pH MEASUREMENTS AND GLP

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ABSTRACT

Good laboratory practice mean among other things, specifically for pH measurements, that measurements are done correctly and to have evidence that this has been done correctly. The several components of the pH System has to be considered. The pH meter has to provide raw data (mV) as well as the sample results. This is specially important during the calibration. The choice of the electrode is important as well as the sample type is critical. Once proved and validated the correct electrode and its correct filling solution the refilling procedure should be a part of the correct SOP (Standard Operating Procedures). Calibration points and frequency together with maintenance procedures also need to be included in the SOP's. Calibration standards must be traceable to international Standards, and therefore identifiable and dated. The various factors to affect the pH need to be understood. In particular the temperature has different effects which need to be worked out on the proper way to arrive at a reproducible and accurate result. In particular the patented Ross technology compensates several of the temperature effects, using an innovative galvanic pair, which is proven to have a faster answer to temperature changes and is more precise on the readings. Finally, recording and submitting data in proper records is very important in GLP environments as adherence to this procedures enhance the relevance of documentation.

Key Words: GLP, pH measurements, Ross electrodes, temperature effect.

INTRODUCTION

The pH measurement is the most common laboratory procedure because many chemical processes are dependent on this parameter. Many chemical reactions can be altered significantly in its speed by changing the pH of the solution. In our modern life, virtually everything has been tested for pH: from the water to the food and medicines we have. The Good laboratory practice procedures, are related to the organisational processes and the conditions under which laboratory studies are planned, performed, monitored, recorded and reported.
The measurement of the pH is one parameter that is subject to this regulatory environment. Some of the issues affecting this measurement will be discussed.

Background and History of GLP.

In the early 1970's the Food and Drug Administration (FDA) of the USA identified significant problems with the way in which safety non clinical studies were performed. A series of experiences has led to the GLP (GOOD LABORATORY PRACTICE). Adherence of the Laboratories to the principles of the GLP ensures the proper planning of the studies and the provision of the adequate means to carry them out. It facilitates the proper conduct of studies, promotes their full and accurate reporting and provide means whereby the studies can be verified. The application of GLP studies

assures the quality and the integrity of the Data generated and allows its use by regulatory authorities in hazard and risk assessment.

The Regulatory Environment

The regulatory environment has a top level definition of a controlled working environment, audited by a third part, which then branches into several sub-levels. This structure is shown in Figure 1.
All the top levels are auditing bodies of the regulators. Whilst these may be internationally recognised organisations as ISO (International Standardisation Organisation) or the USA FDA (Food and Drug Administration), they could equally well be a company's own internal Quality Department. However, all bodies require evidence of compliance in all the lower sub-levels.

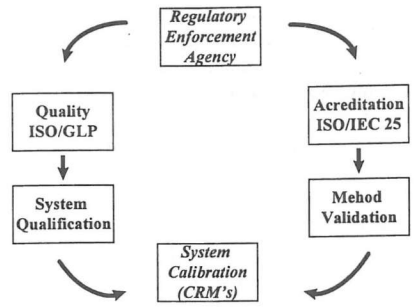

Figure 1. The Regulatory Environment

Good laboratory practice is intended to promote the quality and validity of test data. It is a managerial concept covering the organisational processes and the conditions under which laboratory studies are planned, performed recorded and reported.

pH Measurement - Critical Issues

There some parameters affecting the pH measurements, that will have a direct effect on the accuracy and precision of the data generated. These parameters are :

- 1- Temperature effects
- 2- Calibration procedures
- 3- Selecting the right electrode
- 4- Electrode maintenance
- 5- Sample handling

These parameters are discussed in the following sections.

1. *Temperature effects*

As is well known the temperature affects the pH data as seen in the Nernst equation

$$E = E_0 + \frac{2.303RT}{nF} \log (H^+)$$

The electrode slope is affected by the change of temperature. The value of E_0 changes and is affected by temperature changes.
At 25 °C the ideal value is be 59.16 mV/pH.
Actually as seen in Table 1 the temperature influences the slope. The error can be quite important if this factor is not taken into account. The value is approximately of 0.003 pH/°C/pH units from the calibration point.

Table 1 . Temperature effects on Slope

Temp °C	mV/pH
0	54.20
5	55.20
15	57.18
30	60.16
40	62.14

Another important factor of variation, and that will affect the measured value of the potential is the E_0 potential. This value is very affected by temperature. The Ag/AgCl electrodes show a marked shift of this potential with the temperature.
To avoid this effects, we must calibrate and measure at the same temperature. But there is another option, which involves a new and patented technology. This electrode known as the ROSS electrode, uses a different and innovative Galvanic pair which does not exhibit variation of the E_0 with the temperature. The precision is enhanced as well as the velocity of response.
The temperature affects as well the solution in which the Hydrogen ions are measured. As the temperature affects the activity of the ion, or other physical parameters of the solution (as viscosity), the proper Quality Control procedures must take in account the eventual differences in temperature of the sample and the calibration procedures.

2. Calibration Procedures

As any calibration procedure, we use a reference standard, with a known value at a certain temperature, where we can adjust the instrumental answer to that pre-known value. The buffer solutions used for that intention, must bracket the samples as much as possible. The 2 point procedure starting at pH 7 and combining with buffers of pH 4 or 10, is the most commonly used and enough for most of the applications. However the use of more points with buffer solutions is important to bracket the most extensive region possible, as slope values have slight variations in different parts of the pH scale. These Standard solutions should be primary Standards, traceable to international Standard Reference Materials.
As the integrity of the information during the process is compulsory in the GLP processes, the date, validity, lot codes and traceability data must be available for all the measurements and ready to use on the proper SOP's. The buffers should be properly stored, preferably in the refrigerator at 4°C if they are prepared in bulk solutions. Never reuse or pour back the Buffer into the bottle. The most

convenient way of handling the buffer solutions and avoiding any occasional problem is using Buffer Sachets or pouches, that contain only the necessary volume for one calibration on a closed environment. This pouches solutions are discarded after each calibration procedure and the envelope stored for data integrity purposes.

3. Selecting the right pH Electrode

The sample where the measurement will be done will have probably some particular issues and the right electrode has to be chosen. Certain samples will affect the electrode, clogging easily the ceramic junction, slowing the answer and making the measurement to drift. For example there are special electrodes for the following applications:

- Proteins, sulphides, Tris may precipitate the silver of the Ag/AgCl . A Ross electrode is used for those cases for example.
- Pure water samples cover a wide range of waters, and the common problem is slow response, drift and irreproducible results. A second difficulty is the difference in strength of the sample compared with the calibration buffers. After calibrating with a high ionic strength buffer a long stabilisation period will be needed for measuring any low ionic pure water. The use of special electrodes, Ionic Strength adjusters, and low ionic buffers are necessary for reproducible readings.
- Colloids, suspensions, slugs and slurries may clog the electrode junction. The ceramic standard junctions are not recommended for these applications. Using a special sleeve junction or the "Sure Flow" type minimises the clogging of the electrode response and improves reproducibility. In addition the sleeve type junction electrodes are easy to clean.
- Solids and flat surfaces use a special electrode design to adapt to the surface in samples like paper, cheese, etc.
- Extreme high salt content pose special problems on the reference electrode site. Outside within the working conditions a liquid junction potential can be a problem as wrong results can be obtained. The use of a proper electrode with double junction should alleviate this problem.
- Viscous samples have difficulties because are difficult to stir. Note that the electrode answer will be slower if the samples are not stirred.
- Non aqueous solutions. Unstable readings are commonly observed in non aqueous solutions, mainly due to the high resistance of the sample solution. A pH electrode membrane constructed from low resistance glass can avoid this problem. The Ross electrodes uses bulbs with lower resistance than any other electrode. A quaternary salt can be added if the problem is severe. Addition of a salt may affect the Ion activity of the hydrogen and therefore the pH value, but the error will be small compared with the drift of the measurement. Other errors can appear if the non aqueous sample adheres to the glass surface and carries over to other samples. Rinsing with the proper solvent and adequate cleaning will prevent contamination. Calibrating can be a problem as comparing aqueous buffers with non aqueous ones can be so different as comparing apples and oranges.

Theses are some examples where we can see that proper method validation is important on the sample measurement process.

4. Electrode Maintenance

A regular maintenance schedule and proper storage of the pH electrode will maximise performance, help extend the life of the electrode, and avoid the cost of replacements. On a weekly basis is recommended that the electrode is inspected for scratches, cracks, salt crystal build up, and membrane junction deposits. The reference chamber of refillable electrodes should be drained and flushed with new electrode filling solution, and newly refilled. The electrode must be stored in

proper storage solutions to keep it in order. The use of this type of solutions keep it ready to work in a fast and stable manner. Please note not to leave the electrode to dry out.
As the electrode may become coated with some samples, it may be important to clean it properly periodically. Proteins can be cleaned with pepsin in HCl; fats can be cleaned with a detergent; oils and grease with a proper solvent. After cleaning, the electrode must be refilled and immersed in the storage solution.

5. Sample Handling

The proper sample handling will affect directly the results. The integrity of the sample has to be assured and has to be well documented in the SOP's of the sample handling.
When measuring the pH, the effect of stirring speeds up the response, but the electrodes are flow sensitive, so the proper method has to adapt the sample. We should try the same method with the samples as we do with the calibration, whenever possible.
We shall remember as well all what we have already said about temperature.

- pH measurements and GLP - Conclusions

The use of GLP in the modern laboratory, as well the participation of many company in accreditation or certification schemes, is a trend on nowadays. But the pH measurement, most of the time considered the simplest or the easiest, has a lot of special difficulties that have to be reviewed. Some examples of this issues were given in this brief. The integrity of the data and the proper results will be guaranteed, as this is the objective of any Quality management system on the several forms that may appear, following some of these indications. The method validation, can assure the proper handling of this parameters as a whole.

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