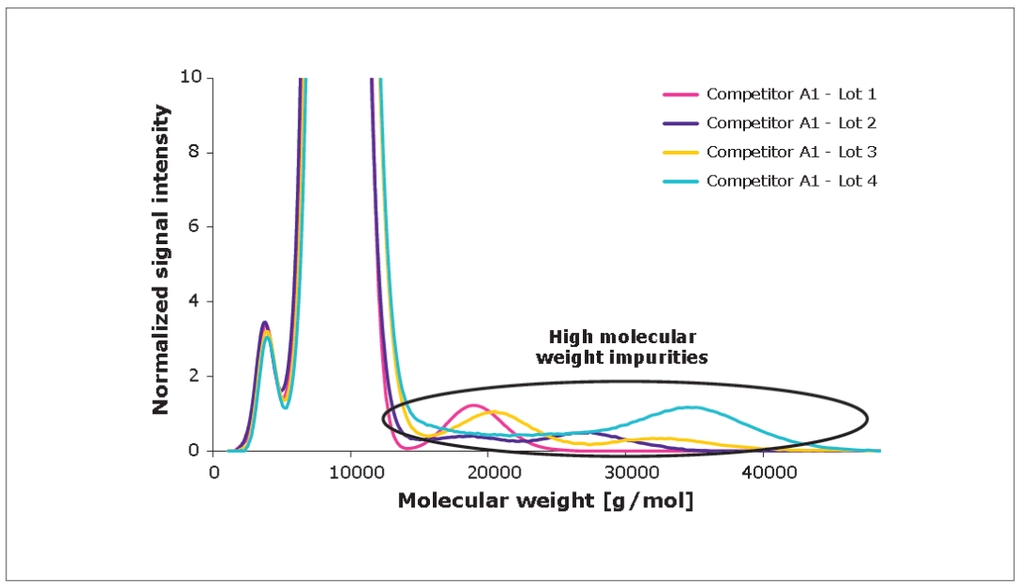
Out with the old

Although animal serum traditionally was an important component of cell culture media, concerns around variability and infectious agents have forced the industry to seek other alternatives and to develop chemically defined cell culture media. One of the challenges was finding a non-animal derived substitute that could withstand hydrodynamic stress in the bioreactor. The industry explored various options and discovered that poloxamer 188 works very well; it increases the robustness of mammalian cells to shear from sparging, which is the strongest contributor to hydrodynamic stress in a bioreactor. This is why poloxamer 188 became a standard ingredient in the industry's cell culture processes.

Over time, however, with process intensification through increasing cell densities and productivities in fed-batch and perfusion processes, a new challenge has emerged: variability between poloxamer 188 lots started to be reported in the industry. Biopharmaceutical



Poloxamer 188 EMPROVE Expert cell culture optimized assays to prove shear stress protection. High molecular weight species have a negative impact on performance - our Poloxamer 188 contains no high molecular weight material.



manufacturers began to approach Merck, explaining that they were seeing significant variation in their processes that caused unexpected loss of cell density and viability in their manufacturing operations. One manufacturer told us they had seen a 30 percent loss in yield, which was finally identified as a result of bad lots of Poloxamer 188. You can imagine how variable manufacturing runs result in much higher manufacturing costs for companies to produce biotherapeutics.

It was very rewarding for us to have our customers open up to us about their problems, which highlights the good relationships we have built up with them over the years. We decided that it would be valuable to investigate the issue in detail – in collaboration with our customers – to learn what makes a good or bad lot of poloxamer, and whether it would be possible to develop a well-characterized product for biopharma applications that would result in more consistent quality and cell culture media performance.

In 2015, Merck purchased Sigma Aldrich and the two cell culture teams became one. Now, it was possible to globally work on the problem together – a huge benefit as the project ultimately benefitted from different insights, approaches, and combined knowledge in chemical and biological areas.

The science of variability

First of all, we had to understand why different lots of poloxamer 188 were negatively impacting biopharma processes. What was the correlation between the polymer, the material-chemical properties, and performance in cell culture media? It made sense to include our customers in our investigation, as we wanted to ensure that we tackled the issues that were important to them; we worked alongside them to exchange samples, characterizing good and bad lots of poloxamer. Over time, we compared our results to ensure we were reaching the same conclusions. We tested almost 200 different blind samples, which led to a reference library that can be used to reliably classify Poloxamer 188. Not only were we able to identify variation, but we began to understand what impact different poloxamer variability would have on cell culture media.

Ultimately, we were able to develop and validate two orthogonal biological and analytical methods for both evaluating the critical quality attributes of poloxamer 188 and identifying lot variability that may impact cell culture media. We now have a highly sensitive cell-based assay that classifies the shear protective effect and a cell test for standard product release. We also used size exclusion chromatography and liquid chromatography-mass spectrometry to identify high and low molecular weight impurities, and hydrophobic impurities, respectively, and have developed an analytical method for reliably separating good and bad lots of poloxamer 188. Since then, we have found a partner to manufacture the polymer in a specific way to ensure that it has consistent quality and cell culture functionality.

Our customers have been very excited to see a new source of poloxamer 188 on the market – particularly one that guarantees quality and performance. But perhaps what's most exciting is that our poloxamer was developed with direct insight from customers based on real-world problems. As well as benefitting from improved consistency, customers also have better security of supply. Previously, many of our customers relied on a single supplier, which can create difficulties – particularly as poloxamer 188 is such a critical component for cell culture media. A new source of poloxamer gives customers greater flexibility, and I am very proud of the robust supply chain that we have developed.

We have put a lot of effort into our quality management systems to ensure that our suppliers meet certain standards – but we also perform regular audits to make sure those high standards are adhered to. The supply chain is also very transparent; our customers know where the polymer is manufactured, where it is released, where it is filled, packed and so on. Our assay also ensures that only product with material functionality is released and shipped to customers.

If you need further proof of our commitment to poloxamer 188 quality, we use the same product in our own cell culture media! Just like you, we are only satisfied with qualified, high-quality components when it comes to all our media products.

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