Osmolality as a valuable tool in formulation studies for advanced therapies

Paul Butler[1] , Linda Buck[2], Kristeena Wright, PhD[2]
[1]Advanced Instruments, Horsham, West Sussex, UK; [2]Advanced Instruments, Norwood, MA, USA

Abstract

Whilst osmolality is a compendial QC test for final product release, it's also useful in formulation development and stability studies for drug products. It provides the excipient concentration and can confirm the soluble content of formulations. In this poster, the rapidly advancing field of cell therapy is discussed, where cryoprotective storage strategies are common. Data is presented which supports the value of osmolality measurements, derived via the freezing point depression method, for characterizing cryopreserved formulations.

Background

Freezing point depression osmometers measure the freezing point of a provided sample and report the corresponding concentration in terms of osmolality (mOsm/kg H_2O). This measurement has long been considered a quick and accurate way to gather information about key bioprocessing solutions. This includes drug formulations that are becoming increasingly more concentrated and more complex as the industry rapidly expands. There is growing evidence that this measurement provides valuable stability information within monoclonal antibody production platforms[1].

Osmolality is also a key property of advanced therapy medicinal products (ATMPs), from a clinical and developmental aspect. Cell therapy formulations typically contain a cryoprotectant component that will preserve the integrity of the cells during storage[2].
Particular attention needs to be paid to osmolality because CPA addition/removal during the freezing and thawing process affect the water content of the cells (and thus osmolality). So care should be taken to ensure that osmotic gradients don't damage the cells(via use of osmotic buffers such as sucrose or mannitol)[3].
Historically, the osmolality of such formulations was determined by calculation or other means. Data shown here presents the case for using freezing point depression osmometry in formulation development and stability testing of protein and cell therapies.

Figure 1. Recipes of common cryo-preservatives in cell therapy

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

Materials and Methods

To mimic common protein formulations, protein (BSA) and sucrose solutions were made across a range of concentrations. CryoStor 5S (STEMCELL Technologies) and Prime-XV (FUJIFILM Irvine Scientific) were purchased from the manufacturers. Cryo-preservatives made in-house were prepared using the formulas in Figure 1. All solutions were tested five times (n=5) for osmolality on an Advanced Instruments single-sample osmometer.

Results

BSA	100 mg/ml			150 mg/ml			200 mg/ml		
Sucrose	**100 mM**	**200 mM**	**300 mM**	**100 mM**	**200 mM**	**300 mM**	**100 mM**	**200 mM**	**300 mM**
Mean	378	386	376	378	377	380	377	379	384
Std Dev	1.34	1.00	0.71	0.84	1.52	1.00	1.82	3.59	1.48
%CV	0.35	0.26	0.19	0.22	0.40	0.26	0.48	0.95	0.39

Table 1. Osmolality (mOsm/kg H_2O) of protein and sucrose solutions
Solutions of increasing BSA and sucrose concentrations were tested for osmolality to mimic increasingly complex mAb formulations. Results showed typical formulation osmolalities and repeatability across concentrations.

	CryoStor 5S	Prime-XV FreezIS	Trehalose	Glycerol	PEG/ DMSO 1	PEG/ DMSO 2
Mean	1410	2091	2122	2083	1318	1117
Std Dev	4.10	26.91	4.10	5.10	1.30	1.80
%CV	0.29	1.29	0.20	0.25	0.10	0.16

Table 2. Osmolality (mOsm/kg H_2O) of common cryo-preservatives, outsourced and made in-house
Osmolality testing showed repeatability for different solutions using a freezing point depression osmometer.

Discussion

Due to the high and growing number of ATMPs in development and approaching filing for market authorization, cryopreservation is an increasingly popular formulation strategy. Osmolality is key in developing cell therapies, as it comes into play multiple times during development:

- Reagents interacting with cells must be within an acceptable osmolality range
- Cryopreservation: cooling (reducing osmotic shock) and storage are typically at a set osmolality range[2]
- Clinical administration: final product must be isotonic for injection

The data in this poster demonstrates that the freezing point depression method of measuring osmolality aids the ATMP formulation development process as a reproducible method of checking a variety of commercially available and "homemade" cryo-preservative formulations.

References

1. Vimpolsek, Maja, et al. Assessing the Extended In-Use Stability of the Infliximab Biosimilar PF-06438179/GP1111 Following Preparation for Intravenous Infusion. *Drugs in R&D* (2019) 19:127-140.
2. Li, Yan and Teng Ma. Bioprocessing of Cryopreservation for Large-Scale Banking of Human Pluripotent Stem Cells. BioResearch Open Access (2012) 1.5.
3. Hunt C.J. Technical Considerations in the Freezing, Low-Temperature Storage and Thawing of Stem Cells for Cellular Therapies. Transfus Med Hemother 2019;46:134–149

Presented at the 9th Annual MIBio Conference, 2019, Cambridge