TECH BRIEF

Validation Of Inline Photometers For Improved Measurement Confidence

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Abstract

One of the primary concerns when using an inline process analyzer for real-time liquid or gas analysis is validating that the instrument is working to specification. Process photometers are often calibrated using liquid or gas calibration standards or directly to the process. These calibration methods can require considerable resources, so the preferred maintenance approach is often to validate performance as opposed to full recalibration of the instrument. The most typical validation method is verification of instantaneous reported instrument reading against process sample measured offline. This method is limited to only one sample concentration at a time and may not provide a true indication of how the instrument will perform under changing process conditions. This brief discusses the pros and cons of various photometer validation and verification methods and the impact photometer instrument design has on those methods.

Introduction

A photometer is an optical instrument designed to measure the absorption of light at a given wavelength after it passes through a fixed distance of sample. Different chemicals absorb light at different wavelengths, and through the application of Beer's law, the concentration of a chemical in solution can be accurately determined. Photometers are highly applicable to a wide range of applications in process analytical chemistry.

Filter photometers are instruments designed to operate at one or more discrete wavelengths e.g. 280, 300, 330, 400nm, while spectrophotometers allow monitoring of a range of wavelengths within the specifications of the instrument e.g. 400-800nm.

Spectrophotometers are commonly used in chemical laboratories as they provide flexibility when working with a wide range of different samples. They are precision instruments designed to be manually configured and operated by a trained technician in the relative comfort of a laboratory. Spectrophotometers measure individual samples that are prepared and typically placed in a standard size 12.5mm square glass cuvette before being inserted into the measurement path of the instrument. Laboratory spectrophotometers are, in nearly all cases, operated with an offline sample-based measurement methodology and feedback from measurements taken cannot be considered real-time.

Filter photometers are commonly used as an online, real-time process instrument. They are a simpler and therefore more cost-efficient instrument configured to continuously monitor a fixed process stream as part of an overall plant supervisory control system. The expectation is that they will operate unattended over long periods of time without operator intervention or the need for maintenance and servicing. Process photometers use specialized industrial in-line measurement cells or immersion probes which are designed to withstand harsh process conditions e.g., extreme pressures and temperatures, hazardous/explosive and radioactive environments.

The expectation of a filter photometer in a process is that it will continuously and in real time reproduce measurements that correlate directly to samples of that same process measured using a laboratory spectrophotometer.

Photometer Method And Design

A photometer requires a light source to generate light that is introduced to one side of a fixed path length occupied completely by the sample to be measured. The light passes through the sample where some is absorbed. The remaining light emerges at the opposite side where it is converted to an electrical signal using a suitable photodetector. In order to obtain a signal that is proportional to concentration, the light reaching the photodetector must be monochromatic (single wavelength), and Beer's law is applied as follows:

$$A = \log \frac{I_0}{I} = \varepsilon \, l \, c$$

where:

A	=	Absorbance (AU)
I	=	Photodiode signal with sample
I_0	=	Photodiode signal with no sample (zero)
ε	=	Molar absorption coefficient
l	=	Optical path length (cm)
c	=	Concentration of absorbing substance

Because process photometers continuously measure the same sample stream (ε) and use a fixed optical path length (l), Beer's law can be simplified to a simple linear equation as follows:

$$c = a \cdot A$$

where:

a	=	calibration constant

Laboratory spectrophotometers almost exclusively use broad band light sources such as halogen for visible (VIS, 400-700nm) and near-infrared (NIR, 700-2000nm) light and deuterium for ultra violet (UV, 200-400nm) light. Monochromatic light is obtained by splitting broad spectrum light into a spectrum using a diffraction grating or prism, and using either a single photodetector with mechanically moving grating or prism, or on more modern instruments, a diode array detector (hundreds of photodetectors arranged side by side on a monolithic component). Figure 1 contrasts the optical bench arrangement of these two methods. Single photodetector spectrophotometers are more sensitive than diode array spectrophotometers and can typically measure up to 5AU. However, they are slow in operation as they contain moving mechanical components. A diode array spectrophotometer has no moving parts and takes an instantaneous spectrum snapshot, however due to detector "pixel" size, sensitivity is typically limited to approximately 2AU.

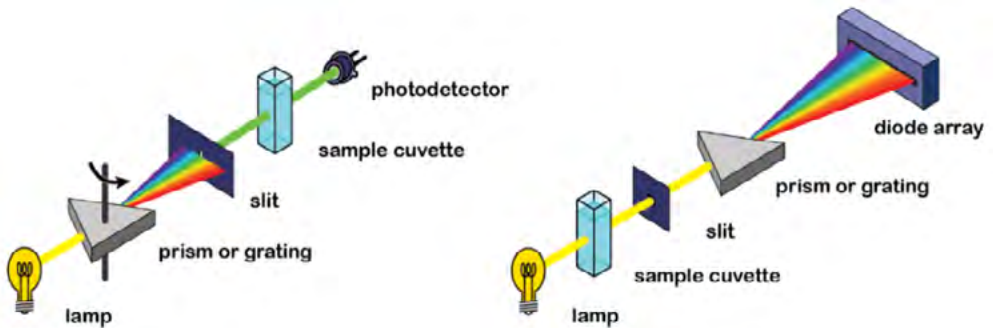
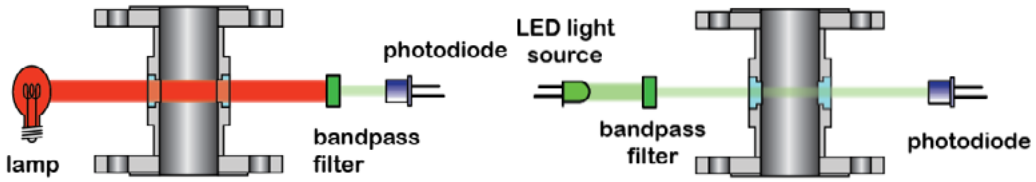


Figure 1.
LEFT: traditional mechanical spectrophotometer or variable wavelength photometer, RIGHT: diode array spectrophotometer

Traditional process filter photometers use narrow bandpass optical filters in conjunction with broad spectrum incandescent, halogen (VIS, NIR) and mercury vapor (UV) light sources to generate a facsimile of monochromatic light. Modern filter photometer designs utilize high performance specific peak wavelength light emitting diodes (LEDs) instead of broadband light sources. Figure 2 illustrates traditional and modern filter photometer optical bench designs; On the left is a traditional process photometer where broad spectrum light is passed through the sample followed by an optical bandpass filter to obtain monochromatic light at the detector; Figure 2 right depicts a modern design where the optical filter is placed before the sample with a specific wavelength LED light source. The primary difference in the two arrangements is the positioning of the optical filter; in traditional designs, the light source is hot and a filter in close proximity to it is subject to potential damage over a relatively short period of time whereas in the modern design the LED source is cold and non-damaging to the filter. A further advantage of the modern design is that the process itself is no longer exposed to broadband light outside of the wavelength of interest that can be potentially damaging to the process itself. This is particularly true for applications such as protein detection in bioprocessing that utilize UV light for the measurement.

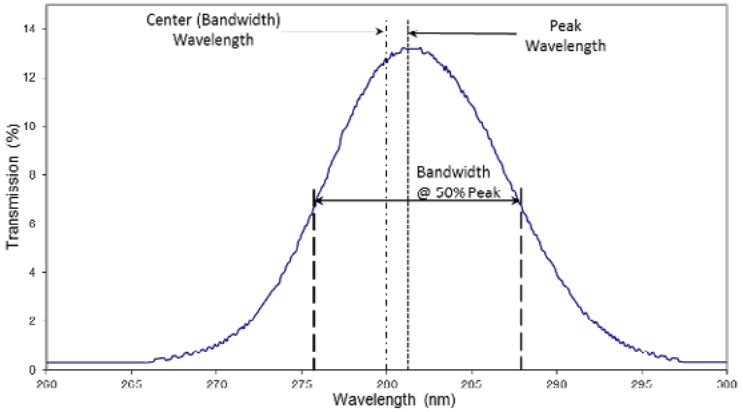
Figure 2.
LEFT: traditional lamp type industrial photometer. RIGHT: modern low power led type industrial photometer



A problem with process filter photometers is the issue of stray light. It is the effects of stray light that often lead to dissatisfaction with photometer performance when deployed into a process environment. Narrow bandpass filters are constructed to allow light to pass through a very small region, typically 10nm wide, around a specific peak wavelength (the wavelength of interest), while blocking light from passing at all other wavelengths. Typical bandpass filters only block to approximately 3 AU (0.1% of the total transmitted light) outside of the pass band and all remaining light below this level passes freely through the optical filter. A typical band pass filter specification is shown in Figure 3.

Process filter photometers that utilize LED light sources combined with narrow bandpass filters operate with virtually no stray light outside of the pass band region around the wavelength of interest. Due to this, LED light source photometers adhere much more closely to Beer's law and provide native units of absorption as their baseline measurement without the need for additional absorption calibration. This means that any engineering unit can be correlated to absorbance while leaving a possibility for confident validation of the instrument's performance.

Figure 3.
Typical band pass filter specification



Example Filter Specification

Peak Transmission: 280nm +/-2nm, >13% transmission

Center (Bandwidth) Wavelength: to be at peak transmission +/- 2nm

Bandwidth: 10nm +/-2nm at 50% of peak

Blocking: 5 OD average, >3OD from peak transmission, at 300nm point

Where broad spectrum light sources are used, the amount of stray light passing through the filter can be significant and therefore the light reaching the detector is not monochromatic. Due to this, such photometers do not adhere to the essentially linear nature of Beer's law and photodiode signals must be calibrated in order for the instrument to work in units of absorption, noting that absorption is proportional to concentration. This requirement for signal correction presents some specific challenges when validating instrument performance.

Figure 4 highlights the significant difference between a typical broad spectrum phosphor coated mercury lamp and a narrow band LED light source.

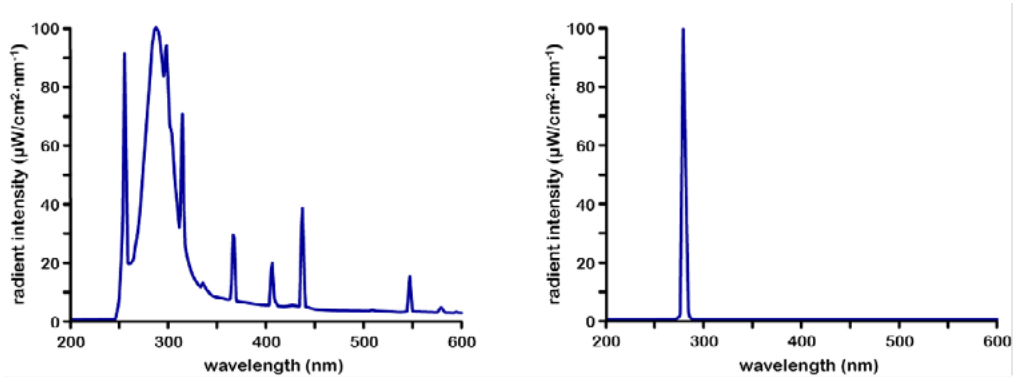


Figure 4.
LEFT: phosphor coated mercury lamp, RIGHT: 280nm uv led light source

In general terms, spectrophotometers and LED filter photometers display absorption units as native units. Traditional filter photometers, due to stray light issues caused by broad spectrum light sources, must be calibrated to read in absorbance units.

In the field, a process photometer is commonly calibrated directly towards a sample or reference color standard using a fixed wavelength and optical path length (OPL) in the desired concentration or engineering unit of the calibration sample e.g. ppm, g/L, Saybolt color units.

The selection of measurement wavelength and OPL depends upon the substance being measured and the desired concentration range of the measurement. Concentrated samples are normally diluted in a laboratory to ensure absorption is within the operating performance of the instrument when a 1cm OPL cuvette is typically used (although shorter OPL cuvettes are available from some manufacturers). Sample dilution is not possible on process photometers working in-line. For cases where the concentration of the measured fluid is high, industrial inline measurement cells with a shorter OPL can be used. Further strategies of selecting an "off-peak" wavelength as shown in Figure 5 can be employed when the process fluid is highly absorbing.

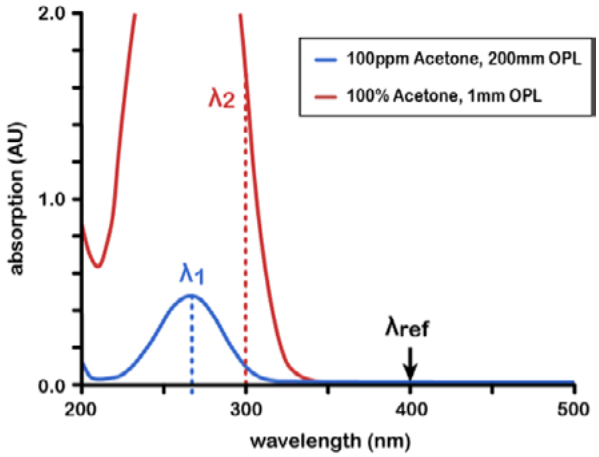


Figure 5.
Absorption of acetone in water. For 100ppm acetone, a 200mm opl measurement cell and measurement wavelength (λ1) of ca. 265nm is recommended. For 100% acetone, a 1mm opl measurement cell and measurement wavelength (λ2) of 300nm is suitable. For both samples, a reference measurement wavelength (λref) of 400nm is suitable as there is no influence from acetone at this wavelength.

Industrial photometers can also use a second non-absorbing reference wavelength to compensate for baseline shifts due to turbidity, air bubbles or optical window fouling and therefore ensure a continuous measurement of high reliability.

For the majority of applications where absorbance characteristics obey Beer's law, a simple zero plus one sample point will be sufficient to calibrate an online photometer to read in the desired concentration or engineering units. It should be noted, however, that Beer's law does not describe the behavior of concentrated solutions (greater than 10-2 M) due to interactions between the absorbing molecules, likewise when measurement is made at a wavelength where the slope of the sample absorption curve is very steep. In such cases a non-linear calibration should be used. Industrial photometers typically include non-linear and piece-wise linear calibration models.

Validation

One of the primary concerns of an operator is to be sure an instrument is working to specification and therefore providing a correct measurement to an overall control scheme. The ideal way to validate a process photometer is to vary the process concentration itself between certain maxima and minima, taking samples at cardinal points and comparing the process photometer reading to laboratory data. However, this may not be practical for a continuously running production system. An alternative method is to remove the in-line process photometer measurement cell from the process line and introduce a series of verified samples or standard solutions in a controlled manner to confirm agreement. However, this method is not always desirable where the process line must be shut down, where line fluids are potentially hazardous to health or it creates disposal and other challenges. Both of these methods require a considerable labor overhead.

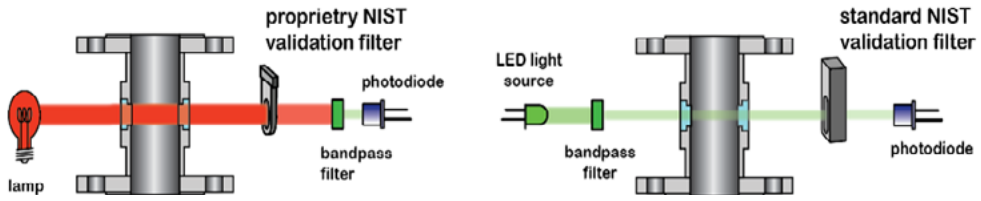


Figure 6.
LEFT: traditional hot lamp type industrial photometer with proprietry reference filter supplied by manufacturer. RIGHT: modern low power led cold light industrial photometer using stanard 12.5 Mm certified filter cuvette

A process photometer can be validated using standard non-intrusive traceable reference standards that will verify photometric accuracy and linearity, both of which are critical to measurement result quality. Figure 6 illustrates traditional and modern filter photometer optical bench designs comprising support for validation filters which are placed in the optical light path independent of the process line.

As process photometers can be verified without the need to interfere with the process line, confidence as a result from regular trouble-free validation is assured while saving valuable time and resources. Internationally recognized quality systems such as GLP, ISO9000, ISO/IEC Standard 17025 and US Pharmacopeia chapter <857> require that systems are certified using traceable reference materials. The US National Institute of Standards and Technology (NIST) and the United Kingdom National Physical Laboratory (NPL) have developed a number of convenient traceable reference standard materials for verifying the accuracy of the absorbance (or transmittance) and wavelength of a photometer. These materials are certified for absorbance at a number of wavelengths in the ultraviolet (UV), visible (VIS) and near infra-red (NIR) spectral regions, using national reference spectrophotometers built and maintained by NIST and NPL.

Validation Of Wavelength And Blocking

Bandpass filters essentially define the operating wavelength and performance of a process photometer so it is important to ensure they meet or exceed the original specification from the manufacturer. Bandpass filters, while generally stable, can exhibit signs of decay over time with changes in peak transmission and stray light blocking capability caused by environmental factors e.g., moisture or erosion from the light source itself. Figure 7 shows an example of the type of damage that can occur to a bandpass filter over time when installed in close proximity to a hot mercury vapor gas discharge light source.

Figure 7.
Bandpass filter damage. New filter is shown on the left, eroded optical filter due to prolonged exposure to a hot mercury type uv lamp on the right.

Validation of the wavelength accuracy of a spectrophotometer is undertaken using a liquid sample containing a number of distinct peaks such as a solution of holmium perchlorate or a holmium oxide and/or didymium doped glass filter. If the spectrophotometer is equipped with a deuterium or mercury lamp then the emission of the lamp itself can be measured due to distinct emission lines that will not change with time.

For example, a deuterium lamp has a distinct emission at 656.1nm and a weaker emission at 486.0nm. Mercury vapor lamps have a number of principal emission lines at 253.7nm, 313.25nm, 365.48nm, 404.66nm, 435.83nm and 547.07nm (all clearly visible on Figure 4) which can be used for wavelength validation. A number of spectrophotometers use such emission peaks to validate wavelength accuracy (even automatically calibrate themselves) and generate validation reports.

Liquid photometer wavelength reference standards include a solution of holmium oxide in perchloric acid (holmium perchlorate), didymium in perchloric acid (a mixture of praseodymium and neodymium) which is recommended by the U.S. Pharmacopoeia (USP 24), samarium perchlorate and rare earth sulphate which all have distinct wavelength peaks in different ranges of operation. Liquid reference materials are normally permanently sealed into standard 12.5mm UV quartz cuvettes to prevent contamination. The most common glass type reference filter used for wavelength validation is holmium oxide as this filter provides a range of distinct absorption peaks in the visible region. Solid glass type validation filters provide a convenient and safe method to quickly validate wavelength accuracy.

It is not possible to validate wavelength accuracy of a filter photometer using wavelength calibration standards because most filter photometers operate at one or two fixed wavelengths and are therefore not able to resolve the distinct peaks provided by the wavelength validation standard.

For filter photometer designs that utilize fiber optic connections to the inline measurement cell, wavelength accuracy can be validated using a portable fiber optic type spectrophotometer. As shown in Figure 8, a fiber optic based industrial photometer is connected directly to a portable spectrophotometer using standard fiber optic connectors, making the task of wavelength validation straightforward. The portable fiber optic-based spectrophotometer is validated using nationally recognized and certified wavelength standards and used as a transfer standard. Stray light issues, should they exist, can also be assessed. The entire fiber optic circuit can be checked and tested in this way and a high degree of confidence obtained on the measurement circuit integrity.

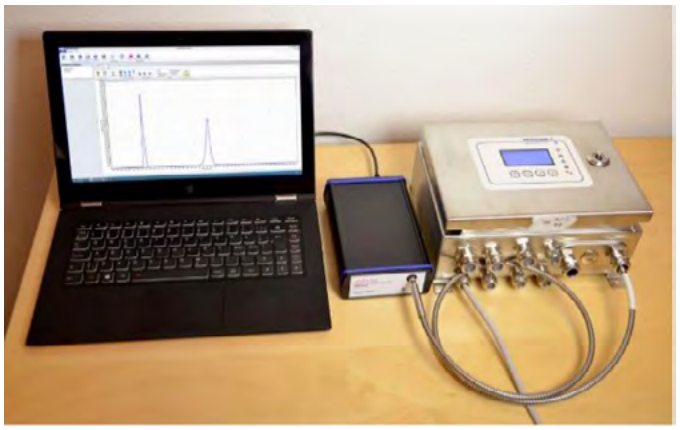


Figure 8.
Wavelength validation of an industrial photometer (right) using a portable usb based fiber optic spectrophotometer (middle).

Lamp based filter photometers typically have their photodetectors and filters installed in housings directly on the measurement flow cell so it is not possible to verify wavelength accuracy in the field. In this case, the band pass filters must be removed from the assembly and scanned in a traceable spectrophotometer to determine whether their passband characteristics are within specification and whether there is any stray light in the out-of-band areas. Figure 9 shows two scans of a bandpass filter. The first measures the actual dimensions of the passband itself. The peak wavelength and bandwidth at 50% of peak transmission can be measured and checked against specifications. The second scan is a wider scan that looks for potential stray light breakthrough points in the filter. Any filter measured out of specification is an indicator that a lower degree of confidence in the performance of the photometer is warranted and the bandpass filter should be replaced.

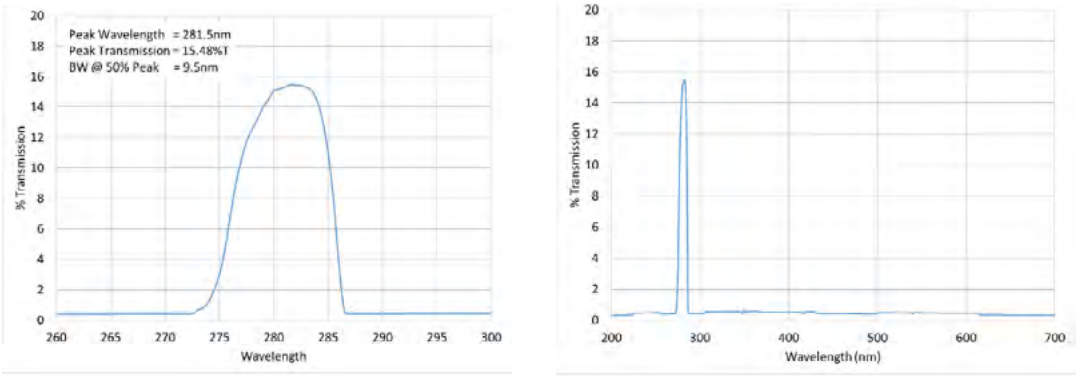


Figure 9.
LEFT: wavelength validation of 280nm bandpass filter using a laboratory spectrophotometer
RIGHT: stray light search in out-of-band areas

Validation Of Photometric (Absorbance) Performance

There are a number of methods for validating photometric performance:

- Process samples
- Certified optical filters
- Certified standards

Process samples

This is probably the most typical validation method employed in the field. A sample of the process stream is taken along with a simultaneous reading of the photometer output on the instrument display. The process sample is analyzed offline and the photometer reading is compared to the result of the analysis.

If the readings are in accord, the instrument is deemed to be operating within established acceptance criteria and no further action is required. If there is a deviation, the action often taken is to adjust the zero offset of the photometer to bring the readings into alignment. This method is, however, limited to only one sample concentration and may not provide a true indication of how the instrument will perform under changing process conditions. To fully validate the performance of the photometer, the process itself should be adjusted so the concentration of the sample stream changes enough to provide a new sample point(s). In most cases, this is neither possible nor desirable in a production environment, making validation a waiting game – taking advantage of a process upset to produce additional validation points to determine the performance of the instrument.

Certified optical filters

NIST have specified a range of robust glass type validation filters that are suitable for use with both laboratory spectrophotometers and field process photometers that use a standard 12.5mm type square cuvette filter holder. A NIST traceable reference material is defined as "a reference material produced by a commercial supplier with a well-defined traceability to the National Institute of Standards and Technology (NIST). The traceability is established via criteria and protocols defined by NIST" [1]. The glass type validation filters specified by NIST exhibit long term stability and are simple and convenient to use. A set of certified glass type photometric validation filters is shown in Figure 10.

Figure 10. Nist traceable metal on fused silica glass absorption filter set for 12.5mm standard cuvette holder.

NIST specify the use of two different types of glass reference filter, namely neutral density [2] and metal on fused silica glass filters [3]. Neutral density type reference filters are made from a type of glass which blocks a known percentage of the light passing through it. The amount of absorption at a given wavelength is determined by the type and thickness of the filter glass. Metal on fused silica glass filters differ in that a layer of reflecting metal is deposited on the glass surface of the filter, reflecting rather than absorbing a percentage of the total light passing through the filter.

A benefit of using metal on fused silica glass filters is that these filters exhibit a relatively flat transmission profile over a wide wavelength range of 250nm - 2200nm, compared to the neutral density type filters that are limited to 360 – 1100nm. As metal on fused silica glass filters reflect light, it is however, important to confirm these filters are suitable for use with the instrument under test and that the positioning of these filters in the sample holder is consistent. It is recommended to validate an instrument using a set of a least three certified glass type validation filters plus a blank for measurement of background absorption. Typical certified filter nominal absorption values include a blank, a filter in the range 0.01-0.30 AU (low), 0.50 AU (medium) and 1.0 AU (high) however higher filter values up to 2.0 AU are recommended for most filter photometers. The range of the validation should also represent the range of the instrument operation. It is a further recommendation by NIST that traceable filters should be recertified on a two-year cycle.

Certified filters are used to validate that a filter photometer is working to its absorbance specification. Inserting a certified filter into the light path of a filter photometer should cause the instrument to read the absorbance of that filter at the configured wavelength of the photometer within the specified uncertainty of the instrument.

In the case of industrial photometers with broad band light sources, proprietary filters supplied by the instrument manufacturer are often used to calibrate the instrument to correct for deviations from Beer's law rather than validate the instrument. When calibrating a photometer using such filters, great care must be taken as any correlation of engineering units to absorbance may no longer be valid after such adjustments, especially if a single point linear or piece-wise linear curve-fit method (essentially joining calibration points with straight lines) is employed.

Where possible, traceable filter standards such as those in a 12.5mm cuvette form factor that can be used to validate both offline devices such as laboratory spectrophotometers and online devices such as photometers should be employed. This greatly simplifies the expectation that offline and online measurements should be essentially similar and thus improve confidence in the online measurement. A typical implementation of standard 12.5mm square certified filters for validation of a process photometer is shown in Figure 11. The non-intrusive cuvette filter holder allows a traceable validation filter to be placed directly in light path without interruption of the process line.

Figure 11.
¾" Sanitary tri-clamp type in-line measurement cell with validation accessory using certified filters in a standard 12.5mm size cuvette format

Validation becomes even more important in systems where disposable measurement sample cells are used as the equipment is constantly being replaced/renewed. In biotechnology separation in particular, use of single use systems is becoming increasingly popular, so it is necessary to connect to sample cells preassembled into readymade tubing sets. More innovative systems include cell "docks" to insert these replaceable items rather than risking the connection electrical or fiber optic cables to them. To validate such a system, all that is required is to insert a test fixture into the cell "dock" that accepts standard 12.5mm cuvettes. Figure 12 shows a typical single use measurement cell "dock" with validation fixture inserted.

Figure 12.
Single use cell "dock" with validation fixture using certified filters in a standard 12.5mm size cuvette format

Certified liquid standards

Liquid standards are commercially available to calibrate and validate a wide variety of optical instrument measurement units. Typical examples include ASTM color (ASTM D 1500), Saybolt color (ASTM D 156), APHA/Hazen/Platinum cobalt color (ASTM D 1209 and ISO 6271-1:2004), Gardner color (ASTM D 1544) and Rosin color (ASTM D 509). There are also a wide range of certified standards for gas measurement instrument calibration and validation.

For chemical concentration or absorption measurements, standards can be prepared in a laboratory and used with online photometer instruments. For example, a commonly used liquid photometer absorption reference standard is potassium dichromate because it has distinct peaks in the UV and VIS spectrum. Instruments measuring alcohol content might use a series of different alcohol/water concentration samples. Whatever the standard used, the absorption of a set of different liquid concentrations are measured and then applied to the instrument to check for linearity and absorption accuracy.

Liquid standards can be introduced into a photometer light path in one of two ways:

1) Directly into the sample cell

2) Using standard cuvettes or proprietary insertable liquid holding devices

Some process photometers are installed in a side stream/fast loop sample line where they can be isolated and standard fluids introduced into the sample cell for validation purposes. Aside from the problems of hazardous material handling in some instances, flushing the cell between one sample and the next is key to obtaining good results. The practice of flushing with a zero solution between standards should be avoided as the potential of diluting effects of a zero solution to any one standard solution are greater that the effects of one standard solution to the next. This method may require the consumption of a significant volume of standard which can be costly as standard solutions should always be discarded once used and not reused.

An alternative to inline side stream validation is to remove the flowcell from the line, blank one end and introduce standard fluids directly into the optical flow cell. This is a perfectly valid method of validating a photometer, notwithstanding any issue surrounding materials handling. Care should be taken to ensure that ambient light is prevented from entering the cell while measurements are being taken and that the fluid completely fills the cell and is free of bubbles (and “microbubbles”), particularly on the surfaces on and around the optical windows.

For process photometer measurement cells fitted with validation filter holders, standard fluids can be used to validate the instrument without the need to remove the cell from the process line. It is important to take into consideration the optical path length of the measurement cell compared to the optical path length of the cell used for the liquid validation sample. For example, if the optical path length of the measurement cell is 10cm and the liquid sample cuvette is 1cm, then the reading of standard in the 1cm cuvette will be 10x

Figure 13.
Industrial 2” ansi flange wafer type in-line measurement cell with validation accessory using 12.5mm liquid sample cuvettes.

lower than if measured by the longer path length of the measurement cell. In accordance with Beer's law, it is also possible to use a 10x more concentrated sample in the 1cm cuvette to provide the same reading as a 10cm path length. Figure 9 shows how liquid filled cuvettes can be used for validation purposes.

Optical Pathlength And Error Assessment

Industrial process measurement cells are typically assemblies of robust metal bodies e.g. stainless steel, nickel alloy, titanium etc., scratch resistant optical windows and elastomer seals. All the mechanical parts have general tolerances related to the limitations of the CNC lathes and milling machines used to manufacture them. Normal engineering tolerances for such operations are typically ± 0.1 – 0.05 mm (± 0.004 – 0.002 in).

When using short path length measurement cells, the error due to manufacturing tolerances can be significant and must be taken into consideration. If the measurement cell is calibrated using a liquid calibration solution directly in the measurement cell, then any inaccuracies in the optical path length will be automatically taken into consideration and compensated for in accordance with Beer's law.

Certified validation filters only validate the performance of the photometer and cannot be used to compensate for inaccuracies in the measurement path length. For that reason, it is always recommended to validate fixed measurement cells using liquid solutions. If necessary, an optical path length correction factor should be applied to the process photometer. As the optical path length is fixed and will never change, this correction factor need only be measured once and will be unique to the specific measurement cell.

For single use/disposable measurement cells, assessment of measurement uncertainty should be made using the manufacturers optical path length tolerance as a guide.

Conclusions

Filter type photometers provide valuable real time measurements into an overall control scheme and assist in reducing production costs by improving product consistency and quality. Current proprietary methods of validating performance in the field may not necessarily guarantee that the instrument is performing to specification.

An understanding of the stray light issues surrounding filter-based photometers can greatly improve the approach to field validation and can enhance measurement accuracy. The ability to use photometric standards certified by third parties to nationally maintained standards for measurement and performance validation allows a transparent correlation between offline and online measurements and therefore provides a greater confidence in the results provided by inline photometers.

Validation of a photometer is necessary at more than one measurement point. Bandpass filters used to generate a single (monochromatic) wavelength can decay over time, particularly in the presence of high power, hot light sources. This can create non-linearity of measurement at higher concentrations that can lead to product quality problems. Using multiple validation points can help describe non-linearity exhibited by a photometer and allow action to be taken to correct it.

The use of filters and absorbance standards to validate photometers removes the need to take photometers offline and handle potentially hazardous materials as test samples. Provided stray light impact is understood and any error in optical path length is assessed, highly reliable validation measurements can be obtained with a resulting high confidence in the measurement results of the instrument itself.

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