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Scientific Resource

8 Ways That Osmolality Testing Improves Cell And Gene Therapy Process Development And Manufacturing

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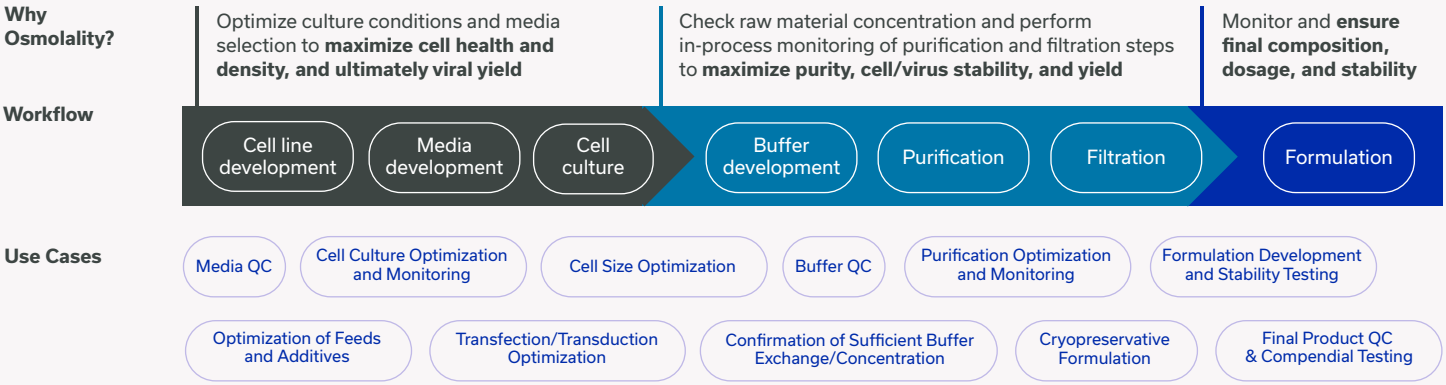
Graphical Abstract



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Background

Gene and cell therapies have the potential to revolutionize the treatment of incurable diseases which are often debilitating, if not fatal. Compelling clinical successes and recent drug approvals have fueled ongoing investments in this exciting area of therapeutic research, with over 1000 therapies in clinical trials at the end of 2019 [1]. Despite the forward momentum in the clinic, challenges remain to be solved in order to make these novel drugs accessible to larger patient populations. As it stands now, downstream recoveries are as low as 5-30% for viral vector production, depending on the process [2]. Manufacturing capacity, production yields, and supply chain logistics continue to be an area of focus for improvements to ensure customers get safe and affordable therapies.

The key to improvement and success is implementing high quality process design and process parameters to ensure robustness and reproducibility. What tools and checks can be implemented to ensure constant product and process control?

Osmolality is a measure of solute concentration and has long been considered a critical measurement in Biopharma [3, 4], describing how much of a solute is present in a given solution. From simple to complex solutions, osmolality represents a consistent and valuable concentration that is insensitive to temperature and pressure, which makes it a unique and beneficial parameter during bioprocessing. Osmolality directly affects the movement of solutes across a membrane [5], making it a necessity during cell processing and any subsequent testing of injectable products. However, there are numerous uses for testing throughout any bioproduction workflow, and cell and gene therapy development will certainly benefit from additional exciting applications. Eight emerging and novel uses for osmolality testing in advanced therapy production are outlined below.

What Is Osmolality?

Osmolality is a property of any liquid that describes how much of a solute is present. For more complex solutions, it reflects the overall concentration and provides a valuable measurement standard within the clinical and biopharmaceutical realms. Osmolality is concentration as a function of mass, which is not sensitive to temperature and pressure, which is particularly beneficial during bioprocessing changes. Osmotic pressure, which is very closely related, is commonly used the Biopharma industry to describe solution concentration in terms of the pressure required to prevent a solvent from osmosing into a solution. Both are frequently used to describe the concentration of a solution as it relates to the properties of osmosis. As a compendial release test for biological manufacturing, osmolality testing is prevalent within bioproduction; however, there are numerous applications for testing throughout the workflow, not just for release. Eight emerging and novel uses for osmolality testing in advanced therapy production are outlined here.

1. Media Quality Control (QC)

Optimize cell growth/expansion and eventual yield

Cell media osmolality must be controlled to optimize and maintain cell growth and vector production. Media vendors measure and report the osmolality range for their solutions, and it is common for scientists to check the osmolality of each media batch against specifications to ensure the proper environment for a specific cell line or type. Whether it is later used in flasks or bioreactors, media must meet pre-determined osmolality specifications to ensure optimal cell growth and expansion [6]. It has been established that falling out of specification could result in lower growth rate and viable HEK 293 cell concentrations, leading to growth inhibition and ineffective vector production [7]. Productivity was up to 10-fold higher when the cells were exposed to an osmolality of 330 mOsm at infection, compared to 480 mOsm. Drug manufacturers are trending towards purchasing off-the-shelf media, a cell culture segment that was valued at $1.4B in 2017 and predicted to grow at about 8% over the next few years, according to the GM of BioProcess at GE Healthcare Life Sciences, Oliver Loeillot [8]. This growth means that quality control is more important than ever.

This clear need and market trend show that osmolality is an essential parameter for the preparation of cell media to ensure optimal growth and productivity in the early stages of cell and gene therapy.

2. Culture Expansion and Monitoring

Provide an ideal environment for cells to reach optimal attributes (optimal production, size, growth rate

Osmolality continues to serve as a Critical Process Parameter (CPP) in cell culture monitoring. Consumption of media substrates and production of waste by the cells create a dynamic osmolality profile. Additionally, different feeding and supplementation strategies, as well as pH control strategies using specific gas levels, will impact the osmolality differently [7, 9].

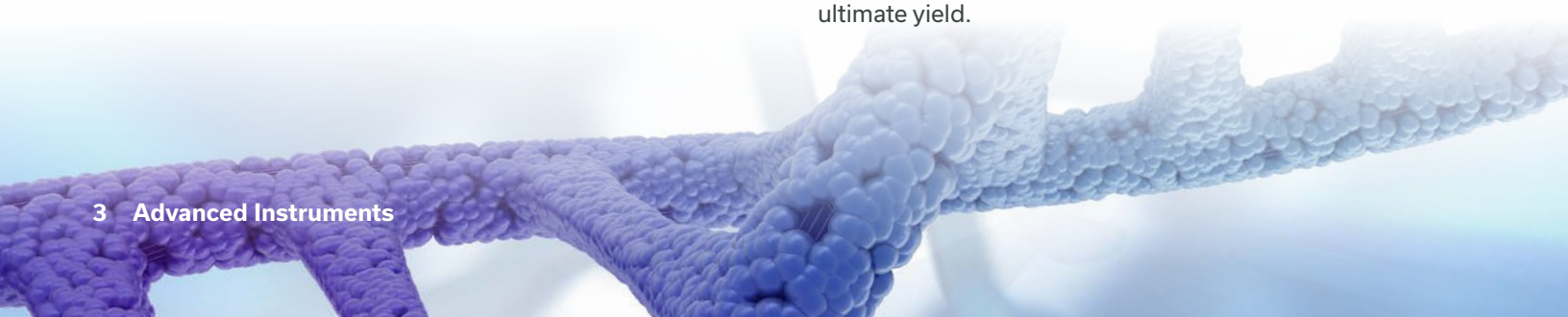
Osmolality is a critical indicator of lactate production (influenced by pH) and media base addition, ultimately impacting glycosylation in cell culture. Monitoring this parameter is essential to understanding and optimizing growth and productivity. Multiple sources have established the importance of osmolality when establishing cultivation conditions for stem cells to preserve optimal critical quality attributes (CQAs) like cell viability and productivity. Higher osmolality has been used to increase the specific production rates of waste and/or slow cell growth to maximize productivity [10, 11].

On the higher end, one study found that chondrocyte expansion in serum-free media at 450 mOsm had a productivity 0.65-fold lower than that at 400 mOsm [12]. Overall expansion was determined to be optimal in a media of 290 to 350 mOsm. This specific osmolality range must be established for each cultivation and expansion while taking the afore-mentioned strategies in mind. Not monitoring the osmolality could compromise CQAs of the upstream process and eventual drug product.

3. Transfection Efficiency and Osmotic Shock

Establish a stable transfection system and for optimal cell titer

Achieving high transfection efficiency is critical for high viral titer yield, but it is common to see subpar transfection efficiencies in gene therapy development. Regardless of the transfection method, osmolality is important when transfecting cells with plasmid to initiate viral production. HEK293 cells, which are highly transfectable, commonly undergo polyethyenimine (PEI)-mediated transfections due to the known effectiveness. Many theorize that osmotic pressure plays a role in the stability of the internalized PEI:DNA complex. The rate of in situ complexing and dynamics of the PEI:DNA complexes is dependent on a number of parameters, including osmolality [13]. The increase in osmotic pressure caused by entry of the complex protons into the trafficking endosomes is hypothesized to allow for the release and delivery of the DNA to the target cells. This, along with other variables, must be considered and characterized early in process development to establish reliable and reproducible methods. While not well developed within the cell and gene therapy space, there is evidence to suggest that osmolality is a major factor in effective transfection for a variety of systems, including immunoporation [14] and electroporation [15, 16]. Osmolality influences the ability of buffer solutions to cross cell membranes. As such, it is critical to set specifications around the osmolality of media and transfection cocktails. A delicate balance is required when considering the stability and permeability of the cells to be transfected. The same can be applied during cell lysis, when the goal is to release cell-associated adeno-associated virus (AAV). Osmotic shock is one method to access the genetic material and, as the name implies, it relies heavily on the osmotic pressure of the cellular environment. Incubation at high salt concentrations (high osmolality) can expose the viral genomes but it could also potentially damage them [17]. Again, establishing a tight specification range for the osmolality is essential to develop a controlled and repeatable process. These early upstream process controls are established to ensure optimal titer and ultimate yield.



4. Vector Production and Stability

Influence the membrane composition and structure to maximize vector stability

High viral vector titer is highly dependent on vector stability, which is inherently compromised in retroviruses (and the subset of lentiviruses) by an outer envelope consisting of a lipid bilayer interspersed with envelope proteins.

There is evidence to support the idea that vector stability, particularly for enveloped vectors, improves at higher media osmolality [18]. In a series of studies using Fly cell lines to generate RV vectors, high cell media osmolality corresponds to low cholesterol-to-phospholipid ratios in the viral membrane, resulting in increased vector stability [19]. Production of the membrane lipids is greatly influenced by the sugar metabolism of the cell. Amaral et al. discovered that enhanced sugar metabolism and the resultant increase in medium osmolality (due to the presence of sorbitol) correlates positively to retroviral stability and productivity [18, 20]. This evidence strongly suggests that a stable retrovirus is heavily dependent on an established media osmolality. Osmolality specifications of media and buffers should account for these effects on vector production and stability, as process repeatability and product yield depend on this process control.

5. Buffer QC and Purification

Provide comprehensive control of downstream buffers and in-process purification

We recently described osmolality as an orthogonal property of downstream buffers, alongside pH and conductivity [21]. Failure to identify composition issues or deviations in these buffers could result in process and/or product deviations down the line. Osmolality testing should occur during buffer QC and as an in-process parameter during purification. This applies to in-process testing during downstream processing as well. A recent publication described osmolality as a predictor of protein concentration during purification, in conjunction with UV absorbance [22]. This offers a quick and accurate predictive measurement alternative to standard concentration methods that require more time and technical expertise. Earlier identification of impurities saves operational costs and eventual product yield. It remains to be seen how this translates in the gene therapy production workflow, but the role of osmolality as an orthogonal parameter in downstream processing is emerging as a valuable tool.

6. Concentration and Buffer Exchange

Support high filtration recovery and yield for high product yield

Ultrafiltration/diafiltration (UF/DF) in cell and gene therapy depends on tangential flow filtration (TFF), which has proven advantageous in multiple applications because of the fickleness of advanced therapies [2]. Viruses, especially lentiviruses (LVs), are much larger than mAbs, so getting high recovery and consistent process performance can be difficult. Additionally, achieving high concentrations of viral vector in a drug substance and drug product lends itself to aggregation for enveloped (retrovirus) and non-enveloped (AAV) viruses [2]. Aggregation is especially likely in enveloped viruses, which tend to have a stickier exterior. Filtration operations can damage the membrane of large enveloped viruses, greatly impacting the stability and transduction efficiency of the vector [23]. Osmolality plays a crucial role in reducing the potential for AAV aggregation as well and has been used to check levels of excipients that prevent build-up of AAV2 vectors [24]. UF/DF is also used for buffer exchange within cell and gene therapy workflows. Osmolality testing would help verify the efficiency and completion of these exchanges. Due to the properties of some common buffer components, conductivity and pH meters may not have the resolution to capture differences between the starting matrix and final formulation buffer. We have shown that osmolality provides orthogonal value in these situations [21]. Osmolality describes the ability of a solute to cross a membrane, so it is required to give a comprehensive view of solutions during filtration processes.

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7. Pre-formulation Processing

Optimize potency and stability of the product during the final filtration step into formulation

Osmolality testing is commonly used as a release specification for final formulation buffers, and because cell and gene therapy products are extremely delicate, strict control is required. More novel indications for osmolality, which require additional investigations, are also possible. As mentioned above, sterile filtration of LV is a known pain point due to low recoveries [2], impacting the final filtration and formulation of the drug. Osmolality can serve as a possible indicator for the purity and safety of the end process and final product, as higher osmolalities could eb indicative of unwanted solutes and impurities.

This is particularly useful for viral processing where there is no terminal sterile filtration [25]. Additionally, osmotic pressure could play a role in the hydrodynamic radius of virus and help formulation development teams optimize the molecular weight cutoff for further filtration steps. The role of osmosis and osmotic pressure in viral properties [26], along with evidence with other cell types [27], provide promise for this application. This may be of interest if additional studies are completed to illustrate that variables like osmolality impact filterability for LV through a 0.45 µm or 0.22 µm filter. There is clear evidence that osmotic pressure of the pre-formulation drug substance impacts its filtration and recovery and its characterization could provide the control needed to deliver a high-quality drug product.

8. Final Product Testing

Promote high quality drug products by optimizing CQA

Osmolality testing has long been considered a compendial measurement for the release of biological drugs (FDA), as any substance entering the body must be physiologically isotonic and iso-osmotic. How does this translate to cell therapy, where the cells are the starting and ending material? When specifically discussing cell banking and expansion, comprehensive testing platforms are required to promote optimal identity, safety, purity, and stability [28], key CQAs for which osmolality offers valuable information. These CQAs must be maintained and protected upon cryopreservation, the process of freezing cells for storage of drug intermediates and product [29]. Prior to freezing, adherent cells must be cleanly detached without damaging them.

There is evidence to support the hypothesis that the osmolality of a dissociation agent influences the size of cell aggregates that detach [6]. Freeze/thaw cycles can be detrimental to the cells' stability and recovery, so cryopreservatives (such as those in Figure 1) must meet osmolality standards to ensure cell quality and retainment of critical quality attributes are throughout the entire bioprocess. A study was recently completed to test various homemade and commercially available cryopreservatives for osmolality using freezing point depression osmometry.

Trehalose Solution
- Base DMEM media
- 10% DMSO
- 10% FBS
- 50mM trehalose

Glycerol Solution
- Base DMEM media
- 10% glycerol

PEG and DMSO (1)
- Base DMEM media
- 7.5% DMSO
- 2.5 PEG
- 2% BSA

PEG and DMSO (2)
- Base DMEM media
- 7.5% PEG
- 2.5% DMSO
- 2% BSA

	CryoStor 55	Trehalose	Glycerol	PEG/ DMSO 1	PEG/ DMSO 2
MEAN	1410	2122	2083	1318	1117
STD DEV	4.10	4.10	5.10	1.30	1.80
%CV	0.29	0.20	0.25	0.10	0.16

Figure 1. Recipes and osmolalities of common cryopreservatives in cell and gene therapy. Summarized data are shown for osmolality testing (n=5) on various cryopreservative solutions with a freezing point depression osmometer. Despite high concentrations and DMSO integration, osmolality testing showed sound reliability and repeatability. CV, coefficient of variation; PEG, polyethylene glycol; DMSO, dimethyl sulfoxide

Understandably, introducing cryoprotectants and freezing cells has a considerable impact on their osmotic pressure [30-32], and repeating this process becomes increasingly complex. Testing the osmolality of the cryopreserves before freezing ensures that the composition is correct and provides confidence in the upcoming preservation steps. Preservation can become critical at multiple stages of therapeutic development, from cell banking through shipping to the clinical environment for injection (Figure 2). Consequently, osmolality testing should be performed at these stages throughout the workflow to ensure cryopreservative properties and protect product CQAs.

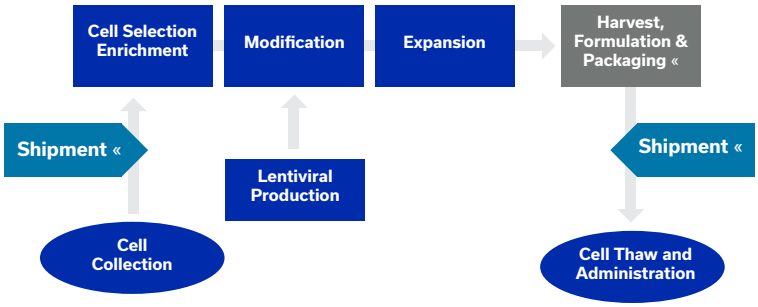

Figure 2. Common workflow for cell-based gene therapies. Indications for cryopreservation are indicated by «

Conclusion

The success of cell and gene therapy development is heavily dependent on the strength of its process controls for repeatability and/or scale-up. As the field matures, there will certainly be increased scrutiny from regulatory bodies as expectations around these qualities, as well as product quality, increase. Drug developers must overcome the obstacles of poor process performance. The increased need for robust process control strategies demands reliable and accurate parameters, and osmolality testing fits the bill. Osmolality testing provides a reliable and accurate concentration measurement for solutions throughout the bioprocess. Several applications for this quick and easy test have been outlined here and there is an expectation that more will be uncovered as this field continues to grow. Past studies into osmolality and viral vector development were significant, as shown here, but not previously pulled into a story of why osmolality must be tested at each stage. As these themes gain traction, there has been a consistent interest in tying osmolality to key CQAs. The ability of this parameter to support optimal yield, purity, and stability is well established and supports its implementation within any advanced therapy lab. And the advancements in osmometer technologies and capabilities will lend itself to a continued increase is osmolality testing in bioprocessing. Cell and gene therapy has come a long way in a short amount of time and there is a desire to identify ways to consistently improve manufacturing workflows. Osmolality offers not just one, but eight ways to build confidence in your bioprocess.

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