OBSERVATIONS OF AN OPERATOR IN THE LAB – IMPROVING LABORATORY RESULTS

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ABSTRACT

The expectation of an Operator in the water industry sometimes requires them to be an expert in Mechanical, Electrical and Microbiological processes. Operators also need to be proficient in laboratory analysis and have skills which ensure they meet daily EPA discharge licence agreements from their waste water treatment plants or meet Australian Drinking Water Guidelines with the water produced from their drinking water treatments plants.

As we know, very few operators in the industry have backgrounds in Chemistry or Microbiology and have very little experience in executing Good Laboratory Practices (GLP). My 10 years experience in the industry and 10 years prior experience in a NATA Certified lab, has led me to being able to identify common, re-occurring errors in an Operators laboratory techniques, particularly when performing analysis of critical parameters using a Photometer or Spectrophotometer. I write to share these experiences and identify ways of improving general Operator laboratory analysis techniques, and their results.

1.0 INTRODUCTION

Operators of waste water treatment plants, in most cases, analyse daily, both influent and more importantly, the effluent from their plant to ensure firstly, that they are producing effluent in accordance with their EPA discharge licence agreement and secondly, so they can make immediate process changes in their plant to correct any out-lying results. Critical parameters that are analysed in waste water plants include all nitrogen compounds (Nitrates, Nitrites, Ammonium and Total Nitrogen), phosphorus compounds (including organically bound Phosphorus – Total P) and reactive Ortho-phosphates and COD (Chemical Oxygen Demand)

Similarly, drinking water treatment plants need to monitor raw and finished water for critical parameters such as Manganese and Iron which are found in many raw water sources in Australia. These can affect water treatment processes and finished water quality. Other parameters that are daily analysed for, are free and total chlorine and in some cases mono-chloroamines (all added as disinfection processes), fluoride (if fluoridation dosing is utilised) and Aluminium (present naturally in the raw water or to ensure minimal Aluminium floc break-through during the treatment process).

All of the above parameters monitored in both drinking water and waste water treatment plants are generally tested by an Operator using photometric or spectrophotometric analysis techniques. I have observed many *common errors* in analysis which seem to be repeated in the Water Industry across the country.

2.0 DISCUSSION

2.1 Theory Of Spectrophotometric/Photometric Analysis

Spectrophotometric analysis utilises the *absorbance* of a light source (generally a visible light source) at a specific wavelength by a specific chemical complex.

The chemical complex is normally a bounded/bonded compound that contains the target analyte.

For example, in the analysis of reactive ortho-phosphate (PO4 – the target analyte), ortho phosphate ions react with molybdate ions, in an acidic medium, to form molybdophosphoric acid. Ascorbic acid reduces this complex to phosphomolybdenum blue (PMB). The concentration of PMB and in turn the concentration of ortho -phosphate is then determined photometrically at a wavelength of 690 nm.

The relationship between the concentration of ortho–phosphate in the sample being analysed (or the complex formed –PMB) is linear over a certain range, so therefore, the Absorbance measured has a linear relationship with the concentration of ortho- phosphate. Spectrophotometric or Photometric Analysis follows the Beer-Lamberts Law:

$$A(\lambda) = \varepsilon(\lambda) \cdot b \cdot c$$

Where:
A = Absorbance at a specific wavelength (λ)
ε = co-efficient of absorptivity at specific wavelength **(λ)**
b = path length of light passing through sample (in cm)
c = Concentration of the Analyte

Commercially produced test kits that are used by the Operators in conjunction with their photometer or spectrophotometer are designed for an Operator with a non chemical or analytical background to produce accurate and reproducible results for the critical parameters mentioned. All test kits specific to the parameter of interest are supplied with all reagents, dosing tools and measurement cells in exact quantities so the intended formation of complexes is achieved and reactions go to completion.

Most commercially produced photometers used in the Water Industry are pre-programmed to be used with the manufacturers consumable test kits. The instruments thus, have Beer – Lamberts equations pre–programmed into the instrument, specific to each individual test. The Operator does not need to perform their own calibration data, zero points (in some cases), nor programming on the instrument, which is the case with a traditional open Spectrophotometers.

2.2 Observation No. 1 – Poor Quality Analytical Water Source

In the typical laboratory, an Operator will require a quality de-ionised or de-mineralised water source for two reasons. The first is to produce standard solutions (for quality assurance purposes) or make dilutions to samples to bring them into the measuring range of the test kit. If a poor quality analytical water source is used, there is the real potential to produce false high results on standards and diluted samples, by adding concentrations of the target analyte.

The second reason is that many photometers in use today, require the Operator to produce a reagent blank, prior to the analysis of the sample. This is a process common with the measurement of critical parameters in both drinking water and waste water.

A reagent blank, is required to set the zero point (**ε -zero)** on the calibration curve for a particular test kit (Zero concentration = Zero absorption). A reagent blank should be prepared using analytical grade water with known, low trace levels of impurities.

If a water source contains high levels of the parameter you are about to test, then the result is, that you will obtain a false LOW reading. This occurs due to the fact that you have zeroed your instrument with a "reagent blank" that had a level of the target analyte present in the first instance.

Many Water Authorities and Shire Councils use commercially available "de-ionised or distilled water" sourced from a supermarket or petrol station. These cheap DI water sources are accompanied with NO specifications or guarantees as to their quality. If using these un-specified waters for "reagent blanks", ask yourself what the levels of Al, Cl, Mn Fe or F that are present. Commonly found in these un-specified, cheap water sources (particularly distilled water) are significant levels of chloro-amines. If you are operating the lab of a waste water plant ask yourself what is the level of COD. Un-specified analytical water sources, particularly in HDPE containers can have COD levels of 20ppm or more. This common error in lab analysis is amplified if the Operator is measuring relatively low concentrations in the sample, at the lower end of the measuring range of the Test kit ("Lower Detection Limit" of the test kits).

An example of one of the worst cases I have observed in the industry, which produced false low results, occurred when preparing a reagent blank for Manganese prior to analysis of the final filtered water. Their instrument was zeroed using a water source generated from the wet rack inside their lab (adjacent to the plant). The final filtered water (produced in the drinking water plant of a Regional Water Authority) was passed through a single, 10 micron paper cartridge filter (which had not been changed for some years). It was discovered that the water from this filter was identical to the water that they were testing. Therefore, they always had fantastic low results for Manganese, because they were in fact, zeroing the concentration of Mn out, in the analysis. But in reality, results for Mn were alarmingly high.

Even if a new 10 micron filter was used on their analytical water system, it still would not have been an adequate system to remove any level of Manganese.

2.3 Observation No.2 – Incorrect Deliveries Of Sample Volumes Or Reagent Volumes In Photometric Analysis

When performing Photometric analysis of critical parameters it is essential that correct volumes of reagents and correct volumes of sample are used; according to the correct analytical procedure of that particular test. Relatively small sample and reagent volumes are required in photometric test procedures (often as little as 0.1 ml or 100ul is required to be delivered accurately).

To deliver a specified volume of sample or reagent, most Operators use one of two types of pipettes – positive displacement pipettes and air displacement pipettes (old school Operators may use glass pipettes and pipette bulbs).

The incorrect use of these pipettes is common place across the entire industry and is the biggest reason for both systematic errors in results (due to incorrect delivery of volumes) and cross contamination of sample and reagents, resulting in random errors in the photometric determination of these critical parameters.

All air displacement pipettes have single use, non-re-usable tips, yet Operators may use the same tip for months or years, which potentially could (and has been proven to) compromise the accuracy of their lab results.

Re-usable tips are worth approx $30 per pack of 1000 and so the simple solution is to replace tips on use.

Another very common error when air-displacement pipettes are used is that most brands have a dual stage action. To draw up a volume of sample you ONLY use the first stage of the action and to deliver the volume to your Cell or test tube, you use the first and second stage of the pipette action to deliver the correct volume.

The simplest way to check your action and accuracy of your pipetting (and also to calibrate your pipettes) is to simply utilise a set of analytical scales in the laboratory, and weigh a volume of DI water (at 20°C). 1.00 ml of DI water at 20°C weighs approx 1.00 gram. What volume are you delivering? I have often checked Operators pipettes on their own set of scales and volumes have been out by up to 20%. This in turn explains why their lab results are 20% different to their external NATA certified testing results.

2.4 Observation No.3 – Incorrect Filtering Or Pre-Treatment Of Samples Prior To Photometric Analysis

This has been observed, not so much as an error in execution of the filtering process, but the incorrect filtering sequence, the absence of filtering or the filtering of samples where it should not be performed prior to photometric analysis.

Filtering samples is generally performed using a syringe and syringe filters that are approximately 30mm in diameter with a pore size of 0.45um. The larger the diameter of the filter, the easier it is to push through influent waste water samples.

It is a fact that if the measurement solution (reagents plus sample) is turbid or cloudy at the time of measurement in a photometer (in most cases) a false reading (generally a false high reading) will be recorded.

Without entering into the chemistry of each individual test procedure to explain why, we can apply a few simple rules when it comes to filtering samples prior to analysis.

WHAT NOT TO FILTER:

All waste water samples to be analysed for TOTAL phosphorus, TOTAL nitrogen or COD (Chemical Oxygen Demand) undergo a digestion phase at elevated temperature and pressure (120° C or 148°C) to break down organically bound target analytes. The Nitrogen, Phosphorus and Carbon compounds are chemically bound in the organic matter in a waste water treatment plant sample. Generally, the sample, prior to digestion is turbid (this is fine) but after complete digestion has occurred the measurement solution should be clear. If side reactions have occurred (in rare examples) and the sample is still turbid after digestion, then filtering maybe applicable. But never filter prior to digestion when analysing these parameters or else FALSE low results will be recorded, because you will remove most organically bound target analytes.

Additionally, if you are aiming to analyse for Total Aluminium in your final filtered drinking water which requires an acid digestion step, then definitely – do not filter sample prior to digestion.

WHAT TO FILTER:

When analysing soluble anions or cations in drinking water, for example Aluminium, Manganese, Iron, Chlorine or Fluoride (Al, Mn, Fe, Cl, F) or a waste water parameter like Ammonium/Ammonia, Nitrate, Nitrite or reactive Ortho-Phosphate (NH4/NH3, NO3, NO2, PO4 etc.), then as a rule of thumb, prior to any analysis steps, filtering of sample is recommended through a 0.45 um syringe filter. It is particularly important if your sample is turbid to the naked eye.

If unsure whether or not to filter soluble target analytes, then analyse a filtered sample and an unfiltered sample and if a significant difference is noticed (and the unfiltered sample is significantly higher due to turbidity of the final measurement solution) then implement a filtering Standard Operationally Procedure (SOP) for this lab process.

2.5 Observation No.4 – Calibration And Maintenance Of The Photometer (Or Lack Of)

As we have already identified, a photometric and spectrophotometric measurement is a precise optical measurement of light, of a specific wavelength. These instruments should not be left for years without calibration checks or basic maintenance.

Many newer photometers on the market have inbuilt diagnostic features which are initiated on start up, but many instruments will still require a regular baseline zeroing of the light source through the optical pathway (in an optically perfect cell), recommended at least every 3 months, which corrects for any changes to the internal optics and light intensity across the entire wavelength range that the instrument operates at. This process is completely different to measuring a "Reagent Blank", as described earlier. A "Reagent Blank" is specific to setting the zero point on the calibration curve of a particular test parameter.

All laboratories, no matter how little testing they may be performing on their photometer, should implement some level of a quality assurance program that gives confidence in their measurement processes. The simplest form of quality assurance is to measure a known standard solution of the parameters that they test; treating the known standard in the identical way they would measure a sample. NIST Traceable certified standards, where no dilution is necessary, are now commercially available in concentrations suitable for direct use with most test kits of the critical parameters mentioned. If any of the results from measuring standards are outside accepted tolerances, further investigation as to the reasoning should be initiated.

To identify if the measurement and chemical reaction of a particular parameter is being interfered with by another substances in the sample, a matrix check or standard addition should be performed on all critical parameters, on a routine basis.

A matrix check is simply executed by measuring your sample, as per the normal procedure of the test kit and at the same time measure the sample, plus the addition of a known concentration of the target analyte. For example, if the result for PO4 on your sample was 4.0 mg/l and you made a standard addition of 1.0 mg/l of PO4 standard to the sample, then the sample plus addition, should read exactly 5.0 mg/l. If the increase in concentration is significantly less than 5.0 mg/l (in your Sample + STD) then potentially you have an interfering substance affecting your analysis. Further steps and sample pre-treatment should then be investigated. Serial dilutions of sample prior to measurement is an effective way of removing interferences.

If any of the system or instrument checks fails, then it is always recommended to have the instruments serviced by your local service agent or manufacturer immediately and routinely cleaned and serviced on an annual basis.

3.0 CONCLUSIONS

By implementing relatively simple quality assurance checks, paying attention to analytical water sources used in the analysis, ensuring your pipetting techniques and volumes delivered are accurate then an Operator will be well on the way to being proficient and confident in the laboratory analysis performed daily.

Take the time to read the detailed packaging inserts of each individual test kit and the detailed manuals of the instrument that you use for measurement of critical parameters. There is every reason that your results will match identically to your NATA externally analysed results and process changes can be made with confidence.

4.0 ACKNOWLEDGEMENTS

To the Operators in the water industry as I have learnt more from them, than they have learnt from me.