WHITE PAPER

Powder Culture Media Packaging, Preparation and Market Trends

A Study of Manufacturing and Market Trends

BioPlan Associates, Inc.
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Introduction:

This White Paper reviews the impact from recent trends in biopharmaceutical processing on the usage of culture media and its packaging. We discuss current media preparation problems, future trends, and alternatives to media prep practices being implemented. We cover end-users' perceptions of the adverse effect of these problems on their facilities and their operations, and we estimate the value of alternatives to current media prep processes that are being introduced.

Over the past 10 years, advances in large scale bioprocessing have begun to necessitate changes be made to the way culture media are supplied. These changes include basic physical forms of delivered culture media, as well as its packaging.

For decades, large-scale bioprocessing has used ground powders prepared at end-user sites into liquid media. This nearly universal in-house hydration of powders by end-users is changing as alternative powder packaging and preparation methods advance.

Culture media are some of the most complex bioprocessing essentials. Yet relatively few end-users appreciate this complexity. Culture media prepared by end-users from ground powders are one of the only bioprocessing components that come into contact with cultured cells and end products that are not fully sterile.

The most basic factor differentiating culture media is its physical state. The two common options are ground powder for end-user hydration to prepare finished liquid culture media, and bulk prepared fully finished liquid culture media purchased directly from media manufacturers. As discussed below, over 90% of large-scale culture media is purchased from manufacturers as ground powders for in-house mixing; less than 10% involves bulk finished liquids from manufacturers. Most bioprocessing facilities are currently designed to handle powders. However, as discussed below, alternative methods for packaging and, related to this, alternatives for end-user powder hydration are increasingly becoming available.

It is normal for culture media (formulations) to be changed multiple times during a product's development. Essentially, process development uses liquid culture media at small scales, such as media packaged in 1 liter bottles for agent and process discovery, R&D and, perhaps, preclinical manufacture. Culture media are typically further screened and optimized, including switching to powdered culture media, during early clinical development stages. Most every facility switches to using powders as bioprocessing scales increase, particularly, before the start of Phase III and commercial manufacture.

Today, companies tend to prefer 'platform' or standardized media. However, culture media optimization can be very attractive and cost-effective, allowing savings through process efficient at larger scales. In this context, some companies attempt to specify a standard culture media powder formulation, then supplement this on a customized basis, as needed. But the general pattern remains customization/optimization of culture media on a product-by-product (process specific) basis, with each product's culture media individually customized for its bioprocessing. It is rare to change or optimize media during Phase III development or near or after commercialization. Aside from time delays, validation testing at large scale can be expensive. So, in general, there is little standardization in the purchase of culture media.

Method:

For this study, we conducted an on-line survey of 24 large-scale culture media end-users in December 2013 and January 2014. This research was supplemented by in-depth phone interviews to assess the changes occurring in well established media preparation processes, and the long-established attitudes associated with these operations. The findings and commentary are presented here to evaluate relevant industry trends, and their impact on cell culture media selection.

Interviewees and respondents include relevant members of the Biotechnology Industry Council (BIC™) advisory group. These culture media end-users are all significantly involved within their facility cell culture media operations, and for this study, essentially all were from major/large biopharmaceutical manufacturers and CMOs. None of the in-depth interview respondents were from small- or mid-sized companies.

Research Survey and Interview Results

Powder Media Prep Problems and Rating

In this study we first evaluated the problems facilities encounter as a result of current media preparation processes. Methods for large scale preparation and hydrating of powdered media have been developed over decades, and operators tend to be comfortable with these operations.

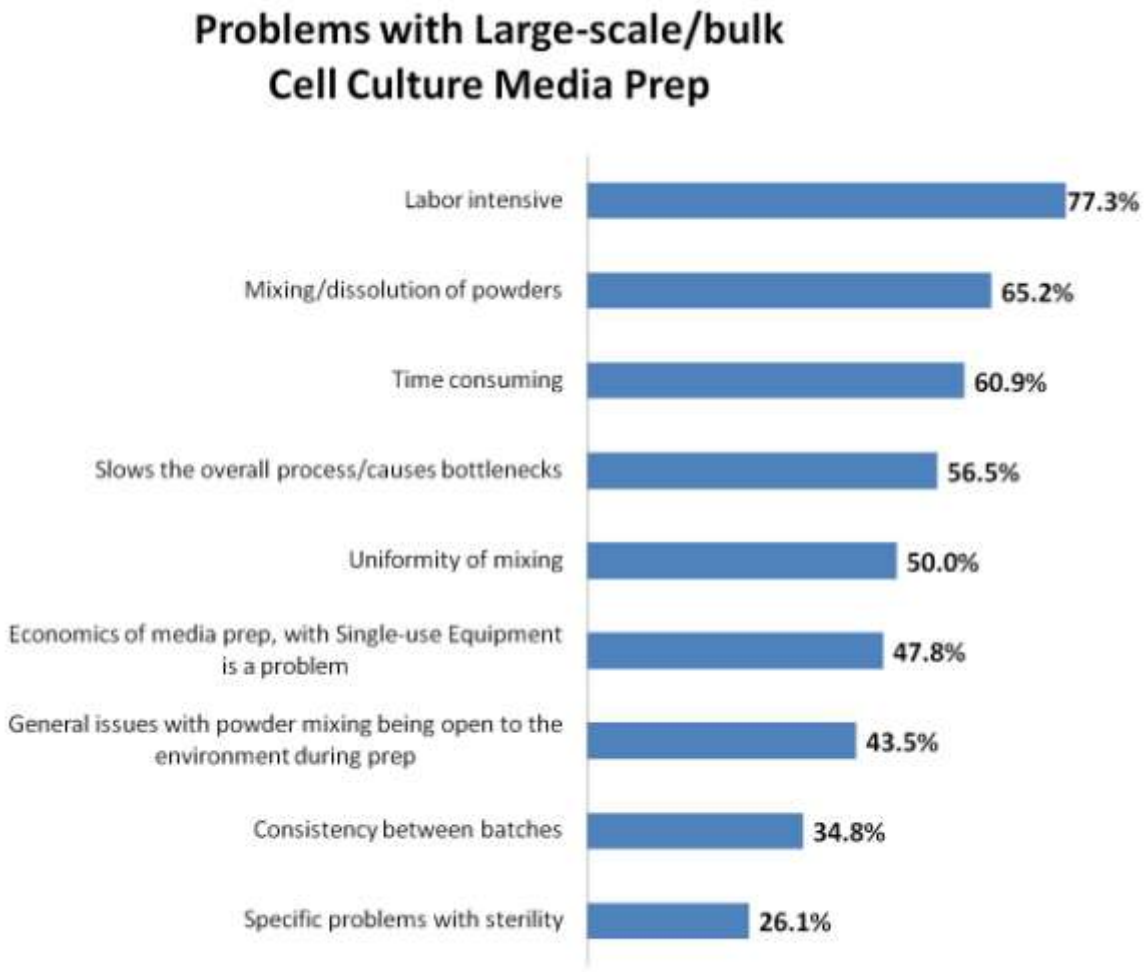
We presented a variety of related problems to be rated in terms of severity, from 1 to 10, where 10 would be considered a 'severe' problem. As expected, the labor involved in media preparation topped the list of problems associated with large scale operations in terms of both the average 'severity', and the percentage of respondents indicating the problem was a 'significant' (5) problem or worse. Table 1 indicates that the critical problem areas include the labor/time problems of media prep, followed by problems associated with mixing, uniformity, and dissolution of powders. Nearly half of respondents indicated concerns regarding open environment preparation, and over a quarter were concerned regarding sterility.

Table 1: Rating of Problems in your facility preparing bulk powder media (scale of 1-10)

	Rating Average "Severity" 1-10 Scale	% ≥"Significant" Problem Top (5-10 Rating)
Labor intensive	6.00	77.3%
Mixing/dissolution of powders	5.91	65.2%
Time consuming	5.22	60.9%
Slows the overall process/causes bottlenecks	5.26	56.5%
Uniformity of mixing	4.41	50.0%
Economics of media prep, with single-use equipment is a problem	5.17	47.8%
General issues w/ powder mixing open to environment during prep	4.04	43.5%
Consistency between batches	4.48	34.8%
Specific problems with sterility	3.13	26.1%

A number of the problems listed are rather closely related, e.g., mixing/dissolution, uniformity and consistency; and labor intensive and time consuming. Overall, it can be seen that mixing is perceived as involving significant problems, such as with powder dissolution, and it being time, labor and facilities infrastructure-intensive.

Fig 1. Percentage of Respondents' Indicating Large-scale Cell Culture Media Preparation to be a "Significant" (5) or Greater Problem



It is interesting that problems with sterility have the lowest rating, here confirming that process stream and product contamination are not serious concerns with end-users mixing powders (although, as discussed below, respondents also commented about advancing microbial detection and inactivation technologies, perhaps, leading to culture media being fully sterile prior to use).

Problems with Powder Media Preparation

Powder mixing protocols, conditions and equipment can have huge effects on the resulting hydrated media's chemistry, performance and shelf-life. For example, the order of addition of ingredients, temperature, pH, different technicians, etc. can result in variations in resulting liquid media characteristics and performance. For end-users to get it right, it is often a matter of finding what works best, with consistency, with this often requiring much experience and experimentation. Problems reported in our interviews as typically encountered include difficulty in achieving uniformity and consistency in mixing, both with specific batches/lots and when comparing different batches/lots. Specifically from interviews we consistently found that mixing and other media prep are seen as:

- Generally not well-studied, e.g., PAT is rarely applied to media preparation; as a result, variations can be expected
- Not standardized, e.g., different facilities use different equipment and protocols for preparing much the same media from powders
- Done in non-specialized equipment
- Labor- and time-intensive
- Requires extensive training, often lacking in many facilities and/or these tasks are left to lesser-experienced operators/technicians
- Potentially toxic- hazardous to workers
- Difficult to follow in terms of mixing protocols, with powder mixing involving much art and experience, along with much variability
- Preparation-intensive, requiring mixing, particularly with fixed stainless steel systems, complex and lengthy, including cleaning, steam sterilization and validation of equipment
- Equipment-intensive, requiring costly mixers, holding bags or tanks, and sterile filtration, including single-use equipment that must be (re)purchased with each process run
- Challenging to maintain low bioburden, including minimizing exposure of powder to air and contact with non-sterile objects

Further, in our online survey we asked for comments concerning powder media-associated problems, and what specifically the respondents would like to avoid. In general, responses elaborated on time and labor issues, and expanded on difficulties in terms of consistency and the need for greater homogeneity. Here we also see further confirmation concerning problems of lack of sterility of powders and powder mixing, and materials transfer and dust problems.

Comments included:

Consistency Problems:

- *We see inconsistent performance with powdered media. This includes when we go up in scale, such as from 5,000 to 15,000 L. We see inconsistencies at different scales, the 'same' media from different manufacturers' facilities, in manufacturer's and our own mixing.*
- *Consistency of culture media components [is a problem] when hydrolysates are used.*
- *Consistency between lots of media powder particularly with hydrolysates. Need more consistent quality of components*
- *Media performance changes when moving from liquid (development stage) to powder or AGT (in clinical or commercial).*
- *Variability [with our powdered media] could be due to inconsistent mixing. Luckily, this has not affected our bioprocessing [commercial manufacture at this site]. Sometimes we redo a process and the results are different.*
- *Lot-to-lot variability [from the manufacturer] -- Some vaccines we manufacture are more sensitive to media variations than recombinant proteins.*
- *We've spent a lot of time conforming our in-house processing, including with vendors, and are confident, the vendors too, that variability is not at our end, not due to our powder mixing.*
- *Variation in powders is normal. We see differences [inconsistencies] between the media we purchase for GMP manufacturing and non-GMP research…grinding is likely done at different scale, likely using different mixing equipment.*
- *We need predictive and QC tools to spot any culture media problems, variances, such as NIR.*

Mixing and Handling Problems/Needs:

- *I would like to avoid the long mixing times required to mix the components.*
- *The manipulation of powders for packaging is not easy and powders are volatile.*
- *[There is a] need for better connectivity between mixing, sterile filtration and delivery into bioreactor or storage bags.*
- *Improved dust control during preparation.*
- *Improved top-down mixing as the media powder tends to float where mixing is at its weakest.*
- *Powders are hygroscopic and this generates strict conditions of storage and processing.*

- *Multiple additives with powders [are a problem].*
- *Extensive mixing time/consistency with making concentrates.*
- *The manipulation of powders for packaging is not easy and powders are volatile.*
- *Weighing of powders is the one operation I'd most like to avoid.*
- *Raw materials management, getting and then storing powder media in-house.*
- *Long lead times by the manufacturers.*
- *Mixing is something we'd prefer to avoid. This involves using tons of buckets, and it is hard to keep track. Powder often floats to the top.*
- *We sometime see clumping in bags [containing powdered media]. We sometimes see inconsistencies in media manufactured at different scales.*
- *End-users are not provided with a full understanding of the products they receive.*
- *We need to prepare media from powders in-house as a way to keep the operators/technicians busy, to even-out their schedules, with manufacturing tending to involve campaigns, with crunch and slow times.*
- *We need to have a certain number of operators [technicians] and still want full on-site control. We need to have work to keep operators busy [with in-house media prep filling this need].*

Lack of Industry Information, Support

- *Media companies are not adding value. All they do is fill our orders. Never any investigation or discussion of the powders we ask them to make. They never suggest how we could be doing things better.*
- *Everyone, including ourselves, all consider everything related to culture media to be proprietary*
- *Vendors are not providing data in a transparent manner.*
- *There is a need for better supply chain and certifications information about media components. Suppliers never proactively provide this information. It's like pulling a thread on a sweater, you just keep on pulling and it keeps on coming, with the process taking a long time.*
- *Media companies keep too much information proprietary, not disclosing this in patents or to us…they've never provided us with information about what has been changed.*
- *Vendors need to provide more information and guidance concerning our media purchases. We give them a formulation and its delivered. We never get guidance about how we should make changes that would improve their grinding and other operations, result in a more stable and better performing product.*
- *Media companies need to work with us to suggest the best ways we could implement savings, process improvements, etc.*

Need for Closed Systems:

- *Regulators want more closed processing or cleaner air in media prep. If all powder media components were mixed together without exposure to the environment, these would be preferred and a big advantage.*
- *Keeping bioburden low [is a problem] - keeping other biological contaminants from media and buffer prep.*
- *Open environment with human borne mycoplasma contamination risks, dust of powders in the room, and dissolution problems which clog unit operations which follow.*

Virus and Other Contamination Problems:

- *Potential for virus contamination from media raw materials remains a threat. Media vendors need to implement better quality control and potentially some terminal treatment of their product to reduce this risk*
- *Trace impurities from lot-to-lot can change cell culture performance (e.g., bivalent metal ions.*
- *[I would like to] avoid excessive microbial growth in media prior to filtration.*
- *Would like to minimize the amount of bioburden present in the bulk powders.*
- *Exposure to animal-derived raw materials is a problem.*
- *With powders, the biggest challenge is virus protection. Companies with legacy facilities need this most, but it is hard to implement with established bioprocesses and facilities.*
- *The biggest problem with media is bioburden of the powders. We currently only test for bioburden and mycoplasma. With viruses…there could be 1,000s of others we are not testing for.*

HSTS and Other Viral Treatments

HSTS and other antiviral culture media treatments are coming. Amgen, Genentech and other large companies will adopt it and others will have to follow. Comments on HSTS include:

- *[In our company] We will be selectively applying HTST to new processes and will slowly phase it in...HTST has relatively little impact on new processes. It's simply the next step [in media evolution]...*
- *HSTS may not be for everyone. But it will be implemented at large facilities. Legacy and other major manufacturers must defend their facility against contamination.*
- *We definitely need to eliminate viruses from our culture media. Yes, this is not needed for product safety. Rather, we need it to protect our facility.*
- *We have already started implementing HTST of culture media…taking a staged approach, product-by-product.*
- *Our facility recently did a risk assessment, and we are thinking of adopting HTST. We would not use this with our single-use systems [where contamination simply means disposing of the equipment], but would use this to protect large stainless steel legacy systems [from virus contamination].*
- *Viral barriers are needed. Media companies should be providing more technical evaluation and guidance in this area.*

Future Changes in Culture Media Preparation

The online survey and our interviews also questioned how the current trends in bioprocessing may change the way companies will be large-scale preparing their media in the future. We asked, *"Consider current biopharma industry trends (e.g., single-use, smaller scales, flexible facilities, perfusion, expectations for complete sterility, including viruses). How will these trends change the way facilities manage their media prep in, say, 5 years?*

In general, in projecting future changes (five years out) , the largest portion of responses included a greater recognition of the fact that powders and culture media are not fully sterile, with one or more technological fixes likely to be applied. Others noted that use of single-use equipment for mixing and culture media preparation from powders will increase. There is also an apparent recognition that media preparation from powders will only get more complex, including as expectations for sterility increase. Based on these responses, at least among knowledgeable/expert end-users, there is definite recognition that current methods for media preparation from current powders are not ideal, although these will remain the dominant method at least for the foreseeable future.

Responses Regarding the Future of Media Preparation Included:

Future: Regulatory Pressures and Closed Systems:

- *Likely need closed processing or high quality environmental air in media prep*
- *Expect pressure on vendors to provide cost effective single use disposable bags/filters/tubings...and design bags which house the raw material that can be directly connected to mixing bags in a fully closed way. This requires being able to dispense the correct amount...so either the vendor needs to be told a specific quantity, or be able make multiple additions from the same connection.*
- *Recent [regulatory] agency focus on microbial control will lead to tighter control on raw material bioburden levels, and reduced tolerance to microbial growth in media preparation vessels. These will likely reduce preparation times, necessitating more aggressive mixing to quickly solubilize material. Bulk concentrates still have formulation and stability challenges, but can be viable with additional work on the chemistry.*
- *Use of single-use will allow facilities to prep in Class D.*
- *There will either be powders in bags (gamma sterilized) which will involve addition of water for rehydration, or liquid media pre-filled in bags aseptically connected to the bioreactor. It could also be that basal media are already filled in bioreactor bags and feed streams are connected to it.*

Future: Better Mixing

- *Better mixing capabilities of single-use products will enable the preparation of concentrated buffers/solutions requiring less footprint and simpler transfer from preparation room to processing floor.*
- *Media prep activities will become even more time consuming. Companies will start using new technologies like granulation processes, or will work with vendors for preparing high concentrated solutions to be diluted prior to use in a sterile environment.*
- *I think as long as media preparation is not the run rate limiting step, the current method of preparing media will not change for established facilities. However, new facilities may benefit from already prepared media solutions [bulk liquid culture media].*

Future: Virus Reduction

- *Larger facilities (and those who can afford) will evaluate virus reduction techniques such as HTST as the terminal step before formulated media goes to the bioreactor*
- *Virus concerns will increase, will very likely lead to routine use of HTST or virus filtration of prepped media.*
- *[There will be] heat treatment of liquids in addition to filtrations. Reduced preparation times may be challenging.*
- *There may be a push to have media powders treated in some way to minimize media risk from bacteria and viruses - may involved irradiation of powder or HTST of media concentrates.*
- *Use of HTST on media concentrates will increase.*
- *[There will be] greater acceptance of just-in-time delivered liquids from suppliers.*
- *Five years from now, powders will come sterile, e.g., be gamma irradiated and pre-measured.*

Future Vendors' Role

- *Flexible and adaptable systems will be developed*
- *For smaller bioreactors, up to 2000L, expect more liquid media supplied by vendors. For larger scale (>10kL) still be using powders, expect to build pasteurisation kits into the plant.*
- *[Expect more] control of media quality and consistency. Once this goal is achieved, outsource media prep or move to single-use platform.*

Future: Single Use Role

- *We will use more single use equipment [in 5 years]. Flexible facilities are required*
- *As long as media preparation is not the run rate limiting step, current methods of preparing media will not change for established facilities. However, new facilities may benefit from already prepared media solutions*
- *Single use equipment is too expensive to allow their use for the production of powder media. Without a decrease of the prices, we won't be able to use them within 5 years.*

Slow Change Expected

- *Media concentrates and processes will become more common, but the traditional manufacturing methods will still remain the primary method.*
- *Don't expect rapid changes: The industry trends over the last 10 years do not indicate a rapid change in practices and I would suspect the next 10 years to be similar with incremental improvements.*
- *Most facilities will expend more resources on development of media formulations than improving manufacturing efficiencies of media production.*
- *The need for larger quantities of media (e.g., for perfusion or ATF) will be somewhat offset by the smaller size of bioreactors.*

Facility Differences in Valuation of Media Preparation Problems: Stainless Steel vs. Single-use Facilities

Our research shows that decision-makers at facilities designed to use primarily stainless steel, fixed equipment considered the problems associated with culture media preparation differently from those whose facilities are, or will be, designed to use primarily single-use, or disposable equipment.

Because the critical factors of mixing and labor are involved, we evaluated the differential between attitudes by asking how valuable certain types of packaging might be in terms of addressing facility problems. We asked, *"For your facility's media prep operations, how valuable would the following be in [current or future Stainless vs Single-Use] operations"*

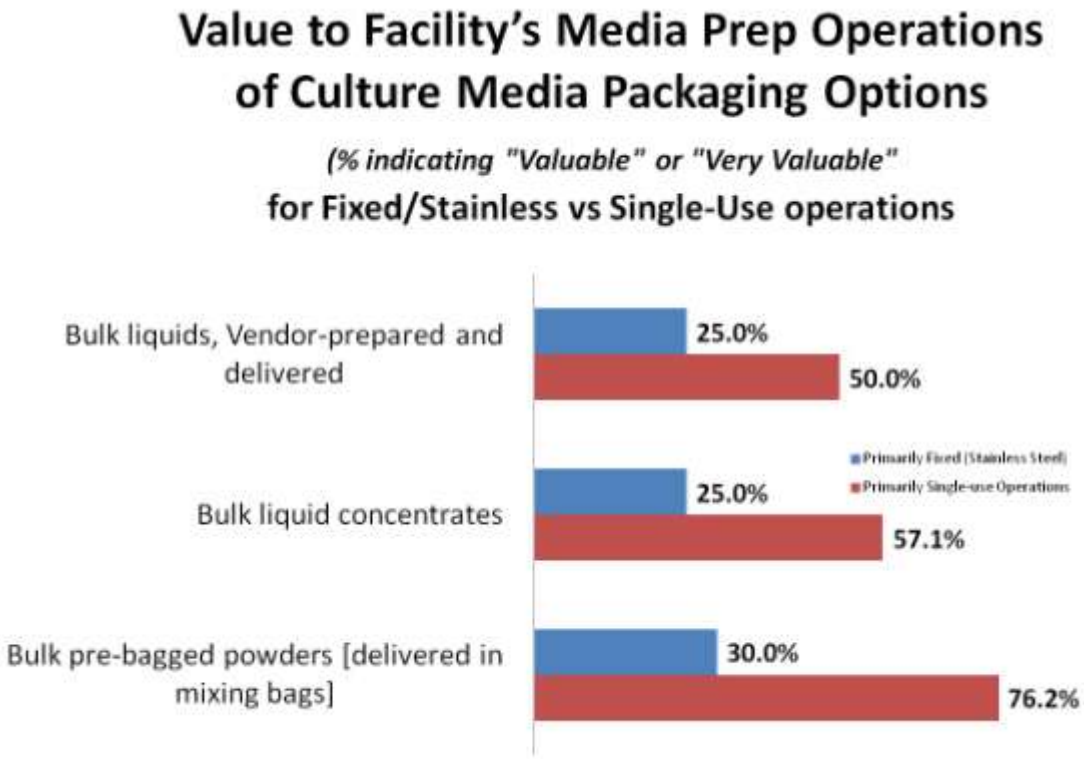
In the online survey, we found that there was a substantial gap between decision-makers when considering single-use vs stainless steel. In every alternative packaging option suggested, respondents noted, by at least a factor of two to one, that these alternatives were at least 'Valuable' for single-use applications. Specifically, the largest percentage of respondents (76%) indicated that, for single-use operations, "Bulk, pre-bagged powders, delivered in mixing bags", would be valuable. This compares with 30% of respondents considering this type of packaging for fixed/stainless steel facilities. In fact, for single-use facilities 43% indicated that pre-bagged powders would be 'Very Valuable' (compared with just 15% indicating the same for fixed, SS operations).

Similarly of interest were bulk liquids, either prepared and delivered by vendors, or provided as bulk liquid concentrates. These were seen as at least 'valuable' by 50% (interested in bulk liquids) and 57% (interested in bulk liquid concentrates) for single-use operations. We also noted that 50% of respondents had additional, other alternatives in mind that they felt might be valuable or very valuable in both stainless and disposable applications.

Comments included:

- *For single use CC reactors 1000L we definitely want pre-made Liquid media and feeds. If powder must be used, then we would want pre-bagged additions to single use mixers.*
- *Alternative packaging would need to include virus treatment or control*
- *Any alternative will require better data accessibility*
- *Alternative packaging must also provide better mixing technology, better sterile barriers*

Fig 2: Percent Respondents Considering Alternatives to Current Media Prep Operations as 'Valuable' or Very Valuable; Single-use vs Stainless Steel Applications



Discussion

Culture Media in Strategic Manufacturing Context

Over the past decade, the pharmaceutical industry has shifted emphasis, particularly for new products, more to biopharmaceuticals rather than small molecule drugs. This includes nearly 50% of therapeutics in the development pipeline now being biopharmaceuticals. A recent study by the Center for the Study of Drug Development (CSDD), Tufts University, reported that the number of biopharmaceuticals in clinical development grew 155% in 11 years, from 355 in 2001 to 907 in 2012[1]. CSDD also reported that financing of biotech research increased 10-fold, from $10.5 billion in 2001 to $103 billion in 2012. As reported by *BIOPHARMA*[2], there are currently over 450 biopharmaceuticals approved in either the U.S. or Europe, with worldwide total biopharmaceutical sales ≥$165 billion in 2013, likely reaching $≤$190 billion in 2014. Both increased R&D and commercial manufacturing of more approved products continue to drive ever-larger biopharmaceutical manufacturing supply markets, including for culture media.

The current cell culture media market (based on estimates from our own analysis and from published market research studies) is estimated to be about $600 million/year, or about 0.36% (about 1/3rd of 1%) of the total biopharmaceutical products market. BioPlan Associates has reported that the bioprocessing supplies market basically involves about $6 billion/year each in upstream and downstream expenses, with total bioprocessing market growth tracking that of product sales revenue and growing at a rather steady ~15%/year[3]. In this context, the total culture media market is about 7% of the current $12 billion bioprocessing supplies market.

Nearly all culture media used for biopharmaceutical development and manufacture and, particularly, bulk orders, involves custom manufacture. Thus, in many respects, culture media manufacturers are contract manufacturing organizations (CMOs).

Volumes of culture media that are used increase as manufacturing scale (the amount of product manufactured) and, particularly, bioreactor size increase. Scale of manufacture is associated with the stage/phase of development. Biopharmaceutical manufacturing processes are progressively scaled-up and validated at cGMP, generally to multiple 100s, 1000s or even 10,000s liters, even before commencing commercial manufacture. Culture media use is much higher for commercial product manufacture. About 90% (as with other bioprocessing supplies) of culture media by volume is used for commercial product manufacture.

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[1] Biotech Products in Big Pharma Clinical Pipelines Have Grown Dramatically," CSDD press release Nov. 14, 2013

[2] See www.biopharma.com

[3] Langer ES, "Perfusion Bioreactors Are Making a Comeback, Industry Misperceptions Persist," BioProcess J., 2011; 9(2): 49-52.]

Alternative Powder Media Packaging and Preparation Technologies

The difficulties and downsides with powders and their mixing are leading some media manufacturers to develop alternative packaging, and through this, novel media preparation methods. Powder media currently is packaged within single-use plastic bags held within drums or other rigid shipping containers. Although some media manufacturers commonly manufacture powders in ≥10,000 L batches/lots (i.e., grinder capacity), the powders are generally packaged in 200 L (~55 gallon) or smaller drums for ease of shipping and handling. However, new, alternative media packaging and, related to these, modified preparation methods are starting to be actively developed and are entering the market.

Newer culture media packaging alternatives include pre-kitted, pre-weighed and packaged, essentially portion controlled, culture media packing already packaged within their own form-fitting bags or within larger appropriately-sized mixing bags/containers. Powders are increasingly becoming available in such custom "pre-kitted" forms from many vendors. This essentially involves the manufacturer pre-weighing and packaging desired amounts of powder media in bags/containers for easy transfer into mixer bags/containers. Bags may be sized to the amount of powder or the powders may potentially (no examples on the market yet) be packaged within larger mixing or even final bioreactor bags/liners. This allows avoidance of powder transfer and weighing operations; and particularly, if the bags/containers have aseptic seals/connectors, eliminates much exposure of the media to air, minimizing adsorption of water vapor and potential microbial contamination.

Powder media could, theoretically, be packaged in final bioreactor bags, rather than common use of intermediary/holding bags. These bioreactor liner bags containing powder media ready for mixing could potentially include these shipped pre-installed within single-use bioreactors, with WFI added and mixing done by the end-user in the bioreactor, again minimizing transfers and related labor, time, costs and risks. Delivery of powder media already weighed out and transferred into mixing bags allows avoiding the all the work and hazards involved in weighing and transfer of powders into mixing bags/containers, plus the culture media manufacturer can presumably do this more accurately with a higher levels of sterility than end-users. However, problems can be expected with this powder-in-a-large-mixing-bag approach, with it more standard practice and feasible to incrementally add powder into stirred liquids vs. adding liquid to powder at the bottom of a bag, with powder clumping and other difficulties in hydration seemingly likely.

Other packaging options reportedly in development or just starting to enter the market include capsules or other containers with end filters or other permeable enclosures allowing fluid movement in and out, with these dropped into mixing bags/containers and WFI added. This could involve packaging powder in containers with filters or series of small openings allowing

WFI and dissolved media to enter and leave the container during mixing operations. The containers could simply be left in their single-use mixing bag and the bag disposed with at the end of bioprocessing. Or media might simply be packaged in inert, cell culture degradable or water-soluble bags, such as composed of starch or other harmless polysaccharides. Here, there would be nothing to remove at the end of bioprocessing, and few concerns about leachables from the powder container.

Powdered culture media can be conventionally mixed and prepared using any suitable combinations of fixed stainless steel or single-use equipment. Whether stainless steel or single-use equipment is used depends on the facility, e.g., if it already has relevant mixing equipment and the preferences and biases of the company, facility and bioprocessing professionals involved. Use of single-use equipment requires consideration of related plastics' leachables/extractables, while use of stainless steel equipment requires consideration of its leachables, e.g., metal ions, and its cleaning, sterilization and validation prior to (re)use. Single-use mixers continue to improve with more variations and more powerful models becoming available. Some single-use mixers now even include heating jackets. But even the use of stainless steel equipment for mixing powders can result in significant variations affecting cell culture. Different stainless steel vessels and piping can lead to culture media containing different amounts and types of metal ions as leachates, with these potentially affecting cell culture performance. Some in the industry are now complaining that the quality and consistency of stainless steel has been going down as more recycled materials are used in their manufacture, with this tending to push them to use single-use mixers if possible.

Problems that remain unresolved with culture media mixing and preparation from powders by end-users include media prepared at different scales, even within the same vessels and following the same general protocols, are often inherently different, variable. This can cause problems as processes are scaled-up, with culture media mixed at say the 100 L scale performing differently than that mixed at say 1,000 L scale, even within the same 1,000 L capacity equipment.

Interview comments concerning alternative powder packaging and preparations technologies:

- *What I would like to avoid is the long mixing times required to mix the components.*
- *Pre-weighed media in mixing bags would streamline our operations. But for smaller volumes, it would be easier to use some standard buckets and weigh out what you need. This provides greater flexibility*
- *Pre-bagged media would be more relevant at up to 2,000 L scale, so it should be of particular interest to single-use facilities. Definitely, not for our 12,000 L manufacturing operations.*
- *A problem with pre-bagged powder [in larger bags for mixing] media is that this introduces yet another product in the upstream bioprocessing.*
- *Other than using a larger bag, it should cost the same as other pre-weighed media.*
- *Using pre-bagged media would be very difficult in large scale [vaccines manufacturing] facilities. We run a very large number of lots in a short time. It is hard to envision the logistics required – receiving, testing/QA, storing, moving.*
- *Bags are definitely of interest, but only for smaller scale, R&D, not manufacturing. Our primary concern would be costs.*
- *There should be a range of sizes [volumes] offered. We'd be most interested in 1 L and 10 L pre-bagged media. That is where I see the best value.*

Powder Culture Media

In-house culture media preparation from powders has long dominated bioprocessing, even among many newer single-use system-based and smaller-scale facilities. Keep in mind that typically, for 100s of years, culture media have been almost exclusively reconstituted and formulated in-house from ground powders. Culture media are the original single-use bioprocessing products; used once and disposed of. In a modern single-use systems context, powdered culture media may not be considered a true single-use product. Bulk powders [i.e., the products purchased - containers containing powder] may be reused many times and stored for long periods. Powder users often prepare many batches/lots of hydrated culture media from the same stored powder container.

Powdered culture media will remain the leading physical form, highly cost-effective and preferred, for all larger-scale bioprocessing (the bulk of the market), at least for the foreseeable future. As discussed below, they can be very cost-effective, particularly at larger scales or with fixed stainless steel facilities already having infrastructure.

However, with powders, the end-user is left to do his or her own extensive process development, dedicated equipment, testing/validation and documentation, designing and evaluating mixing protocols, equipment and QC testing, training staff, etc. End-users must on their own prepare a GMP-grade product, generally without having full knowledge about their culture media powders (in comparison with the rest of their bioprocessing), with media preparation from powders being inherently complex and challenging, particularly for smaller- and mid-sized facilities.

Because bioprocessing professionals are comfortable with powders, at all but the smallest scales, there is an industry-wide bias favoring powdered media. Preparation from powders is a core venerable feature of bioprocessing. The vast majority hold core beliefs that powders are always cost-effective and simply how bioprocessing is done.

Much as with single-use bioprocessing systems, a cultural divide can be perceived among bioprocessing professionals concerning culture media. 'Flexibility" is a key concern. Those favoring traditional powder media technologies tend to have less favorable views, or less hands-on experience with single-use bioprocessing, and tend to view powdered culture media as providing sufficient 'flexibility:' the ability to mix media when needed, and only in the amounts required (generally large-scale fixed stainless steel bioprocessing). However, those favoring alternative media prep approaches also expect 'flexibility,' but as with other single-use products, expect avoidance of labor and capital costs, minimal need for media prep rooms, saving in facility space and infrastructure; avoidance of labor and occupational hazards, etc. Further, these 'early adopters' believe that alternative media prep options should include all work, documentation, validation, etc., being done by suppliers with expertise in these narrow areas.

Discussion of Factors Affecting Culture Media Trends

Commodity vs. Custom Culture Media

Culture media can be considered to be a commodity or a specialty/custom product. "Commodity" culture media include those rather set legacy formulations used extensively at the R&D or desktop scales. Media routinely packaged in single liter bottles may be considered to be commodities (e.g., unmodified DMEM, Ham's F-1 and F-12, MEM, Medium 199 and other "classical" formulations). These "commodity" culture media are very rarely used in bioprocessing beyond the smallest scales where liquid media dominates. Rather, nearly all large-scale culture media are now custom manufactured (and/or end-user customized/modified).

Developed Country vs. Developing Markets

Although the primary markets for culture media, over 70% by bioreactor capacity, are facilities in highly developed Western countries with well-established biopharmaceutical industries, this is changing [see BioPlan's *Top 1000 Global Biopharmaceutical Facilities Index* Web site/database at www.top1000bio.com]. Bioprocessing supplies markets in developing countries are growing as biopharmaceutical manufacturing technology matures, including its core technologies going off patent and otherwise being widely disseminated, (consider the many biogenerics/biosimilars in international commerce). The number of biopharmaceutical companies is rapidly increasing in developing countries where local companies are seeking to develop products for regional markets. Much bioprocessing established in developing countries involves use of single-use bioprocessing systems[4], because building and maintaining large GMP fixed stainless steel facilities can be difficult in these areas. However, unlike in developed countries, where an increasing number of single-use-based upstream process lines are finding use of bulk liquid culture media to be attractive, powders effectively remain the only rational choice for facilities, even single-use-based, in developing countries. Powders must be used, if only because bulk liquids would be too expensive to ship and because many locations lack the cold chain, transportation and other infrastructures required for transport of bulk liquid culture media. Plus, powders can be stored for months, or even longer. So facilities in developing countries will continue to focus on powder media usage, and newer alternative powder packaging and preparation options will first be adopted in highly developed countries.

Variations/Inconsistencies with Powdered Culture Media

Three factors largely determine culture media identity and performance:

1) Ingredients/raw materials
2) Formulation – the complex mixture that is the product
3) Manufacturing processes

[4] 10th Annual Report and Survey of Biopharmaceutical Manufacturing, 2013, BioPlan Associates, Inc.

A change in one of these aspects can result in a different product, or at least one that functions different from the 'same' product from the same manufacturers. Besides desired quantities of each ingredient, basic physical-chemical attributes that must be controlled during media manufacture include physical appearance (e.g., color); pH; osmolality, humidity; and particle size distribution. The media must also meet expectations for cell culture performance. Inconsistencies in powdered culture media, whether due to the manufacturer or preparation the end-user, often result in cell performance (viable cell densities, yields, etc.) for the 'same' media varying by as much as 50%[5], Variability/inconsistencies in culture media manufacturing and in-house mixing from powders are one of the major sources for overall bioprocess inconsistencies. These can lead to product quality issues, including lack of comparability (and inability to market) of different commercial batches or lots and even regulatory agencies denying approvals. So far, culture media preparation, particularly, in-house from powders, has generally not been studied and optimized using Process Analytic Technologies (PAT) or similar approaches to systematically understanding and controlling bioprocessing, but sooner or later this will likely happen.

The 'same' powdered culture media, e.g., DMEM/F12, or same custom formulation from different sources can vary significantly in physical-chemical attributes and cell culture performance. For example, in tests on rather common formulations performed by Merck Millipore, "DMEM/F12 dry powder media produced by various suppliers appear very different...Together, the physico-chemical and performance data from this DMEM/F12 benchmarking study conclude that 'standard' formulation dry powder cell culture media are not in fact standard in their physicochemical and performance characteristics across suppliers"

Ingredients/Raw Materials Inconsistencies

Culture media is typically composed of 50-60 or more individual ingredients, most of which are sourced by media manufacturers from other sources. Many of these are pure organic nutrients and other biochemicals that may have distinct variations in properties due to complexity and variations in their manufacturing, including from different manufacturers. Obviously, variations in ingredients will result in variations/inconsistencies in finished culture media. Media manufacturers must have robust supply chain processes in place so that they can consistently procure and process raw materials to provide batch-to-batch consistent dry powder cell culture media with manufacture scalable from kilograms to tons. Batch-to-batch and all other aspects of consistency begin with consistency of the raw materials used. The proper selection, qualification, and pre-processing of raw materials is essential for ensuring that the starting materials are of the highest quality in support of optimal media performance.

[5] Improving Cell Culture Optimization," Vol. 32, No. 7, *Gen. Eng. & Biotech. News (GEN*), Apr 1, 2012

Many media ingredient powders are hygroscopic, including adsorbing water vapor from the air. Exposure to air, whether in storage or during powder transfer and mixing operations, which is rather common, can increase microbial contamination, and can lead to clumping or otherwise result in inconsistent mixing. The water content or humidity of dry powdered culture media has a significant impact on powder stability and storage. Powdered culture media water content should generally be ≤1%, with higher humidity supporting microbiological growth and causing physical changes in powders while in storage. Variability of humidity is rather routinely observed among powdered culture media, with some approaching or exceeding a water titer of 1.2%, suggesting likely problems.

Formulation Inconsistencies

Inconsistencies in formulation and performance include the 'same' media, e.g., common generic formulations among different manufacturers, the 'same' custom culture media prepared by different manufacturers, etc. And of course, end-user preparation varies even more. Inconsistencies in powdered culture media preparation are apparently normal and unavoidable (without expending effort to study and resolve these issues). Today, just about every culture media is produced custom for the client, with little bioprocessing at large scale using generic, off-the-shelf culture media formulations. Essentially every product is generally unique, custom manufactured, to some extent. This limits the ability to study and make conclusions concerning variations/inconsistencies in culture media, including powder preparation.

Manufacturing Grinding and End-User Mixing Inconsistencies

The manufacturer's powder grinding process largely controls the physical form of powdered culture media, and any change in grinding can result in physical, chemical and/or performance changes in the resulting ground powdered culture media. The production process, including milling, mixing, and packaging, must be controlled and gentle enough to avoid degradation of the raw materials, while also rigorous and extensive enough to support complete mixing. Various types of powder grinding mills and technologies are available, with each providing its own unique powder characteristics. Powder particle sizes and size distribution can greatly influence powdered culture media hydration by end-users and, related to this, cell culture performance. Processing by the manufacturer, often proprietary, seeks to ensure that each component has the proper particle size and surface area for optimal solubility. Different-sized ground particles of some ingredients may mix and dissolve/hydrate differently, providing inconsistencies in hydrated liquid media.

Variations in the grinding process can significantly affect powder media physical, chemical and performance aspects. This includes the same manufacturers manufacturing

the ‘same’ product using the same ingredients with the same processing, but using different equipment, at different scales and/or different mixing protocols. Thus, the ‘same’ powdered culture media prepared at one scale, say a custom order of several thousand Liters [post-hydration equivalent] for Phase I/II manufacture may be significantly different, potentially even to the extent of not being ‘comparable’ or the same by regulatory agency standards, as the ‘same’ powder prepared at larger scale, say lots of 10,000 L [post-hydration equivalent]. Inconsistencies at different scales may be due to the different equipment being used, or earlier smaller orders manufactured using the same equipment but only partially filled.

Grinding, required to produce powder mixtures with the desired physical properties, can introduce chemical and physical changes, leading to product inconsistencies and degradation. The physical act of grinding results in increased temperatures and shear forces that can result in physical changes, chemical breakdown and cross-reactions among culture media ingredients; differences in resulting powders; leading to inconsistencies in powder mixing and cell culture performance.

In terms of liquid media preparation from powders by end-users, there is no standardization, and many end-users lack sophisticated media prep/mixing process analysis, engineering and modeling studies; lack specialized mixing equipment, often using the same generic equipment for mixing media for different processes/products; often lack sufficient knowledge of the chemical and physical characteristics of their culture media; and usually lack staff with specialized culture media preparation training and expertise, such as only can be provided by media manufacturers. End-users using single-use mixers have even fewer options for customizing their mixing operations.

Besides mixing, another problem-prone area in preparation of culture media from powders is “sterile” filtration, generally using 0.2 µM (sometimes, 0.1 µM) single-use filters. With powder media, this is done prior or during filling of the holding bags/containers or the bioreactor. This 0.2 µM filtration only removes down to the bacteria size level, leaving any preexisting contaminating viruses and, potentially, mycoplasma in the culture media. Culture media is not currently terminally sterilized, such as heat pasteurized or gamma irradiated, with these processes causing too much ingredient breakdown and cross-reactions. Luckily, virus filtration using much finer filters prior to or during final fill-finish operations removes any residual microorganisms, so final end-products are “sterile” and the industry has no major problems with product contamination.

Sterile filtration of hydrated powdered culture media is a complex, time-consuming and expensive process, with the filters being rather costly. Further, any live bacteria captured

in filters may break down, releasing endotoxins into the media. Sterile filtration is yet another area where most end-users often adopt generic equipment and protocols, rather than obtaining optimal in-depth analyses, engineering and modeling studies and using customized equipment and protocols. So filtration is yet another area potentially introducing inconsistencies in the preparation of hydrated culture media by end-users.

Cell Culture Performance Inconsistencies

Variations in physical and chemical aspects of hydrated powder culture media, including those cited above, can lead to perceptible and significant inconsistencies in cell culture performance. Besides cell culture performance differences observed with the 'same' comparably-hydrated powder culture media from different manufacturers, end-users purchasing the 'same' powdered culture media from the same manufacturer often see variations/inconsistencies in resulting hydrated liquids and their cell culture performance. This includes differences observed when mixing the same powder in the same container using the same in-house equipment and mixing protocols. End-user variations in mixing equipment, timing of additions of powder while mixing, mixing methods and equipment, physical conditions, different operators, etc. can cause variations in resulting hydrated media that impact cell culture performance.

Currently, in a regulatory context, much or most culture media variations are considered and defined as being normal and acceptable, including related specifications being broadly, rather than precisely, defined (so anything within the broad specified ranges is acceptable). However, FDA and other regulators can be expected to increasingly require evidence of full(er) understanding of media, related inconsistencies and better in-house control of these.

Animal-Free and Chemically-Defined Culture Media Trends

Major trends in recent years have included removal of animal-sourced ingredients from culture media and culture media becoming chemically-defined. Animal-sourced ingredients were originally removed to minimize or resolve issues of prion/BSE/TSE contamination. However, in more recent years, concerns about use of animal-derived products have largely involved animal virus contamination risks, including in terms of product contamination, but mostly in the context of facility-wide virus contamination. Any animal-derived products, such as bovine serum albumin, that are used in culture media, are now often not part of the base media formulation. Rather these, along with other more difficult-to-fully-define natural products, such as yeast or plant hydrolysates are generally added as liquid media supplements or feeds, if required by the bioprocess. As further discussed below concerning culture media sterilization, culture media is simply not manufactured or expected to be sterile, and can be expected to become contaminated

with viruses or other microbes during manufacture and end-user handling. Animal virus contamination of end products are a risk proven to be well-controlled by the existing industry supply chain and QA/QC systems. Virus contamination of products is currently not a serious problem (with final fill-finish virus filtration resolving any contamination that remains after multiple purification steps.

While bioprocessing professionals, mostly concerned with product quality, see no major problems, company executives interviewed by BioPlan saw issues of culture media viral contamination much differently. Executives noted that even if chances of facility contamination are 1 in a million, or much less, there are relevant worst-case facility animal virus contamination precedents (including Genzyme), and they are simply obligated (required by duty to investors) to assume it can happen and to defend their facilities. BioPlan projects decreasing and minimal use of animal-derived products in culture media for mainstream biopharmaceutical products manufacture, particularly with any new bioprocesses being developed for new products. It can be presumed that the great majority of culture media being used for new bioprocesses, for new products in development, particularly monoclonal antibodies and other mainstream recombinant proteins are now and, particularly, going forward will be chemically-defined.

Contamination Issues: Culture Media Microbial Inactivation

Avoiding culture media-sourced microbial contamination and, particularly, related facilities-wide contamination, is a trend that will significantly affect, perhaps even redefine, the culture media market in coming years. Culture media remains the only major part of bioprocessing that is not fully sterile or terminally sterilized, including inactivation and/or removal of all viruses. All bioprocessing equipment is either steam (or peroxide, ozone, etc.) sterilized, while single-use equipment is sterilized by gamma irradiation. Note, we are using a simple, non-regulatory, definition of sterile – absence of replicable (under any circumstances) microorganisms. Plus, culture media should obviously be prion/BSE/TSE-free, generally not any issue, and be retroviral oncogene and other potentially host cell line mutation-inducing oligonucleotide sequences-free.

Rapidly advancing technology is making it much more practical to detect any microorganisms (contamination). For example, Covance is collaborating with Pathoquest to develop next-generation sequencing-based bioprocessing analytical technology capable of detecting *all* viruses, not just those known or for which detection methods are available. It is likely that we will soon start to see a ratcheting-up of both technological feasibility and regulatory and market expectations regarding upstream bioprocessing virus and other microbial contamination. Most of this will be directed to facility-level animal virus, not end-product, contamination.

Various leading biopharmaceutical companies, e.g., Genentech and Amgen, are developing culture media microbial inactivation treatments, including high-temperature, short-time (HTST)

heating and UV light treatment. Combined with better assays increasing detection limits by orders of magnitude, once these technologies start to become adopted for commercial products culture media, their wider adoption will become inevitable, they will be expected by regulators, health care professionals and consumers. *So, in coming years, preparation of culture media in-house from powders (vs. purchase of bulk finished media) may well require use of HTST, UV and/or other virus/microbial inactivation processing.*

Non-sterilized culture media, including media prepared from powders, could become legacy products, an anachronism, not acceptable, for GMP uses (for new bioprocesses).

Worker Safety Risks and Other Hazards

Powder culture media, like essentially all powders, is a potential inhalation and airborne, e.g., ocular, exposure hazard. Transfer and weighing of any quantity generally require technicians to use masks, goggles and, perhaps, other safety equipment. Weighing and transfer of powder media is a common area for complaints. Powders present much less problem in shipping compared to bulk liquid culture media. A broken bag/liner or other powder spill is much easier and cheaper to remediate than a comparable volume of hydrated liquid media. Keep in mind, each 1,000 L of aqueous fluid weighs 2.1 tons, and bulk liquid media is often shipped in 1,000, L bags/containers. The BioPlan Annual Survey of Biopharmaceutical Manufacturing[6] regularly reports single-use bags of all types, including bioreactor, mixer and culture media shipping liners, have a failure (loss of containment/sterility) rate of about 1%.

Impact of Perfusion and Other Bioprocessing Advances

Perfusion may well be an ongoing incremental evolution in bioprocessing being done at smaller scales[7]. There are many types or approaches to perfusion, ranging from Refine Technologies' use of a pump and filter, to centrifugation, variations of tangential flow filtration, and novel membrane filters being used. Most perfusion operations may use more volume of culture media than used in comparable batch-fed bioprocessing (although some may use less). Many perfusion operations involve media being more concentrated and/or containing higher levels of cells, expressed proteins, etc. So, it is not easy to predict the impact perfusion will have on culture media, other than the media likely required to be yet more complex and customized.

Regulatory Issues

So far, despite variations/inconsistencies in culture media prepared by end-users from powders being rather normal, regulators have not raised industry-wide concerns over culture media

[6] Op. cit.
[7] Langer, ES and Rader, RA, "Introduction to Continuous Manufacturing: Technology Landscape and Trends," in *Continuous Bioprocessing: Current Practice and Future Potential* [80-page book] Refine Technology, June 2013, p. 3-8)

inconsistencies. Industry and regulators largely accept and presume that the 'same' GMP-quality culture media can consistently (enough) be prepared by manufacturers and from the 'same' powders by end-users, including powders stored over considerable time (but within their shelf life). So far, regulators do not distinguish or view in-house prepared media differently, of lower quality, than the 'same' bulk liquid media purchased from its manufacturer. So, unless a switch from powder is part of an IND filed during development or supplemental BLA filed after initial approval, regulators have shown little interest in the comparative analytical and performance 'comparability' (defined by regulations) testing of the 'same' media prepared by end-users from powders vs. bulk liquid media from the manufacturer. In the worst case, where a switch between liquid and powdered media/buffers does result in inability to prove sufficient process and product comparability, the facility will do additional work. For example, a company switching from liquid media to use of powders for Phase II or III manufacturing might have to redo some studies done with the prior version of the product, if the switch results in a 'different' product, such as a modified glycosylation pattern, etc. Even if the next iteration/version product is better, e.g., safer, it is different and will require new testing. There are no specific regulatory, e.g., product approvals-related, benefits or risks associated with use of bulk pre-prepared liquid media vs. comparable liquids prepared in-house from powders. There are no specific FDA or other major market country regulations explicitly concerning culture media.

Similarly, to date, microbial contamination of culture media and upstream operations has not been a major concern for regulators, with this simply not a significant problem in terms of end-product quality and safety, which is what FDA and other regulators are primarily concerned with. But sooner or later, it is likely that regulators will want more and better control of culture media contamination, including potentially expecting or requiring new bioprocesses/products to be manufactured with fully sterile culture media.

Conclusion

In conclusion, powder culture media use will, at least in the near-term, remain the preferred and most commonly-used form of culture media. However, particularly for new facilities operating at less than large scales and those using single-use systems, alternative methods for powder packaging and preparation will increasingly be entering the market and capturing market share from conventionally-packaged powder media.

About BioPlan Associates, Inc.

BioPlan Associates, Inc. is in the business of delivering tactical and actionable information the industry needs to make smart, decisions. Understanding markets and trends is especially important in fast-moving biotechnology, life sciences, and health sciences markets. BioPlan Associates, Inc., has worked with life sciences companies of all sizes since 1989. Our extensive market research and management project experience covers biotechnology and biopharmaceutical manufacturing, vaccine and therapeutics development, diagnostics, devices, biotechnology supply, physician office labs and hospital laboratory environments.

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