ADVANCED INSTRUMENTS

Application Note

Osmolality as a concentration measurement method for key buffers in bioprocessing

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Introduction

The manufacturing of biologics is a complex and costly process by which a protein is recovered and purified of contaminants to ultimately generate a drug product. This is accomplished through a chain of filtration and chromatography processes. Achieving and maintaining the desired product quality and purity requires optimization of process parameters as well as precise control of these parameters.

As the biopharmaceutical industry grows, there is greater emphasis on developing robust analytical methods that provide more accurate and reliable measurements to characterize downstream processes. This entails close monitoring of the buffers that maintain purification conditions and stabilize the protein[1]. Standardized testing of buffer components provides assurance of process consistency and reduces the risk of batch failure, improving the overall success rate of manufacturing. Table 1 lists a number of common buffers and buffer components that are used in the downstream processing of biologics. Improper preparation and assessment of buffers can have negative effects during filtration and chromatography steps (e.g. reduced process performance or out-of-specification result)[2,3].

Traditional measurements for the testing and release of buffers throughout downstream processing include pH and conductivity. Currently, the buffer component concentration is ensured through procedural controls (e.g. Standard Operating Procedures, or SOPs) as well as through indirect measurement with a combination of pH and conductivity. Both pH and conductivity are inter-related[4], molecule specific, and dependent on the degree of dissociation of the molecule under the conditions.

While these measurements are invaluable to ensure protein stability and appropriate interaction with chromatography resins, they have a significant dependence on ionization[5,6]. This renders them unreliable as concentration measurements in weakly dissociating buffers. Additionally, both pH and conductivity meters can be affected by temperature[5,6]. For buffers that are prepared and stored below room temperature, these methods could require additional calculations and precautions.

Buffer component	Applications in Downstream Bioprocessing
Tris	pH Neutralization, Chromatography
Tris-HCl	Chromatography
Phosphate Buffer	Chromatography, UF/DF
Citrate Buffer	Chromatography, UF/DF
Acetic Acid	pH Acidification, Protein A Chromatography Elution
Citric Acid	pH Acidification, Chromatography, UF/DF
Phosphoric Acid	pH Acidification, Chromatography

Table 1. Common buffers in downstream bioprocessing and their applications. These buffers were tested for osmolality, conductivity and pH.

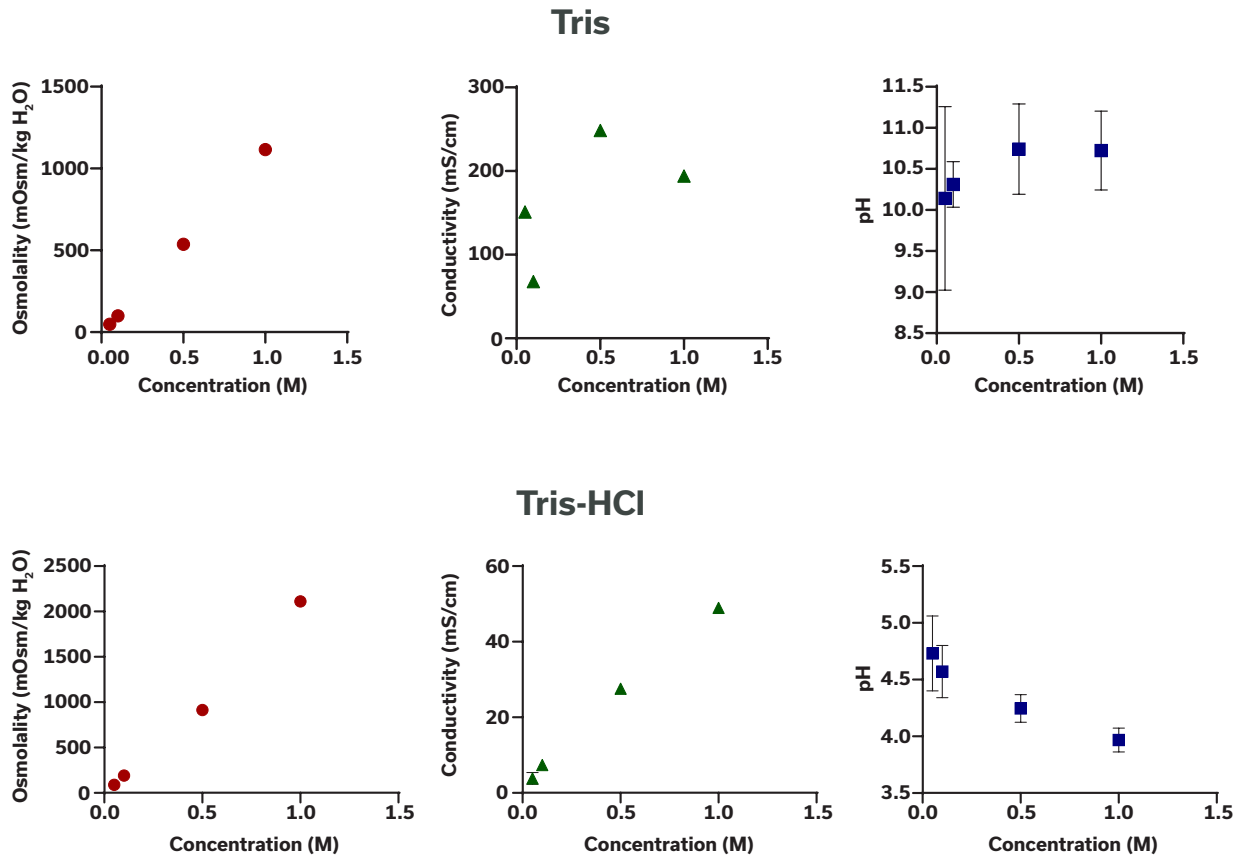
Osmolality has been evaluated in this study as an orthogonal measurement with the potential to add consistency and improved control strategy for bioprocesses. Osmolality is a measurement of the moles of solute per kg of water, and therefore is expected to be relatively unaffected by the type of molecule, the degree of ionization, or the pH. This study was conducted to understand how osmolality can serve as a concentration measurement and how it compares to pH and conductivity measurements.

Materials and Methods

The buffers in Table 1 were prepared individually using a gravimetric method. All buffers were prepared at 0.05 M, 0.1 M, 0.5 M, 1 M, 1.25 M, 1.5 M, 1.75 M, and 2 M and tested within 24 hours of preparation. The lower concentrations (0.05 M to 1 M) best represent their use in bioprocessing. Solutions with concentrations greater than 1 M were incorporated, as the instruments allowed, to account for the growing bioprocessing trend of testing buffer concentrates prior to in-line dilution[2]. Osmolality was measured using the Advanced Instruments OsmoTECH® Single-Sample Micro-Osmometer, which employs the freezing point depression method. Conductivity was measured with the Hanna Instruments HI5321 Laboratory Research Grade Benchtop Conductivity/Resistivity /TDS/Salinity/Temperature Meter. pH was measured using the Thermo Scientific Orion® 8157BNUMD Ross™ Ultra Refillable Triode™ in conjunction with the Orion Star™ A211 pH Benchtop Meter. All measurements were taken 10 times at each concentration. Data were recorded and the mean and coefficient of variation (%CV) were calculated for each concentration.

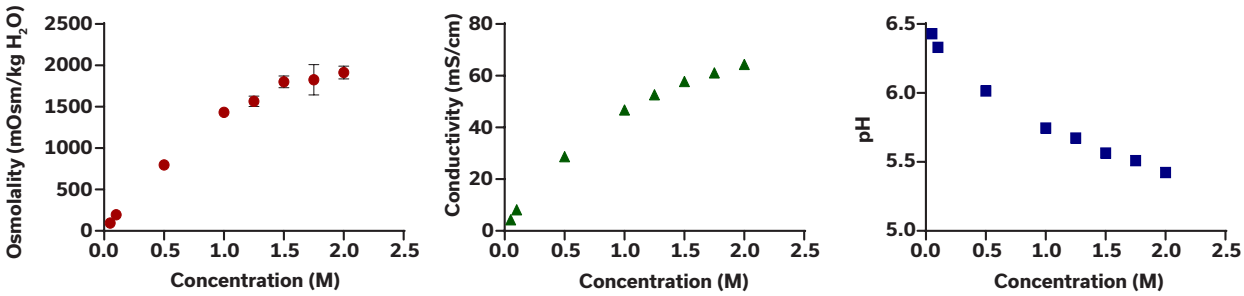
Results

The seven sets of graphs below show the mean and %CV (represented by error bars) for the three properties of each solution. Error bars representing extremely small CVs are not visible. The axes for each chart were adjusted to highlight the trends.

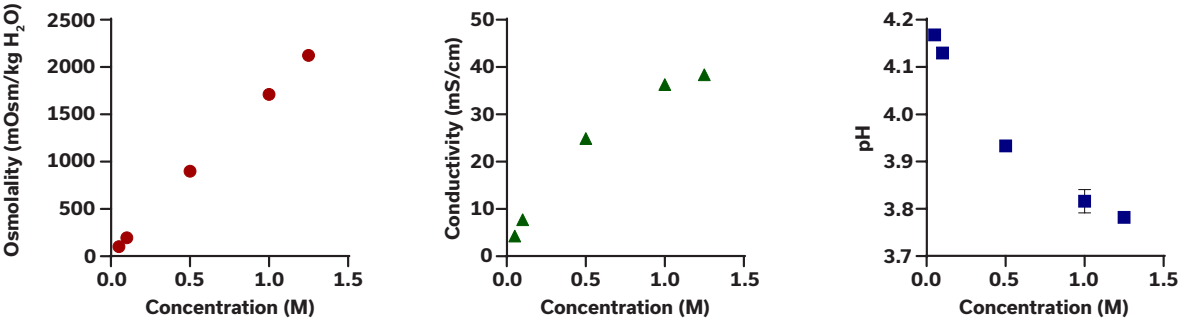


Results continued

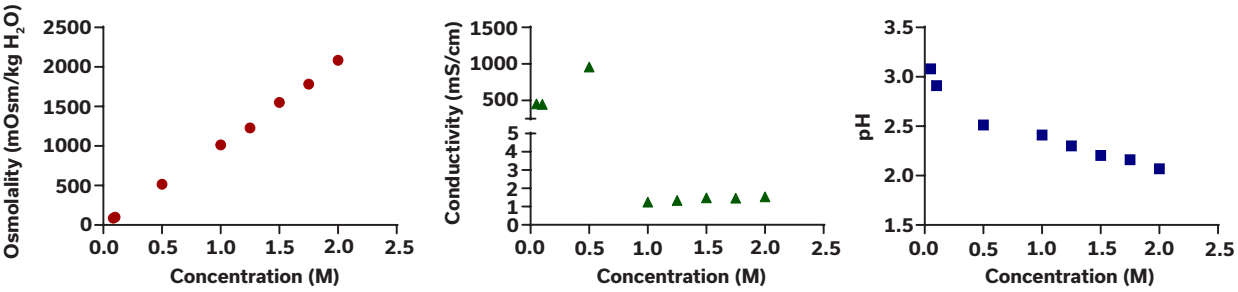
Phosphate Buffer



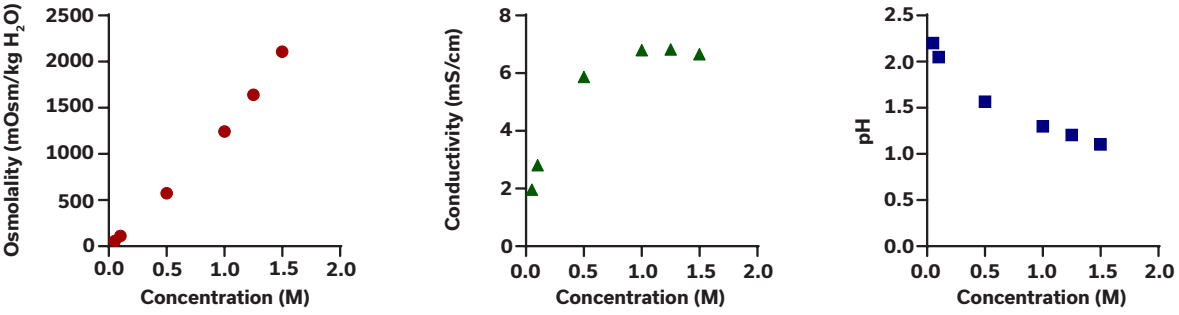
Citrate Buffer



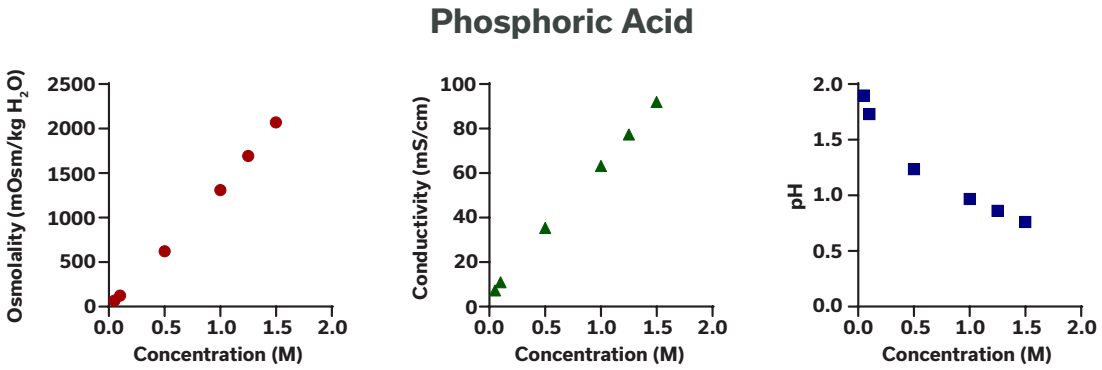
Acetic Acid



Citric Acid



Results continued



Discussion

The data show that pH and conductivity do not provide the best measure of concentration for buffers such as Tris, acetic acid, citric acid, and citrate. Osmolality, on the other hand, provides a strong and proportional measurement for each of these buffers over a wide range of concentrations. While conductivity and pH have clear value within downstream bioprocessing, osmolality can offer additional information about buffer concentrations. Measuring pH is critical within downstream bioprocessing; however, it lacks sensitivity, as the range varies very little across a scale of buffer concentrations. For example, the pH curve for citrate buffer (Figure 1) fits within a range of 0.5, while the osmolality curve spans about 500 mOsm/kg H_2O. This was a common trend across buffers and demonstrates that osmolality provides a wider dynamic range and more sensitivity than pH alone. Conductivity is commonly measured in downstream buffers and reagents because it provides an idea of concentration of ionic solutions. It is not reliable for nonionic and weakly ionic solutions, such as acetic acid. Above a 1 M concentration, the conductivity of acetic acid stayed around 1 mS/cm (Figure 2), but this effect is not reflected in osmolality measurements.

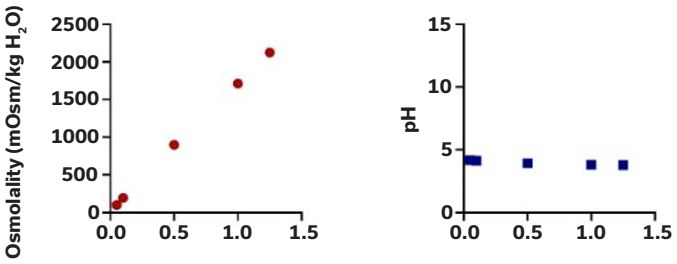


Figure 1. Comparison of osmolality and pH on a citrate buffer concentration curve. The y-axis expands the full pH range to highlight the relatively small change across concentrations of citrate buffer. The sensitivity of the osmometer was much greater than on the pH probe, as shown by the low slope of the pH concentration curve.

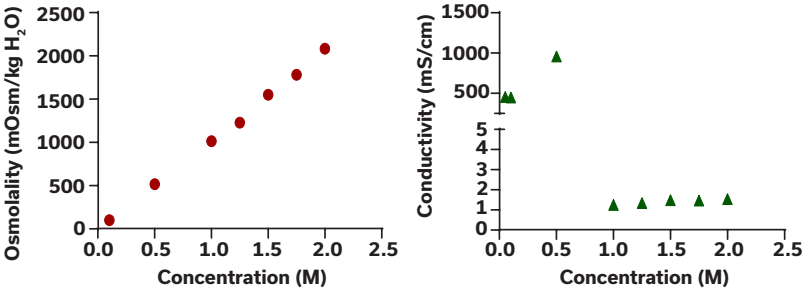


Figure 2. Conductivity of acetic acid, a weakly ionic solution, drops drastically at 1 M concentration and remains constant as the concentration increases.

This study demonstrates the sensitivity of osmolality as a measure of concentration of common downstream buffers. These data provide support for osmolality as a valuable orthogonal property to conductivity and pH. Due to the increased sensitivity of osmolality, it would be an ideal method to help detect potential issues with buffer concentration or formulation. Given the simplicity of the measurement as well as the value it adds, osmolality testing in the downstream workflow should be considered to provide a more complete and comprehensive picture of downstream buffers and processes.

References

1. Bratt, Jennifer, et al. Buffers in Biologics Manufacturing. BioProcess International 15.2(2017).
2. Challener, Cynthia. Behind the Scenes with Buffers. BioPharm International 28.2(2015).
3. Gronemeyer, Petra, et al. Trends in Upstream and Downstream Process Development for Antibody Manufacturing. Bioengineering 1.4(2014).
4. Leveling, Tony. The relationship between pH and Conductivity in a Lithium Contaminated, Deionized Water System. Pbar Note 675: The relationship between pH and Conductivity. Fermilab 2002.
5. West, Jonathan, et al. "PH Measurement." Visual Encyclopedia of Chemical Engineering, University of Michigan College of Engineering, 2018, encyclopedia.che.engin.umich.edu/Pages/ProcessParameters/pHMeasurement/pHMeasurement.html.
6. Manns, Reinhard and Jurgen Schleicher. Information on Conductivity Measurement. JUMO GmbH & Co. KG FAS 624 04.07(2007).

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