Index of Refraction as a Quality Control Metric for Liquids in Pharmaceutical Manufacturing

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This article presents a case study comparison of four analysis system technologies: pH, conductivity, osmolality, and refractive index for nine buffer chemical mixtures.

Introduction

The complex processes involved in the discovery and manufacturing of pharmaceutical products require advanced process analytical techniques for even routine applications. This requirement applies to large-scale manufacturing processes in stainless steel and glass vessels as well as single use disposable bag systems. Processes ranging from media and buffer preparation to sterilization and decontamination, require liquid chemical concentration and temperature monitoring and control to ensure peak process performance. Errors at any of these steps can result in the loss of costly product, compromise of product quality, or loss of time and labor. However, while each step in any pharmaceutical manufacturing process does represent a potential source of costly error, most steps also can be used as points of potential quality control. Close monitoring of key steps in manufacturing processes is therefore a critical part of good manufacturing process design.

Meaningful quality control of liquid chemicals requires reliable, easy to use, high precision, and fast response time analytical instrumentation. Current in-line methods that attempt to meet these requirements include pH, conductivity, and osmotic concentration. Briefly, osmotic concentration is the measure of solute concentration, defined as the number of osmoles of solute per liter of solution. All of these available technologies face limitations of dynamic range, linear-

ity, precision, and Limits of Detection (LOD) and Limits of Quantification (LOQ). Furthermore, none of these methods are fundamental measurements of liquid chemical concentration. In the work presented here, a new instrument based on Index of Refraction (IoR) is presented and compared to pH, conductivity, and osmotic concentration. Since conductivity is in especially high use as a concentration monitor, special care is taken to compare IoR and conductivity measurement results.

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. In pharmaceutical manufacturing, IoR may be used in both upstream and downstream applications, while the results presented here are focused on downstream buffer preparation applications. Conventional refractometers operate by shining a single wavelength of visible light onto a prism that is in contact with the fluid under analysis. The IoR of the fluid is determined from the critical angle using Snell's Law. For most liquids, a simple calibration converts IoR values to chemical concentration in either ppm or wt%. A limitation of conventional refractometers is that they operate in a transmission mode, where light travels through the fluid to an optical light detector. This method has the disadvantage that the light signal is affected by diffraction and absorption effects of the fluid. The IoR analyzer used in this study operates in a reflection mode optical

geometry. This means the light reflects off the back side of an optical window (in contact with the fluid under analysis) and up into an optical light detector. In this way, the derogatory fluid effects of turbidity, diffraction, and absorption are completely mitigated, and the concentrations of opaque fluids are conveniently measured.

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To explore the usefulness of IoR as a quality control metric, a set of experiments was performed involving measurement of known concentrations of commonly used buffer constituents and cell culture growth media ingredients dissolved in water. IoR measurements were compared with measurements of pH, conductivity, and osmotic concentration. From these experiments it was determined that measurement by IoR provided data superior to current methods of measurement of conductivity, pH, and osmolality of solutions with respect to accuracy, precision, linearity, Limit of Detectability (LOD), and Limit of Quantification (LOQ). LOD is defined as the lowest concentration of a substance that can be measured compared to a blank value (one sigma). In this study, LOQ is distinguished from LOD as the lowest concentration that can be determined with a reliability of ten sigma.

Experimental Methods

A comparison of pH, conductivity, osmotic concentration, and Index of Refraction (IoR) was made to determine the best method for routine liquid chemical concentration measurements. The measurements were made at the Bristol-Myers Squibb pilot plant in Syracuse, NY. The pH, conductivity, and IoR measurements were all made in-line and in real-time. Osmotic concentration measurements were made off-line using grab samples. Conductivity, pH, and osmotic concentration were selected based on their common use in the industry and as served as benchmarks for the IoR analyzer.

Each technique operates under differing principles of operation. Conductivity is a measurement of the electrical conductance per unit distance in an electrolytic or aque-

ous solution, and is limited in its ability to measure low or non-conductive liquids. The pH of a solution is a measure of the activity of the solvated hydrogen ion (H+). Osmotic concentration is the number of osmoles per liter of solution. The specific method used here is "freezing point depression" osmotic concentration, where differences in freezing points as a function of solutes added to solvents produces a concentration value of the solution. Freezing point depression osmotic concentration is limited as an off-line laboratory technique with relatively long response times.

Index of refraction is an optical technique that is a direct measure of the concentration of solutions. The IoR instrument used in this study was operated in a reflection geometry; meaning light is reflected off of the backside surface of an optical window in contact with the solution under analysis and into a photo-detector. This geometry offer the advantage that the IoR analyzer monitors the electronic density of the solution without interference from other optical effects, such as turbidity, diffraction, and absorption. Additionally, the IoR instrument includes temperature measurement in a single probe, thereby providing measurement of two key process parameters (concentration and temperature).

IoR, conductivity, and pH measurements were made simultaneously and in series. The pH and conductivity probes were placed in fluidic cells and buffer chemicals were circulated in closed loop. Osmotic concentration measurements were made off-line. Components of buffers that are commonly used in pharmaceutical manufacturing were serially added to various solutions. For each incremental addition of solute, measurements of index of refraction were compared with measurements of conductivity, and the data for the two methods of measurement were compared for accuracy, precision, and linearity. Buffer ingredients used in the studies included serial additions of 1. sodium chloride added to solutions containing fixed concentrations of monosodium phosphates, 2. sodium citrate added to solutions of fixed concentrations of monosodium phosphates, 3. monosodium phosphates added to sodium chlorides, 4. sodium citrate added to monosodium phosphate, 5. HEPES added to sodium chloride, 6. polysorbate 80 added to water, and 7. Triton X-100 added to water. HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid , is an organic chemical buffering agent widely used in cell culture. These seven buffers were chosen to best represent traditional slat buffers and newer buffers expected to realize increasing use in downstream processes. Limit of Detection (LOD) and Limit of Quantification (LOQ) measurements were made for a solution of 50 mM (mM = milliMolar) HEPES solution of pH 7 into which aliquots of NaCl were incrementally added. In these experiments, IoR was pitted against conductivity, pH, and osmotic concentration. LOD and LOQ values were calculated based on the standard deviation of the response and the slope of each instruments response as a function of concentration

Figure 1. Index of refraction analysis system. The analyzer sensor head contains a miniaturized optical sensor that is in contact with the liquid chemicals under analysis.

change. In a separate set of experiments for media preparation, several chemicals were analyzed in a comparison of the IoR instrument and conductivity. These included HAM F10, Dulbecco MEM, RPMI 1640, yeast extract and other media chemicals.

Results and Analysis

Figure 1 is an image of the IoR analyzer used in these studies. The analyzer consists of two fluidic cells (one fluidic cell

is for redundancy), and digital control electronics. The fluid cell contains a miniaturized IoR sensor and thermocouple that is in contact with the liquid chemical under analysis. The digital electronics box performs analysis of the raw optical signal, real-time temperature concentration of the IoR and outputs either IoR or liquid chemical concentration. Figures 2 through 5 are data graphs representative of the results found for all buffer preparation processes studied here. Figure 2 shows an example of typical data acquired in this study for mixing of buffer salt solutions. In the figure, pH, conductivity, and IoR are plotted versus $NaPO_4$ (monosodium phosphate) concentrations. The measurements were performed by adding 20 mM spikes of $NaPO_4$ into 1 Liter of NaCl (sodium solution) 10 times to reach a total concentration of 200 mM of monosodium phosphate in sodium chloride. The pH showed insufficient response to $NaPO_4$ concentration changes. Conductivity and IoR both show excellent response to NaPO4 concentration changes. As was found for other buffer chemicals, IoR showed a higher degree of linearity than conductivity, and has a least squares (R2) fit confidence of 1.00 compared to conductivity's R^2 value of 0.98. The IoR's higher degree of linearity was found for all buffer solutions tested.

Figure 3 shows pH, conductivity, and IoR plotted as a function of HEPES concentration. Neither pH nor conductivity is able to monitor the HEPES concentration effectively. The conductivity data does a linear relationship with HEPES concentration; however, the slope is negative. For an appropriate conductivity response, conductivity should increase with increasing HEPES concentration. The negative slope can be explained by, as the HEPES concentration increases, the solution becomes increasingly less ionic and therefore conductivity decreases. For an appropriate concentration
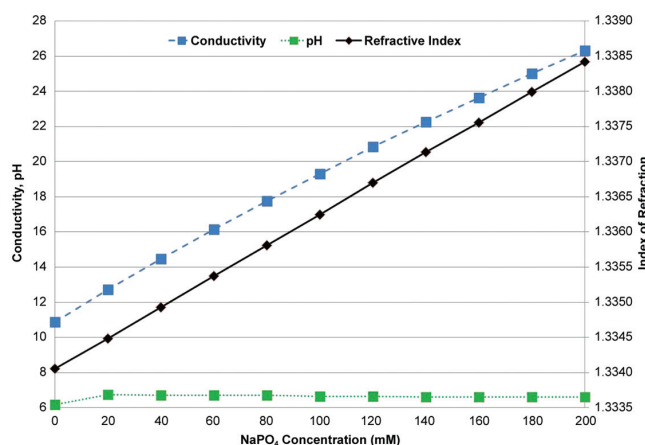

Figure 2. Index of refraction (right hand vertical axis) and conductivity and pH (left hand vertical axis) outputs are shown versus monosodium phosphate concentration in mM. Ten monosodium phosphate concentration spikes of 20 mM were added into a 1 Liter sodium chloride solution.
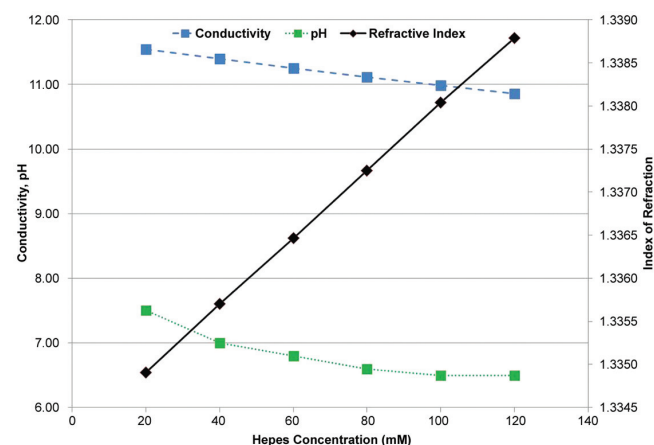

Figure 3. Index of refraction (right hand vertical axis) and conductivity and pH (left hand vertical axis) outputs are shown versus HEPES concentration in mM. Six HEPES concentration spikes of 20 mM were added into the sodium chloride solution for a total HEPES concentration of 120 mM in NaCl.
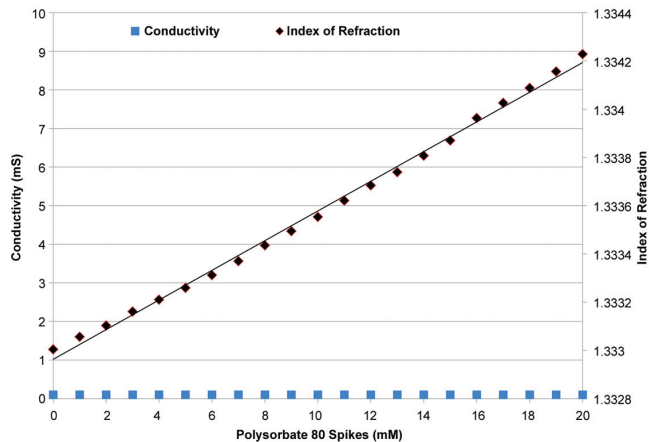
Figure 4. Index of refraction and conductivity are plotted as a function of Polysorbate 80 (P80) concentration spikes added to a 1 Liter NaCl solution. Conductivity shows no response, and is unable to measure P80 concentration.

response, one would expect that as the HEPES concentration increases the conductivity value also will increase, as it does for the salt solution in Figure 2. Therefore, conductivity fails to measure HEPES concentrations because HEPES is a non-ionic solution and the conductivity probe provides false HEPES concentration readings throughout the HEPES concentration range. Conductivity also failed to measure Polysorbate 80 and Triton X-100. IoR measures the HEPES concentration with a high degree of linearity over the entire concentration range studied.

Figure 4 shows IoR and conductivity plotted as a function of Polysorbate 80 (P80) concentration. P80 was added in 1 mL spikes to a 1 Liter NaCl solution. Conductivity fails to measure the P80 concentration. In contrast, IoR shows high linearity over the entire concentration range of Polysorbate
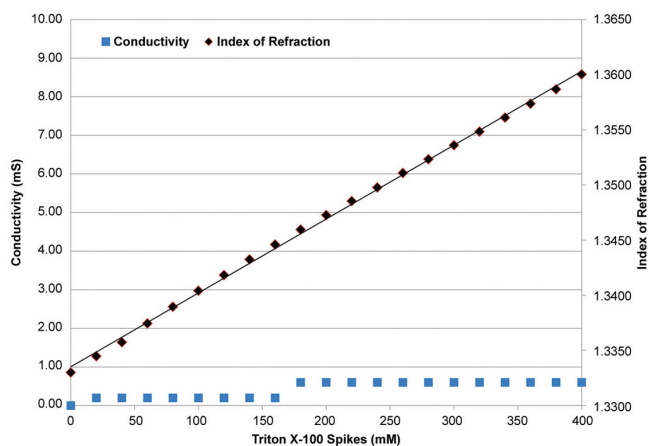

Figure 5. Index of refraction and conductivity are plotted as a function of Triton X-100 concentration spikes added to a 1 Liter NaCl solution. Conductivity shows no response, and is unable to measure Triton X-100 concentration.

Test (Spikes → Base)	Index of Refraction	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_2O	Yes	No
Triton X-100 → H_2O	Yes	No

Table A. Summary of the IoR and conductivity results for concentration measurements of the buffer solutions.

80. Figure 5 shows that IoR performed similarly well for Triton X-100, and that conductivity failed to measure Triton X-100 concentration changes.

Table A, shows a summary of the IoR and conductivity results for concentration measurements of the buffer solutions studied here. Index of refraction showed high linearity and was able to measure the entire dynamic range for all buffer solution tested. Conductivity failed to measure five out of seven buffer processes. Furthermore, the IoR demonstrated concentration measurement accuracies of ±10 ppm compared to ±100 ppm for conductivity.

Figure 6 shows LOD and LOQ for each of the techniques evaluated here. These results show a strong advantage for the IoR analyzer over conductivity, pH and osmotic concentration. For the IOR analyzer, LOD and LOQ were 0.70 and 2.33, respectively. Conductivity LOD and LOQ were more than two times worse than the IoR analyzers with values of 1.76 ad 5.84, respectively.

Figure 7 shows a comparison of the IoR analyzer and conductivity for yeast extract in a media preparation process. The IoR analyzer showed superior linearity to conductivity.
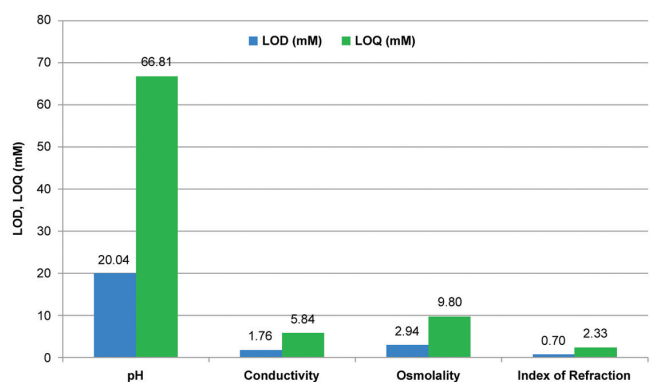

Figure 6. Limit of Detection (LOD) and Limit of Quantification (LOQ) for pH, conductivity, index of refraction, and osmolality concentration measurements.

Similar results were found for all other media preparation processes evaluated here.

For all experiments, solute concentration measurements using the Index of Refraction (IoR) analyzer were superior to those measured by conductivity in terms of linearity (by measured R^2 values of lines generated by the data), precision, accuracy (as determined best least squares linear fit), dynamic range, and reproducibility. In summary:

- For measurement of serial additions of NaCl into a solution of constant [$NaPO_4$], concentration measurement by IoR exhibited superior linearity and precision (as measured by R^2 value).
- For measurement of serial additions of $NaPO_4$ into a solution of constant [NaCl], measurement by IoR exhibited superior linearity and precision (as measured by R^2 value).
- For measurement of serial additions of HEPES into a solution of constant [NaCl], concentration measurement by IOR exhibited superior linearity, precision, and specificity. Since the slope of the line generated by the conductivity of additions of HEPES to solution was mostly flat, and in fact slightly negative, conductivity measurements have little specificity at all for HEPES in a buffered solution.
- For measurement of serial additions of sodium citrate into a solution of constant [$NaPO_4$], concentration measurement by IOR exhibited far superior linearity (conductivity measurement was nonlinear), precision, and range.
- For measurement of serial additions of $NaPO_4$ into a solution of constant [Sodium Citrate], concentration measurement by IOR exhibited superior linearity, precision, and specificity.
- For measurement of serial additions of Polysorbate 80 into water, concentration measurement by IoR exhibited superior linearity, precision, and specificity.
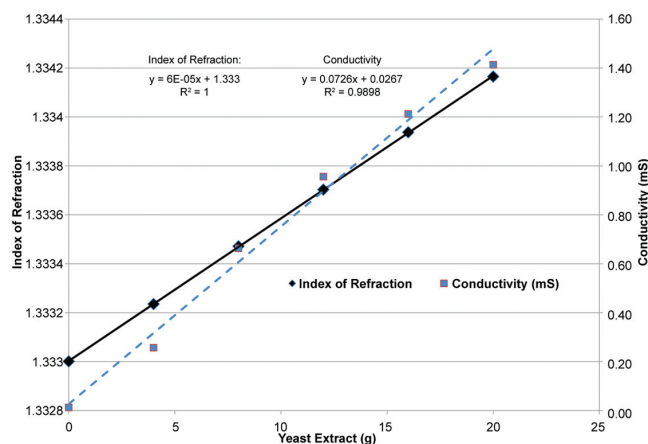- For measurement of serial additions of Triton X-100

Figure 7. Index of Refraction (IoR) and conductivity plotted as a function of yeast extract (in grams). The IoR analyzer showed superior linearity than conductivity.

into water concentration measurement by IOR exhibited superior linearity, precision, and specificity.
- For the LOD and LOQ experiments against pH, conductivity, and osmotic concentration, IoR was 2.5 times more sensitive than conductivity, 4.2 times more sensitive than osmotic concentration, and 28.6 times more sensitive than pH for both limits of quantitation and limits of detection.
- For serially increasing concentrations of complex media, measurement by IoR exhibited superior linearity and precision, with higher R^2 values for data generated.

Discussion

These experiments demonstrate that metrology of process fluids by measurement of index of refraction is a viable and superior means of real-time, in situ quality control in pharmaceutical manufacturing as compared to pH, conductivity, and osmolality. Furthermore, the experiments demonstrate that a currently available device based on IoR exhibit significantly greater linearity and precision, as well as lower levels of detectability and quantitation than currently available fluid measurement devices which measure other fluid properties, such as conductivity, osmotic concentration, and pH. They also demonstrate that, as compared with conductivity and pH, measurement by index of refraction exhibits greater specificity and relevance for measuring the concentration of anionic solutes in any buffer or growth media containing them. As non-ionic solutes do not affect conductivity or pH, measurement of these properties provides little information when measuring the content of such solutes in prepared solutions. Furthermore, the behavior of solutions containing zwitterions (dipolar ions), such as HEPES is highly unpredictable; therefore, using pH and conductivity to measure the concentration of solution constituents that are zwitterionic is also not optimal. These attributes—superior precision, linearity, and specificity for non-ionic solutions and solutions containing zwitterionic (dipolar ion) ingredients—make measurement by index of refraction appropriate for quality control of many buffers and cell culture media essential for pharmaceutical manufacturing. Furthermore, it is likely that IoR also would be a highly relevant metric for validation of Clean-in-Place (CIP) solutions for fermentation and isolation tanks. The comparison to conductivity especially is based on the need for improved real-time, in situ concentration monitor, and control. In fact, in nearly all cases IoR is a superior method to conductivity for concentration measurements. However, conventional IoR is limited by its inability to speciate a chemical mixture. Therefore, absorption spectroscopy techniques such as Near-infrared (NIR) and Fourier Transform Infrared Spectroscopy (FTIR) are powerful concentration speciation tools for complex chemical mixtures.

Measurement by IoR has additional advantages. Because refractive index measurement only requires a beam of light

reflected off the surface of a process fluid, it is minimally invasive. As a consequence, it effectively eliminates any potential risks of contamination caused by fluid sampling, or potential damage to sensitive media contents due to application of voltage potential or other means of measurement. Furthermore, index of refraction measurement is virtually instantaneous, whereas other methods, such as sampling require more time. Measurement of index of refraction is also more likely to reduce human error as well.

This is not to say that index of refraction measurement has no disadvantages when compared with other quality control metrics. Specifically, conventional IoR measures the average concentration of a multicomponent fluid mixture, and lacks the capability to speciate the concentration of specific components in said mixture. IoR is also temperature dependent, and state-of-the-art IoR analyzers have real-time temperature compensation as a built-in feature. It is not to be inferred that index of refraction measurement should replace measurement by pH, conductivity, or osmotic concentration in all cases. On the contrary, pH, while currently a significantly less accurate measurement metric, is nevertheless a physical property of fluids which in and of itself can have a very direct impact on product quality. A solution's osmotic concentration or conductivity also might have a direct effect on product yield and quality; however, this is not true in all cases. Indeed, given the fact that critical constituents of many buffers and nutrient media are anionic and therefore have no impact at all on conductivity, it could be easily argued that, in such cases, index of refraction would be a metric that is far more directly relevant to overall product quality.

Likewise, equimolar amounts of two very different solutes could yield identical osmotic concentration readings, and the disparity between the two solutes would thus be undetectable by osmotic concentration measurement. It is unlikely that those two different solutes, giving the same osmotic concentration reading, would give the same IoR reading. Thus, for many applications, measurement of index of refraction of process fluids might well be a substitute for other metrics, while for other applications it more appropriately might be an addition to measurement by other physical parameters. In many such cases, the additional metric might be a critical one to safeguard product quality and yield.

Additional applications of IoR as a process quality control metric remain to be investigated, but might hold significant potential. It is likely that index of refraction's superior sensitivity might make it potentially useful in measurement of concentration and/or quality of final product. Less likely might be the ability of index of refraction to detect impurities such as endotoxin. Outside the realm of pharmaceutical manufacturing, IoR is also likely to be a beneficial metric. Additional applications of IoR include related industries such as microchip manufacture, plastics, food processing,

brewing, winemaking, and cosmetics. But for all of these applications, IoR would offer the same thing—superior accuracy, precision, and linearity as compared with conductivity measurements.

About the Authors

Brent Schreiber is an engineer with nine years of manufacturing and process development experience in the biological engineering industry. Currently, Schreiber manages the downstream manufacturing technology team at the Syracuse, NY commercial and clinical manufacturing facility within the Bristol-Myers Squibb Global Manufacturing and Supply organization. In this role, he champions continuous innovation through lean manufacturing, best in class technology solutions, and economic combination of disposable and automated solutions. Schreiber's research focus is on collaborating with innovator companies to develop process analytical solutions to improve manufacturing process robustness and accelerate development and technical transfer.

Christopher Wacinski joined Jetalon Solutions in 2003 as an engineer. Wacinski has helped drive Jetalon's key growth initiatives leveraging his background in product development, testing, and quality assurance, playing a key role in setting strategic technology directions for the company. During his tenure at Jetalon Solutions, he oversaw the development of Jetalon's CR-288™ and ControlMP™ products and managed their transition to commercial manufacturing and product launch. Today, as the Applications Engineering Manager, Wacinski has been instrumental in working with Jetalon's world-wide clients to further develop applications which are mission critical to their business. Prior to Jetalon, Wacinski spent three years as an engineer in the biomedical field working for Navigant Biotechnologies, where he developed spectrometer-based irradiance measurement and calibration equipment. He received a B.S. in electrical engineering technology from Metropolitan State University of Denver, CO, USA.

Dr. Ron Chiarello is an entrepreneur and technology visionary with more than 20 years of experience in developing high technology products and bringing them to market. He co-founded and was Chief Technology Officer of Jetalon from 2002 to 2006 while creating Jetalon's vision to provide product innovations to the world's largest manufacturing companies. He led the invention and commercial deployment of Jetalon's sensor technologies, while continuing to drive innovation in

Jetalon's intellectual property portfolio. In 2006, he was pro- moted to President and Chief Technology Officer and since then has led Jetalon's operations and business development strategies. He has led the charge in this period to triple-digit percentage revenue and net profit increases for the company. Additionally, due to Dr. Chiarello's efforts, the company now serves hundreds of clients worldwide in not only micro-elec- tronics manufacturing, but biopharmaceutical, petrochemi- cal, and solar cell production. In 2009, he was promoted to CEO. Prior to Jetalon, Dr. Chiarello was technical advisor to many leading high technology companies and government agencies on product development, manufacturing improve- ments, and environmental concerns. Dr. Chiarello spent more than a decade as a researcher and program director at Stanford University and the University of Chicago and has published more than 50 technical articles. He is a NATO fellow and has won the Department of Energy Award for Excellence in Research, and the University of Chicago Pace Setter Award. Dr. Chiarello received a BS in physics from the University of California, Santa Barbara (1983) and MS and PhD in physics from Northeastern University (1990). He may be contacted by email: ronc@jetalon.com.

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