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Reliable Osmolality Testing of High Concentration mAb Formulations

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Abstract

Osmolality testing has several unique and essential applications throughout bioprocessing, and new use cases are constantly emerging. As the field of biologics manufacturing matures, osmometers and other analytical devices must keep up and even offer new options to remain valuable. The osmolality of monoclonal antibody (mAb) formulations is typically determined using freezing point depression or vapor pressure osmometers. The wider use of subcutaneous injections; an injection that requires less volume but increased concentration to alleviate patient pain and increase patient compliance has led to a trend in increasing mAb concentrations. This higher concentration, however, can pose analytical issues. Due to much higher viscosities being seen in drug formulations; it is critical to have an instrument that will measure concentration with optimal performance. Although freezing point depression osmometers are the gold standard osmometry method, the previously mentioned higher viscosity samples may signify an issue for older technologies. This would have meant the "go-to" method may have been vapor pressure, even though from a usability and data integrity standpoint, this was not preferred. In response to this, Advanced Instruments has developed a new, intelligent freezing point depression technology that is aimed specifically at these hard to measure drug products. This paper details an evaluation of the OsmoTECH® XT (freezing point) and Vapro® 5600 (vapor pressure) osmometers as a means of measuring concentrated protein formulations. In general, mean osmolality values were similar across a range of saline and monoclonal antibody concentrations. Key differentiations were observed in the accuracy of the salt standard measurements and the variance of the mAb measurements, both of which were preferable on the OsmoTECH XT. In addition, the OsmoTECH XT has key advantages in terms of usability and advanced data integrity features that facilitate implementation into GMP workflows.

Contents

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Introduction

Osmolality represents the concentration of a solution in milliosmoles per kilogram of water, making it a key analytical measurement in many fields. Traditional osmolality testing has been implemented in clinical settings, but there is a clear need for these measurements in the biopharmaceutical industry. From cell culture monitoring to final buffer exchange and product concentration, there are established and emerging use cases for osmolality testing[2,3]. Perhaps the most discussed application of this test is in drug formulation and final drug product. Due to the trend of increasing drug concentrations, owing to the intravenous pathway decreasing in popularity, analytical devices must be able to test these high values within complex formulations[4]. It is known that osmometers cannot always successfully test high viscosity and/or high concentrations, specifically when looking at older freezing point depression technology.

When it comes to measuring osmolality, there are two methodologies most used, freezing point depression (FPD) and vapor pressure (VP) osmometry, both based on different colligative properties of the solution. The freezing point of a liquid solution is directly related to its osmotic concentration, so an increased concentration will present with a lower freezing point[1]. Freezing point depression is defined as the difference between the freezing point of water (the standard solvent) and the sample being analyzed. Vapor pressure osmolality is based on a different colligative property of the solution. It is determined by the concentration of osmotically active particles needed to reduce the vapor pressure of a solution[1]. A decrease in dew point of a solvent is therefore caused by a decrease in the vapor pressure of the solvent by the solute. Dew-point temperature depression is defined as the difference between the dew-point temperature for air in equilibrium with H^2O and the dew-point temperature for air in equilibrium with sample. The latest freezing point depression and vapor pressure technology are evaluated here to show the ability to successfully measure the osmolality of antibody samples.

The FPD osmometer used in this study, the OsmoTECH XT, gives a truly comprehensive measurement of samples with the ability to freeze complex sample matrices such as proteins, with a wide testing range of up to 4000 mOsm/kg H2O. The intelligent freezing technology works significantly better with solutions that have high osmolalities or other complex physical characteristics (such as viscosity) compared to older FPD technology. These samples, such as concentrated sugars, proteins and other large molecules, are commonly seen across various modalities of bioprocessing. The evaluation and findings are discussed herein.

"the OsmoTECH®XT, gives a truly comprehensive measurement of samples with the ability to freeze complex sample matrices such as proteins..."

Materials and Methods

mAb production

A monoclonal antibody was expressed in a DXB-11 CHO cell line in bioreactors operating in fed-batch under an inducible promoter. BalanCD CHO Growth A (Irvine Scientific) with 0.2% Kolliphor P188 and 50 mM MSX was used as the production medium. The bioreactors were initially seeded at a density of 0.3 x 106 cell/ml. Protein expression was induced with 2 µg/ml cumate starting at a cell density of 3-4 x 106 cells/ml and the temperature of the culture was shifted from 37°C to 32°C. The induction medium (BalanCD CHO Feed 4 (Irvine Scientific) with 75 mM MSX) was added to the culture in 1.5% WV bolus over a period of 7 days. The bioreactor was supplemented with 35% WV bolus of BalanCD CHO Feed 4 (Irvine Scientific) during scheduled feedings. Glucose is maintained between 15 and 35 mM with 2 M glucose stock solution when necessary. The bioreactors were harvested at 50% viability and processed by depth filtration. Three coarse depth filters (9 µm to 6 µm pore size) and two fine filters (1 µm to 0.45 µm pore size) were used to process the cell culture medium. Depth filters were initially flushed with DI water, then equilibrated with 50 mM tris/ 45 mM acetic acid buffer to prepare the filters for processing. After depth filtration, the material was 0.2 µm sterile filtered. The titer of the pooled cell free medium (CFM) was measured to be 0.6 g/L by analytical protein A.

"Due to much higher viscosities being seen in drug formulations; it is critical to have an instrument that will measure concentration with optimal performance."

mAb Purification

The monoclonal antibody was purified using a protein A capture column. A Hi-Scale 1.6 x 20 was packed with Tosoh's AF-rProteinA HC-650 F resin. The CFM was purified over 4 cycles based on the maximum load volume in the formula below. The column was first equilibrated with 3 CV of 55 mM Tris, 45 mM Acetic Acid, pH 7.5 then the CFM was then loaded onto the column at neutral pH.

$$\text{EQUATION 1: Max Load Volume} = \frac{\text{DBC} * \text{Column Volume}}{\text{Load Concentration}}$$

After loading the product, a two-step wash was performed with 55 mM Tris, 45 mM Acetic Acid, 300 mM Sodium Acetate, pH 7.5 (Wash 1) and 55 mM Tris, 45 mM Acetic Acid, pH 7.5 (Wash 2) to remove any weakly bound species. The product was eluted using a low pH isocratic elution step with 1.8 mM Sodium Acetate, 28.2 mM Acetic Acid, pH 3.6.

After elution, the column was then neutralized to pH 7 prior to cleaning with caustic buffer. This process was repeated per cycle. The eluate collected from each cycle was immediately pH adjusted and stored at 2 - 8°C. After the last cycle, the eluates from all cycles were pooled and filtered through a 0.2 µm filter. A final protein concentration measurement of the pool was obtained using analytical protein A.

Concentration and buffer exchange

Product concentration and buffer exchange was carried out using a 50 cm^2 TFF flat sheet filter with a 30 kDa pore size. Prior to initial concentration, the filter was DI flushed, cleaned, and equilibrated. The permeate pH at the end of equilibration was 7.7[2], ensuring the filter was sufficiently equilibrated out of the sanitization solution.

The pooled eluate from protein A eluate was initially concentrated to 25 g/L. After this initial concentration step, the retentate was split in half for diafiltration into each buffer system: (1) 10 mM Histidine, 85 mg/ml Sucrose (2) 10 mM Histidine, 85 mg/ml Trehalose. A diafiltration factor of 7 was used to ensure sufficient conversion to the new buffer system.

Table 1 - Initial concentration buffers

How do the Modalities perform in Clinical Isolates[4]

Step	Buffer
WDI Flush	WDI
Sanitization	0.5 M NaOH
Equilibration	55 mM Tris, 45 mM Acetic Acid, pH 7.5
Diafiltration	10 mM Histidine, 85 mg/ml Trehalose
Diafiltration	10 mM Histidine, 85 mg/ml Sucrose

Table 1.

The final concentration of the formulated samples was achieved using conical spin TFF filters with a 50 KDa pore size. A concentration from 15 ml to 250 µl is possible when the filters are spun at 3000 g for 5-60 minutes in a swinging bucket centrifuge.

A target final volume of 1-3 ml was desired to ensure adequate volume for the technology evaluation. Prior to final concentration, both retentates from the initial concentration step were formulated with 0.5 mg/ml of Poly80.

To ensure homogeneity, each formulation was mixed for 30 minutes prior to concentration. The spin filters were prepared by flushing the membrane with 15 ml of formulation buffer (10 mM Histidine, 85 mg/mL Sugar, 0.5 mg/ml Poly80) at 3000 g for 10 minutes.

Enough sample was added to each flushed conical spin filter to obtain a final volume of 1-3 ml of over-concentrated sample. The samples were spun at 3000 g for the time specified in **Table 2**. After concentrating, the retentate was mixed in the reservoir by re-suspending with a pipette. To enhance protein recovery, the upstream side of the membrane was washed with the retentate sample.

The sample was removed and a protein A HPLC measurement was taken to measure the protein concentration of the over-concentrated stock. The stock was then diluted with formulation buffer to reach the project-specified concentration target. To verify the final target concentration, three protein concentration measurements were taken to ensure precision. A ±5 g/L concentration variability around the target concentration was allowed. **Table 3** outlines the formulation of the 6 mAb samples.

Osmolality testing

Performance comparability between the Advanced Instrument OsmoTECH XT system and the Vapro 5600 was tested with the 6 mAb formulations. Each system was calibrated using standard salt solutions. The competitor system was calibrated prior to each testing session. The OsmoTECH XT Osmometer was pre-calibrated by the supplier. Before and after each sample, a Clinitrol™ Reference Solution 290 mOsm sample was tested.

The competitor system was calibrated using the EliTech standards in the following order 290 mmol/kg, 1000 mmol/kg, and 100 mmol/kg. The calibration was repeated until all three standards were measured within the acceptable ranges of 290 (± 3) mmol/kg, 1000 (± 5) mmol/kg, and 100 (± 2) mmol/kg. The calibration values and contamination level were manually recorded. The acceptable contamination level was 0 – 9. If the contamination level was 10 or higher, a cleaning cycle would need to be performed before the instrument could be properly calibrated. At the end of each sampling session, the results logged in the instrument were backed-up onto a USB drive.

Table 2 - Mab formulation results using 15ml TFF conical spin filters

mAb formulation results using 15 mL TFF conical spin filters

Formulation	Sucrose 150 g/L	Sucrose 100 g/L	Sucrose 50 g/L	Trehalose 150 g/L	Trehalose 100 g/L	Trehalose 50 g/L
Spin Time (min)	30	30	12	30	30	12
Sample Stock Concentration	155.427	163.977	73.938	159.895	137.955	56.095
Sample Stock Volume (µl)	2000	1500	2000	1500	2000	2000
Diluent Volume (µl)	72	959	957	98	759	243
Sample 1 Concentration (g/L)	147.5566	99.9114	51.6687	149.4048	96.2583	47.6496
Sample 2 Concentration (g/L)	147.3533	99.6016	53.0822	151.0992	99.3823	47.5522
Sample 3 Concentration (g/L)	150.6831	99.6813	53.8300	152.8857	100.8044	47.8819
Final Average Concentrations (g/L)	148.531	99.731	52.860	151.130	98.815	47.695

Table 2.

Statistical Analysis

Excel was used for initial data entry and analysis (mean, SD, %CV). Minitab® (version 18) was used for the Test for Equal Variances and to generate the mean osmolality bias plots with associated 95% Confidence Intervals, and the histogram plots.

Results and discussion

The study described here was designed to assess the ability to measure the osmolality of concentrated protein formulations. Freezing point depression osmometer (Advanced Instruments OsmoTECH XT) and vapor pressure osmometer (Vapro 5600) were assessed for their ability to test these formulations, the precision of the measurements, and ease of use.

Table 3 - Mab formulated samples

Samples Provided by Jefferson Institute for Bioprocessing (JIB)

Sample Type	mAb Conc.	Sugar Conc.	Histidine Conc.	Poly80 Conc.
mAb Formulations	50 mg/ml mAb	85 mg/ml Sucrose	10 mM histidine	0.5 mg/ml Poly80
	100 mg/ml mAb	85 mg/ml Sucrose	10 mM histidine	0.5 mg/ml Poly80
	150 mg/ml mAb	85 mg/ml Sucrose	10 mM histidine	0.5 mg/ml Poly80
	50 mg/ml mAb	85 mg/ml Trehalose	10 mM histidine	0.5 mg/ml Poly80
	100 mg/ml mAb	99.85 mg/ml Trehalose	10 mM histidine	0.5 mg/ml Poly80
	150 mg/ml mAb	85 mg/ml Trehalose	10 mM histidine	0.5 mg/ml Poly80

Table 3.

Salt Standards

Saline standards were tested for osmolality on the OsmoTECH XT and Vapro 5600. **Figure 1** shows the overall results for both instruments across the concentration range. In general, there was statistically significant lower variance with the OsmoTECH XT as compared to the Vapro 5600 using the test for equal variances, for all salt standards less than 1000 mOsm. The mean OsmoTECH XT values were statistically different from the Vapro, at 100, 300, 500, 1000, 2000, 3000 and 4000 mOsm based on ANOVA with 95% Confidence Intervals.

As indicated in **Figure 2** the overall biases trended smaller for the OsmoTECH XT than the Vapro 5600. The extreme inaccuracy of the 4000 mOsm standard on the Vapro 5600 is due to the lower operating range of 0-3500 mOsm for the instrument, compared to 0-4000 mOsm for the OsmoTECH XT.

Figure 1 - Mean osmolality results

A

Salt Standard (mOsm/kgH_2 0)	OsmoTECH XT	Vapro 5600
100	98	95
200	199	199
290	289	290
300	300	308
500	499	509
1000	999	1009
1500	1496	1498
2000	2010	1996
3000	2998	3093
4000	3975	4622

B

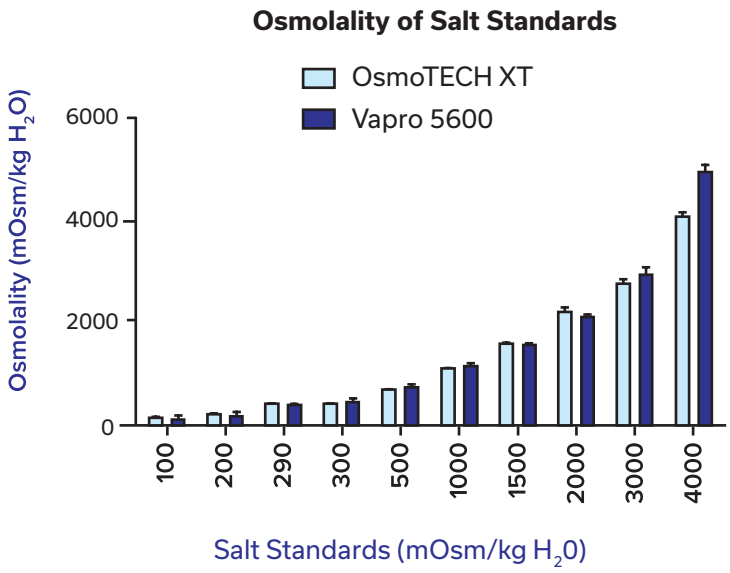

Figure 1. Mean osmolality results (n=15) of saline solution standards across a wide concentration range. The error bars in Figure 1B shows the standard deviation of each data set.

Figure 2 - Mean osmolality results

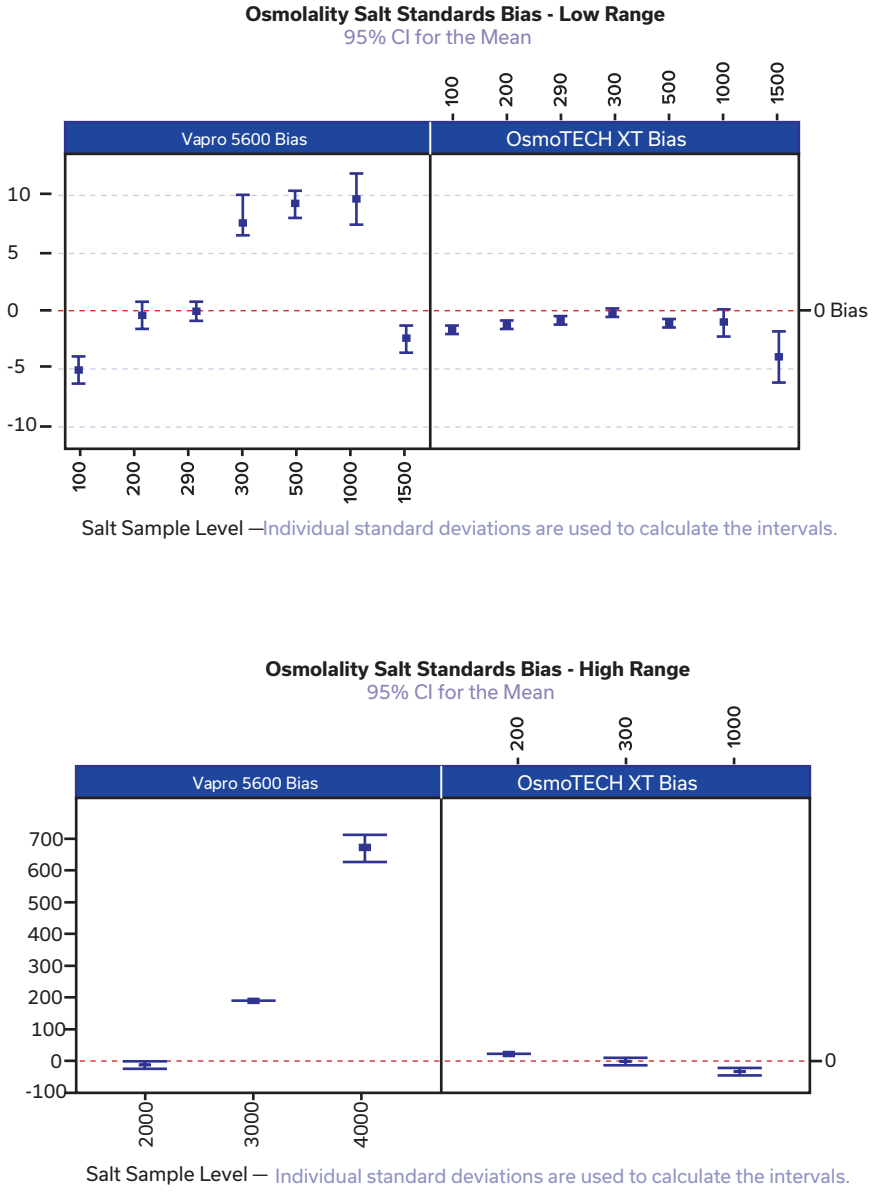

Figure 2. Mean osmolality results (n=15) of saline solution standards plotted to show bias from the accepted osmolality values, as well as the variance for each standard. In general, the bias was greater when using the Vapro osmometer.

"With the inclusion of the OsmoTECH XT, there is now a noticeable improvement (i.e., smaller difference) between the osmolality values determined by two different osmolality mechanisms."

Monoclonal antibody formulations

Testing protein formulations of varying concentrations was a critical component of showing improved performance of the OsmoTECH XT as a freezing point osmometer in the context of high concentration drug formulations. Older technologies show an increasing bias between freezing point and vapor pressure osmolality values as protein concentration increased[5].

Figure 3 shows the osmolality data from both instruments across six unique monoclonal antibody formulations.The mean osmolality values were comparable between the two instruments and there was a slight increase in mean osmolality as the protein concentration also increased. Looking at previous findings, historical biases have been upwards of 100 mOsm for certain protein formulations (Sahin 2016). With the inclusion of the OsmoTECH XT, there is now a noticeable improvement (i.e., smaller difference) between the osmolality values determined by two different osmolality technologies.

Figure 4 confirms this trend, with the overlaid histogram plots highlighting normal distributions for both instruments with similar means. However, the plots also identify the lower variance (the data spread) in the OsmoTECH XT data compared to the Vapro 5600. As this represents the repeatability of the instruments, this supports the finding that the freezing point osmolality determined by the OsmoTECH XT is more consistent than the Vapro 5600 across formulations. The variance for both data sets increased from 50 to 100 mg/ml protein, as expected with a more complex solution. Interestingly, there is no noticeable trend of increasing variance with increasing protein concentration from 100 mg/ml to 150 mg/ml protein.

Figure 3 - Mean osmolality values

Samples Provided by JIB

mAb Conc + Additive	OsmoTECH XT	Std Dev	Vapro	Std Dev
50 mg/ml + Sucrose	298	1.6	301	5.12
100 mg/ml + Sucrose	318	3.27	321	4.17
150 mg/ml + Sucrose	334	3.18	338	6.29
50 mg/ml + Trehalose	298	1.91	296	5.17
100 mg/ml + Trehalose	321	3.45	325	4.63
150 mg/ml + Trehalose	357	2.96	359	4.59

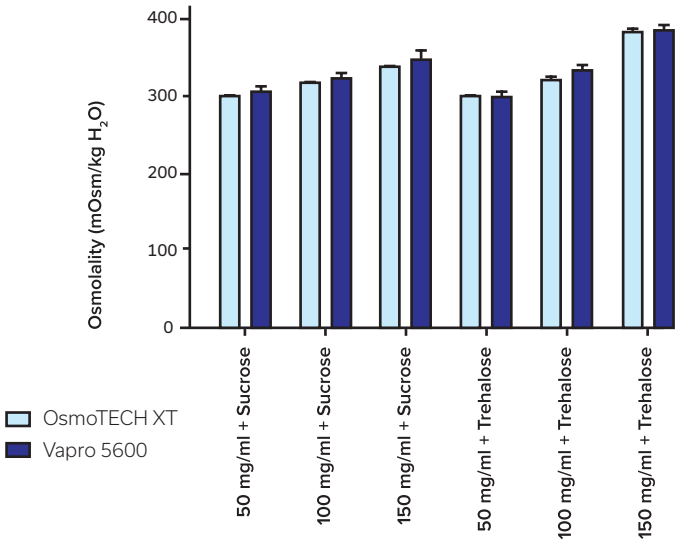

Figure 3. Mean osmolality values (n=15) for various monoclonal antibody formulations using the OsmoTECH XT and Vapro osmometers. The antibody was combined with Trehalose or Sucrose at the given concentrations, among other additives, to generate various mAb formulations. Error bars represent the standard deviation of the data sets.

Figure 4 - Histograms

mAb Formulations osmolality

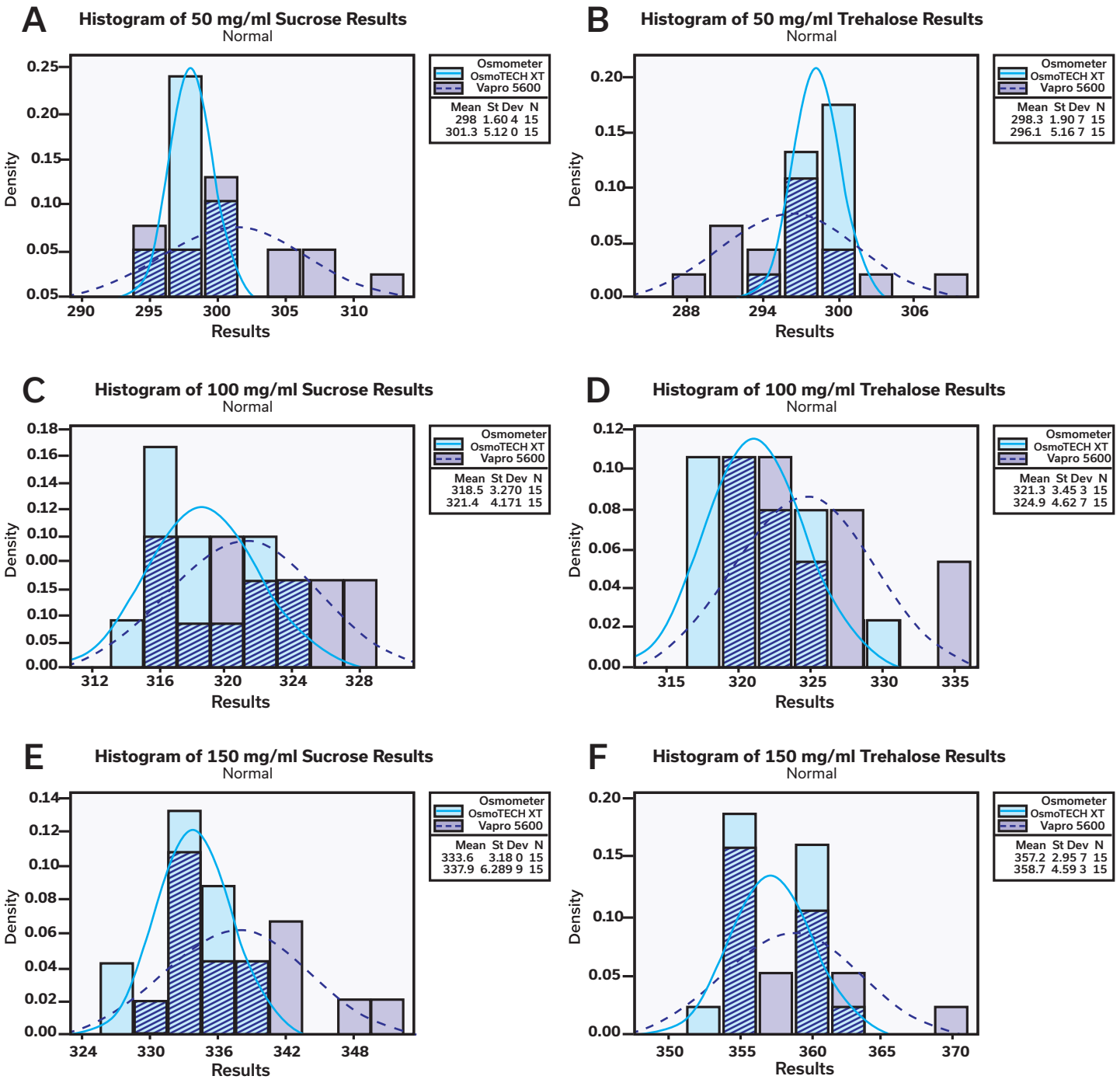

Figure 4. Histograms of mean mAb formulation osmolality values (n=15) on the OsmoTECH XT and Vapro osmometers. The curves' peaks represent mean osmolality, with the curves' spread representing the spread of the osmolality data sets. These quantitative values are also shown in the graph legends.

Table 4 - Osmometer user comparison

	VAPRO 5600	Advanced Instruments OSMOTECH XT
Set-up and Training	Required initialization, several cleaning cycles to reduce the contamination level (dirty from shipping), and calibrating. 1.5 hours to set up. Longer training period required.	**Arrived ready-to-use**, could set up and test immediately. Shorter training period focused on data integrity and usability.
User Interface	Less intuitive with less keys to navigate.	Touch screen, with easy navigation.
Calibration	Must be calibrated before each session. **Calibration can take about 30 minutes to an hour to perform.**	Factory calibrated. **No calibration necessary** before running samples.
Sample Loading	Sample load onto a sample disk which is installed using tweezers. Uses typical / easy micro-pipetting techniques.	Sampling pipette is easy to use – similar to a micro-pipette.
Impact of Common Operator errors	Errors may include inaccurate pipetting, scratching the sample disk holder with the tweezers, under loading the sample disk, and incorrect disk placement. **These errors may lead to major equipment failures** such as damage to the sample disk holder or the thermocouple.	Errors may include inaccurate pipetting, not cleaning the sample tip well, leaving a convex or concave sample in the sample tip, and not cleaning the sample chamber in between runs. **These errors will most likely only result in a sample error** but not damage the equipment.
Storage / Relocation	System stored "on". Required re-initializing, cleaning, and calibrating if the system is moved.	System stored "off". Can easily be moved without requiring additional set-up.
Data Storage	**Limited data storage, instrument will only hold the last 16 data points and will overwrite older results.** Must have a permanent display connected to the instrument to back up data.	**Osmometer holds vast amount of data for the life of the device and does not overwrite when capacity is reached.** Data can be conveniently backed-up via a USB.
Consumables	Consumables include sample disks, micro-pipette tips, 100 mOsm, 290 mOsm, and 1000 mOsm calibration standards.	Consumables include sample chamber cleaners, sample tips, plunger wire and 290 Clinitrol reference solution.
Access	One level of access, no password restrictions.	Three different levels of access; Operator, Supervisor and Admin. Password protected.
Observed Sample Test Time	Standard Run: 97 seconds Viscous Sample: 98 seconds	Standard Run: 121 seconds Viscous Sample: 172 seconds
Standards	3 salt standards (for calibration). Salt standards come in ~200 ml aliquots.	Salt standards; 0, 50, 100, 200, 300, 400, 500, 850, 900, 1000, 1500, 2000, 3000, 4000 mOsm. Standards come in 2 to 5 ml aliquots.
Sample Volume	Uses a sample volume of 10 µl	Uses a sample volume of 20 µl

Table 4. Osmometer User Comparison, inclusive of only some features that were observed during testing. Summary of key comparisons between the freezing point OsmoTECH XT osmometer and the vapor pressure Vapro osmometer. These observations were noted by the researchers over the course of the evaluation and represent the features and benefits of each instrument as they relate to overall usability.

User friendliness and ease of testing

There are obvious mechanical and operational differences between the Vapro 5600 and OsmoTECH XT osmometers involved in this study. The instruments also differ in various features that enhance the user experience beyond presenting an osmolality value.

Table 4 provides a comparison of many features and benefits to the user, from the perspective of the researchers completing the study. Regarding the data presented in this evaluation, it is interesting to note the effects of more frequent calibration of the Vapro 5600 on the testing time and general performance of the instrument. Additionally, some of the features of the OsmoTECH XT (e.g., sample loading, data storage, and data integrity) set it apart from the current vapor pressure osmometer offerings in terms of efficient testing, minimal interruption time, and the ease of integration into a GMP, 21 CFR Part 11 compliant workflow.

Conclusion

An evaluation of freezing point depression (FPD) versus vapor pressure (VP) osmometry is extremely relevant for today's analytical understanding within bioprocessing. The ideal workflow would include a single osmometer type that can be implemented throughout any modality, regardless of final drug protein concentration. The study presented here shows the effect of osmometer type on the quality of osmolality data for concentrated protein formulations. Minimal bias (difference) was observed between the osmolality measurements using the latest FPD osmometer, the OsmoTECH XT, and the latest VP osmometer, the Vapro 5600. This is despite some concerns that the two techniques may have represented vastly different osmolality values for certain concentrated or complex solutions. Moreover, the OsmoTECH XT salt standard osmolality data had less variance, indicated by lower standard deviation values, and greater accuracy than the Vapro 5600 across the operating range of the instruments. The monoclonal antibody formulation data additionally showed less variance on the OsmoTECH XT than Vapro 5600. The evaluation also included a comparison of the usability of both devices. It was suggested that the OsmoTECH XT is the more user-friendly device and also offers several unique features that lend themselves to bioprocessing applications and regulatory requirements. In addition to the "intelligent freezing" technology, the 21 CFR Part 11 compliance means the OsmoTECH XT is better adapted to a GMP environment in comparison to the Vapro 5600. It is therefore easily integrated into electronic batch records and already automated manufacturing systems. Given the similar osmolality measurements, Biotech osmometer users will find that the OsmoTECH XT performs better than the Vapro 5600. It provides significant and extensive features that support the increasing need for workflow efficiency and data integrity in analytical devices.

Evaluation performed by Jefferson Institute for Bioprocessing

References

1. United States Pharmacopoeia 42. General Chapters: 785 Osmolality and Osmolarity. Rockville, Maryland, USA: 2016.
2. Wright, Kristeena. Osmolality as a concentration measurement method for key buffers in bioprocessing. 2019, Advanced Instruments.
3. Wright, Kristeena. 8 Ways that Osmolality Testing Improves Cell and Gene Therapy Process Development and Manufacturing. 2020, Advanced Instruments.
4. Shukla, Abhinav A et al. "Evolving trends in mAb production processes." Bioengineering & translational medicine vol. 2,1 58-69. 3 Apr. 2017, doi:10.1002/btm2.10061.
5. Sahin, Erinc et al. Osmolality Measurements for High-Concentration Protein–Polymer Solutions: Variation Based on Working Principles of Osmometers. Bioprocess Intl 14(6); Jun 2016.

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