BUFFER PREPARATION: INDEX OF REFRACTION IMPROVES PROCESS ANALYTICAL TECHNOLOGY (PAT) IN BIOPHARMACEUTICAL MANUFACTURING

Introduction

Buffer preparation, like many other biopharmaceutical processes, requires liquid chemical concentration and temperature monitoring and control to ensure peak process performance. Accuracy in mixing, precise monitoring of ingredient concentration and the ability to achieve repeatable, consistent product are critical for quality control in buffer preparation.

Implications of an improperly mixed buffer can have wide-reaching consequences in the many upstream and downstream buffer applications. Upstream, quality control of buffers is critical for maintaining pH and a stable cell growth environment. As most organisms only thrive in a limited pH range, pH falling outside of that particular range can cause enzymes to slow, stop working or denature. Downstream, an improperly mixed buffer can negatively affect separation and purification conditions. The impacts overall, upstream or down, include loss of product, loss of revenue and compromised quality and yield.

Currently, there are many methods used to measure chemical concentration including refractive index, conductivity, pH and osmolality. This application note will introduce an index of refraction technology for quality control in buffer preparation. It will then discuss conductivity, pH and osmolality – all of which face process analytical technique (PAT) limitations in terms of dynamic range, linearity, precision, limits of detection (LOD) and limits of quantification (LOQ).[1]

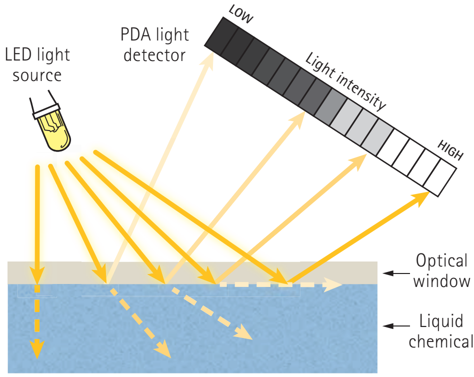
Index of Refraction for Buffer Preparation

Quality control of buffers requires reliable, precise, easy to use analytical instrumentation with fast response time. Utilizing index of refraction – an optical technique that provides a direct measure of the concentration of solutions – this IoR technology provides real-time, accurate concentration monitoring.

Utilizing the miniaturized optical sensor, a light emitting diode (LED) shines light onto an optical window, which is in contact with the liquid being measured. Light from the LED reflects off the optical window into a photodiode array (PDA) detector, and the critical angle (θc) is measured where light is refracted into the fluid and not reflected back to the PDA detector. The index of refraction of the liquid is determined[2] from equation 1, where n_b is the refractive index of the sample, and n_a is the refractive index of the refractometer optics. Concentration is then determined by calibrating the IoR to the liquid's concentration.

$$\sin(\theta_c) = \frac{n_b}{n_a}$$

As the liquid concentration changes, so does θc. The IoR technology measures these changes in θc quickly (100 milliseconds) and accurately (better than ±0.2 wt%). Using internal averaging of the 100 millisecond data rate to increase resolution, it reports an IoR (or concentration) every 1 second. Additionally, because IoR depends on the temperature of the liquid, a thermistor is used to monitor the temperature and automatically compensate the IoR (and thereby the concentration) of the liquid in real time.



Providing real-time, accurate and repeatable concentration and temperature measurements for in-line and off-line monitoring of process liquids, the index of refraction technology provides the necessary monitoring to avoid pH shifts and product variants in buffer preparation applications. The in-line capability provides information in real time, allowing for fast response to process variations, while its small footprint provides the ability to monitor at virtually any point in the process. Additionally, index of refraction measurements offer an advantage over pH and conductivity because IoR is a direct measure of chemical concentration, while pH and conductivity are dependent on the electronic properties of fluids and are therefore by definition an indirect or inferred measurement of chemical concentration.[3]

Testing and Results

To determine the best method for routine liquid chemical concentration measurements, testing was done which involved measurement of known concentrations of commonly used buffer constituents and cell culture growth media ingredients dissolved in water. Index of refraction measurements were compared to conductivity, pH and osmolality. The IoR, conductivity and pH measurements were all done in-line and in real-time, and the osmotic concentration measurements were done off-line using grab samples.[4]

Various buffer components that are commonly used in biopharmaceutical manufacturing were serially added to the solutions, and additional measurements were taken to compare for accuracy, precision and linearity. To provide a sample of the data sets found, Figure 1 shows the results of the test for buffer 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)[5] utilizing an Entegris IoR concentration monitor as well as conductivity and pH monitors.

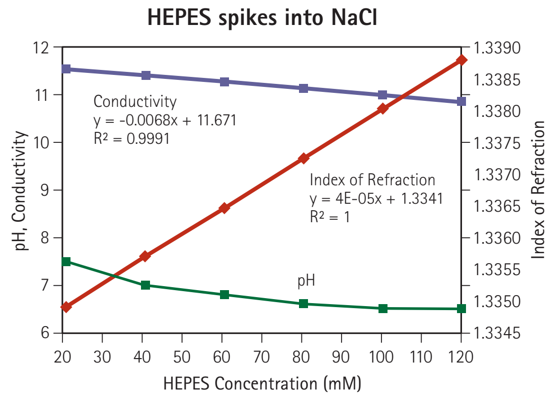


Figure 1: pH, conductivity and Entegris IoR outputs are shown versus HEPES concentration in mM. Six HEPES concentration spikes of 20 mM were added into the sodium chloride solution for a total HEPES concentration of 120 mM in NaCl.

Neither pH nor conductivity is able to monitor the HEPES concentration effectively. The conductivity does change with HEPES concentration, but the slope is negative – an increase in HEPES concentration should provide an increased conductivity measurement. The pH measurement was non-linear with changing negative slopes. HEPES is a weakly-ionic organic buffer which explains the poor performance of the conductivity measurement. The Entegris IoR concentration monitor shows high resolution and high linearity outperforming the other measurement techniques.[6]

This testing was repeated with a number of other common buffers; Table A shows a summary of the Entegris IoR and conductivity results for the buffer solutions tested.

TABLE A: SUMMARY OF THE IOR AND CONDUCTIVITY RESULTS FOR CONCENTRATION MEASUREMENTS OF THE BUFFER SOLUTIONS.

Test (Spikes → Base)[7]	Entegris IoR	Conductivity
$NaPO_4$ → NaCl	Yes	Yes
NaCl → $NaPO_4$	Yes	Yes
HEPES → NaCl	Yes	No
NaCitrate → $NaPO_4$	Yes	No
$NaPO_4$ → NaCitrate	Yes	No
P80 → H_20	Yes	No
Triton → H_20	Yes	No

In all seven buffers, index of refraction showed high linearity and was able to measure the entire dynamic range, whereas in five of the seven buffers, conductivity was unable to provide a measurement. Additionally, IoR typically outperformed in measurement accuracy with accuracies of ±10 ppm compared to ±100 ppm for conductivity.[8]

LOD and LOQ for each measurement technique can be seen in Figure 6. The data show a strong advantage for IoR over the other measurement methods, with the Entegris IoR providing stronger detectability and quantitation levels – 2.5, 4.2 and 28.6 times higher than conductivity, osmolality and pH respectively.

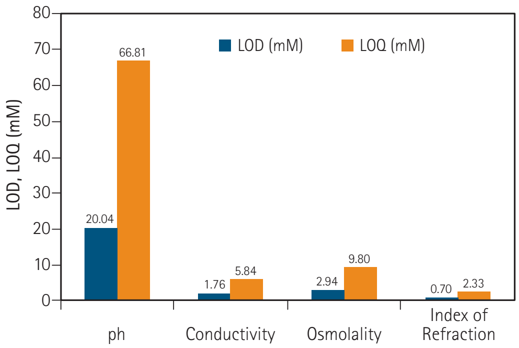


Figure 6: Limit of detection (LOD) and limit of quantification (LOQ) for pH, conductivity, index of refraction, and osmolality concentration measurements.[9]

Conclusion/Summary

For the many critical touchpoints of buffers, buffer preparation requires strong quality control measures to ensure the desired buffer characteristics are accurately and precisely maintained. The additional challenge is to ensure the repeatability of the process. The data from these tests show that using IoR measurement provided superior results when compared to conductivity, pH and osmolality with respect to accuracy, precision, linearity, LOD and LOQ. The Entegris IoR technology's in-line capability provides information in real time, allowing for fast response to process variations. Additionally, it will scale from lab to full-scale production, and its small footprint provides the ability to monitor at virtually any point in biopharmaceutical processes.

References

1. Schreiber, B., Wacinski, C. and Chiarello, Dr. R., *"Index of Refraction as a Quality Control Metric for Liquids in Pharmaceutical Manufacturing,"* Pharmaceutical Engineering, 2013. Print.
2. Halliday, D. and Resnick, R., Fundamentals of Physics, John Wiley and Sons, New York, 1981, pp 693.
3. Schreiber, B., Wacinski, C. and Chiarello, Dr. R., op. cit.
4. Schreiber, B., Wacinski, C. and Chiarello, Dr. R., op. cit.
5. HEPES Molecular Formula: $C_8H_{18}N_2O_4S$
6. Tison, S., *"A New Innovation in Chemical Measurement,"* Production Insight, VWR International, October 2014.
7. Lundblad, Roger L., and Macdonald, Fiona. Handbook of Biochemistry and Molecular Biology. CRC Press, 2010. 4th Ed., illustrated, revised.
8. Tison, S., op. cit.
9. Schreiber, B., Wacinski, C. and Chiarello, Dr. R., op. cit.

ENTEGRIS, INC.
Corporate Headquarters | 129 Concord Road | Billerica, MA 01821 USA
Customer Service Tel. +1 952 556 4181 | Customer Service Fax +1 952 556 8022
In North America 800 394 4083 | *www.entegris.com*

Entegris
creating a material advantage