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ESTIMATION OF UNCERTAINTY ON pH MEASUREMENT RESULTS: A DIRECT PATHWAY TO  
QUALITY ASSURANCE

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by

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Professor Jens Andersen.....

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Date: 23/06/2020

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## **Dedication**

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## **Abbreviations**

BIMP; International Bureau of Weights and Measures

CFR; Code of Federal Regulations

CITAC; Co-operation on International Traceability in Analytical Chemistry

CV%; Coefficient of variation/ relative standard deviation

EURACHEM; a network of organizations focused on analytical chemistry in Europe

FDA; Food and Drug Administration, a federal agency of the United States Department of Health and Human Services

GUM; the guide to the expression of Uncertainty in Measurement

HACCP; Hazard Analysis and Critical Control Point system

H-H; The Henderson Hasselbalch equation

ISO; International Organization for Standardization, develops and publish International Standards

IUPAC; International Union of Pure and Applied Chemistry

JCGM; A Joint Committee for Guides in Metrology

LPU; law of propagation of uncertainty

QA; Quality Assurance

QUAM; The guide to Quantifying Uncertainty in Analytical Measurement, uses uncertainty budget table to evaluate uncertainty

SD; Standard deviation



## Abstract

A technique for estimation of measurement uncertainty of routine pH measurement using pH meters; Thermo scientific Orion Star and Basic 20 is presented. There are issues associated with pH measurements, what we actually measure is not usually what we would expect or even intend, and it is common practice that analysts just take the immediate reading without doing repetitions. The pH measurements made from one chemical laboratory are not consistent with those made in a different laboratory and, the pH meter readings generally do not agree closely with the calculated pH values. The result of first approach, that is single experiment (3 repetitions), uncertainty evaluation was done according to the guide to Quantifying Uncertainty in Analytical Measurement (QUAM) and/or the guide to the expression of uncertainty in measurement (GUM) using uncertainty budget table as a tool. On second approach, we established correspondence between observed and predicted uncertainties with new derived equations to calculate the total uncertainty. At acidic pH -2.08 uncertainty was  $\pm 0.02$  and basic pH 13.3, the uncertainty was  $\pm 0.01$ , the results of calculations similar on both approaches. Results of single experiment over a short period of time confirm that individual uncertainty values at particular pH values correspond to the manufacturer's specification, but not to the expected pH values. Repeatability conditions and pooled calibration method were used for further assessment. Repeated analysis under similar measurement conditions and experimental detail were performed to measure pH of numerous buffers and sample solutions, and then, pooled calibration, the basic statistical calculations, the Horwitz equation, coefficient of variation (CV%) and the law of propagation of uncertainty (LPU) in a spreadsheet model were used for the analysis of uncertainty. Pooled calibration created a satisfactory correspondence between predicted pH values and those observed by experiment. The Horwitz equation constituted an expert judgment on the performance of the meters, it indicates poor performance at pH value -2.08. The results correspond to the CV% of  $[H^+]$  and CV% of pH values as well. HorRat ratio showed significant difference between the coefficients of variation at pH -2.08. At pH 13.3, there was no significant difference between the coefficients of variation and variances are homogenous.

*Keywords: Henderson Hasselbalch equation; Horwitz equation; law of propagation of uncertainty; pH meter; statistical calculations; uncertainty; uncertainty budget table*

## Chapter 1. Introduction

### 1.1 Uncertainty evaluation in analytical laboratories

Analytical laboratories produce analytical results attained from analytical equipment's and there ought to be no doubt about the end results. If there is a doubt about the results its essential to do uncertainty evaluation. The EURACHEM/ CITAC Guide [2, 3] with [4, 5], expresses measurement uncertainty as an explanation of a variety of measured values within which the true value is proclaimed to fit with a quantified level of sureness. According to Miller [4] , the uncertainty should incorporate all foundations of error . It may be expressed as Standard uncertainty ( $u$ ) or Expanded uncertainty ( $U$ ). The EURACHEM/ CITAC Guide to Quantifying Uncertainty in Analytical Measurement [6], alongside [7, 8], suggests that in general practice uncertainty narrates the general concept of doubt. In most cases, individuals have that tendency of confusing an error and uncertainty, but EURACHEM/ CITAC Guide [3] and Tang *et al* [7] made it clear that distinguishing between uncertainty and an error is greatly significant, and uncertainty should not in any means be understood to signify a mistake. Inconsistency amongst a measured value and the actual or true value is a measurement error whilst the effect of those numerous measurement errors is uncertainty [8].

### 1.2 Importance of measurement uncertainty

Measurement uncertainty is a prerequisite of analytical chemistry which is described not in many terms as an acknowledgment of appropriateness for use [9, 10]. In order to avert inappropriate interpretation of results and provide confidence, it is important that a statement of uncertainty should accompany analytical results [11], or it be readily available to those who wish to use the data. The measurement uncertainty is vital for the sake of full comparability and confirmation of fitness of results for purpose, which is fundamental to the quality of potentiometric pH measurements [12]. It is an important part of the reported result [13, 14], and a correct estimate of the uncertainty in measurement is the overall aim for evaluating practices and procedures of analytical chemistry [15].

### 1.3 Quantification of uncertainty

According to the EURACHEM/ CITAC guide 2000, and Araujo [16], there are two types of measurement uncertainty, Type A and Type B. With Type A uncertainty, the best estimate of the input quantity is obtained from repeated experimental measurements using statistical evaluations, examples including among other reliability, linearity, stability, precision, temperature error, pH, calibration uncertainty, etc. Type B uncertainty, on the other hand, is obtained by other means other than statistical analysis. For instance, findings from guides, books, manufacturer's specification, general knowledge, previous measurements, and certificates. Examples of type B uncertainty includes traceability, measurement procedure, calibration procedure, reference electrode, pressure resistance [18- 23] and the EURACHEM / CITAC Guide [2, 3, 6].

### 1.4 Measurement uncertainty model

All the types of uncertainty contributors can be identified with the help of the cause-effect diagram/ Ishikawa diagram which makes it possible to see all possible causes of the result and the root cause of imperfection [13]. However, it is in some way found to underestimate measurement uncertainty partially [18], therefore is very vital to use existing and experimentally obtained data to maximize the probability of including all the possible sources of uncertainty. Examples of the common causes of uncertainties in measurement includes; equipment, unit under test, operator, method, calibration, and environment.

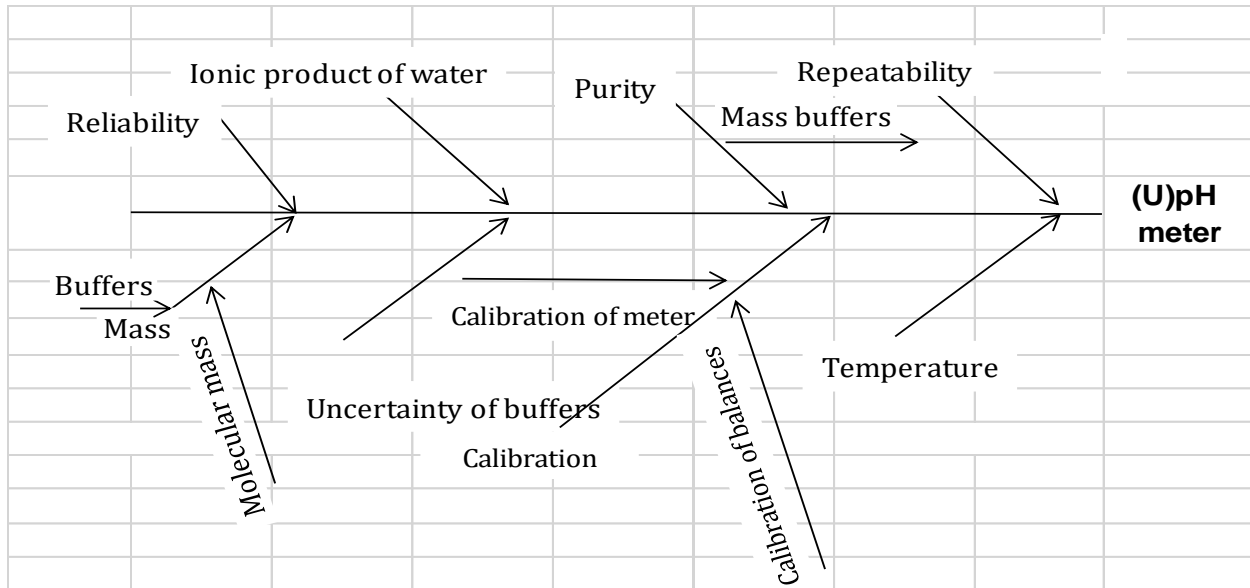


Figure 1: The cause-effect diagram of the sources of uncertainty on pH measurement results

### 1.5 Uncertainty budget

The evaluation of uncertainty should be done according to the guide to Quantifying Uncertainty in Analytical Measurement (QUAM) and/or the guide to the expression of uncertainty in measurement (GUM), This is to confirm that an equipment delivers good quality results, assessing its uncertainty is a process that is vital and needs to be described. The assessment is completed by the Uncertainty budget tool. Uncertainty budget is a tool that explains how the quality of the work was in an experiment depending on the level of confidence [19]. The uncertainty budget is a table which centralizes the size to which uncertainty is associated, the size of the estimate, the standard uncertainty, probability distributions, coefficient sensitivity, contributing to uncertainty / relative standard [20]. One of the benefits of the uncertainty budget is that it reaches and satisfies ISO 17025 requirements which prevent the occurrence of errors in uncertainty analysis, provide evidence that uncertainty analysis was performed, improve quality through evaluation of uncertainty contributors [21]. ISO 17025 is an international standard that has the general competency requirements that aid in testing the capacity to deliver reliable result by testing and calibration laboratories [22]. It explicitly prescribes that “Testing laboratories shall have and shall apply procedures for estimating the uncertainty of measurement”.

## 1.6 How measurement uncertainty should be expressed and/or used

Once the measurement results are obtained they should be described, the measurement uncertainty should be expressed as  $U$ , the combined expanded measurement uncertainty, using a coverage factor  $k = 2$ , at the level of confidence of approximately 95 % [13]. The resulting uncertainty can be used to decide whether there is a difference between results from different laboratories or results from the same laboratory on different occasions. Other means of describing the data set is to do the best estimate of the actual quantity (mean value), characterize the average uncertainty of measurements (standard deviation), indicate the reliability of an estimate based on 95% probability (Confidence interval) according to the ISO 5725, evaluate a linear relationship between test results (regression analysis).

## 1.7 Uncertainty estimation on pH measurements

One of the simplest and easy measurements in research and chemical laboratories is the measurement of pH. Measurement is an experimental procedure of obtaining one or a number of values using a calibrated measuring system that is operated according to the specified measuring procedure including the measurement condition, to give values that can be reasonably attributed to a quantity. BIMP in [23] published by Joint Committee for Guides in Metrology (JCGM), say that determination of the closest true value is not the main objective of measurement in the uncertainty approach, rather the information from measurement is used to set the interval of reasonable values to the measurand. EURACHEM/ CITAC Guide CG2 declare that to allow a statistical data treatment it is a need to repeat a measurement several times. When taking pH measurements, it is a common practice that whoever is doing the measurement just takes the immediate reading without doing repetitions. The practice of repeatability is normally taken lightly although a thorough method validation that provides the correct level of uncertainty on pH measurements has not been done before. It would be possible to perform a single measurement of pH of an unknown and assign the uncertainty from the method validation to it. pH has a greater impact on sensory perception and its control during processing is necessary. The value of pH is a vital quality control parameter in the engineering field [24], in the food and beverage industries [25], it has effects on the activity of enzymes, rate of fermentation, absorption of carbon dioxide,

aroma, taste, physical appearance, tartrate precipitation, malolactic fermentation, shelf-life stability.

When doing repetitions of measurement, the measurement result can either be accurate, inaccurate, true or not true. Measurement accuracy is described as the closeness of the measured quantity value and the true quantity value of a measurand [23]. Measurement trueness, on the other hand, is defined by International vocabulary of metrology [23], as closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value. It is expressed numerically by the mean value. Then precision being the closeness between measured quantity values expressed numerically by standard deviation, variance or coefficient of variance.

### 1.8 Condition of measurement

It is a necessity to do repeated analysis under the intra-serial precision condition of measurement in order to estimate uncertainty of analytical equipment. EURACHEM/ CITAC Guide CG2 says that repeated analysis allows a statistical data treatment. To estimate uncertainties on measurements, more delicate tools of statistics are said to be required [26]. The repeatable data is attained by repeatability conditions such as the same operator, same measuring system, same location, same operating conditions, similar measurement procedures, and repeated measurements over a short period of time. This is put into practice to eliminate inter-laboratory variability [27], and to do away with needless errors as the main aim is to estimate instrumental measurement uncertainty. This avoids confusion on the source of error, in agreement with Andersen [28], who specified that human error and uncertainty of measurement should never be confused. Kuselman *et al* [29], support by stating that human activity is never free from errors, but the fact still remains that those errors are not uncertainties. These repeatability measurements can be done within a day or over several days. And when analyzing data that has been obtained by experimentation over several days, we refer to it as ‘pooled data’, but data that is obtained from short series of repetitions, performed within a day, at a particular time is referred to as ‘single experiment’.

## 1.9 Properties of the measuring device

The indicating measuring instrument that can be used to provide repeated measurement result and is of importance in this study is a pH meter. pH meters give out an indication or quantity value in a visual form. According to Lewin [30] electrometric measurement of pH depends upon the determination of the voltage existing between a hydrogen-ion [ $H^+$ ] sensing, and a reference probe. The pH meter operates with a combination electrode casing both measuring and reference electrodes. According to the manufacturer, pH meters has the ability to measure from pH (-2) which is more acidic to pH 16 which is more basic. The ability of the pH meter to measure this lowest and highest pH can be evaluated. This can be done using the Horwitz equation and the HorRat ratio. According to Horwitz [31], the equation shows the relationship between accuracy of analytical method and concentration of analyte. It is the coefficient of variation ( $CV\%$ ) also known as relative standard deviation or reproducibility standard deviation and is a measure of relative variability under reproducibility conditions [31, 32]. The Horwitz equation;

$$CV\% = 2^{(1-0.5 \cdot \log C)} \dots\dots\dots \text{equation (i) by Horwitz [27]}$$

where  $C$  is the concentration of the analyte expressed as a dimensionless mass fraction.

The Horrat value is used for comparison of the actual measured precision and the predicted precision. The value is a ratio of measured and predicted precision.

$$\text{HorRat value} = \frac{RSD_{R, \text{measured}}}{RSD_{R, \text{Horwitz predicted}}} \dots\dots\dots \text{equation (ii) by Horwitz [31]}$$

If HorRat value evaluates homogeneity of variances through the coefficient of variation. Under repeatability conditions, accepted values are between 0.3 and 1.3. If  $\text{HorRat}(r) < 0.3 \cap \text{HorRat}(r) > 1.3$ , there is a significant difference between the coefficients of variation, so variances are not homogenous.  $H_1$ : If  $0.3 \leq \text{HorRat}(r) \leq 1.3$ , there is no significant difference between the coefficients of variation, so variances are homogenous [33].

A pH meter like any other instrument should be of a specification sufficient for the intended purpose, and it should at all times be kept in a state of good maintenance and perfect calibration that is reliable for its use, this is to ensure that the pH measurement made from one laboratory can

be consistent with those made in another laboratory, supported by EURACHEM/ CITAC Guide CG2 saying that analytical measurements that are made in one location should be consistent with those that are made somewhere else.

#### 1.10 Importance of pH in the industries

- Food industry

pH as a measurement is crucial in the characteristics of food, it is considered a critical stage according to the Hazard Analysis and Critical Control Point (HACCP) system. This is because if the value of pH is not controlled in the process it affects the physical appearance, the mouthfeel and the overall quality of the end products. The methodology that is used in pH measurement is regulated by U.S Food and Drug Administration (FDA) and the procedure is well discussed in the Code of Federal Regulations (CFR) Title 21 Section 114.90 but the uncertainty estimate is not known. To mention a few applications of pH in the food industry e.g. jam and jelly manufacturing, meat and fish processing.

- The wine industry and brewing

Correct pH-values of wine in the wine industries is good for greater stability during aging, maturity and less vulnerability to spoilage. Thus, it is imperative then to continually monitor and control a wine's pH, in order to ensure that it does not decrease below or increase above critical thresholds. The moment wine is open at use it loses the Sulphur dioxide gas. Apparently, there has been a noticeable growth on the demand of wines with low  $SO_2$  content that has been shown by consumers in the past years [35]. This resulted in reduction of  $SO_2$  in wine becoming a subject of focus in the oenological science and a decisive plan for the wine industry [35]. It is known that the same Sulphur dioxide gas that they are removing has antioxidant activity and antimicrobial activity [36], therefore, pH content can be used to monitor the stability and palatability of wine.

In the brewing process, pH is very vital on several processes of production from the brew-house to the filter room. The brew-house consists of processes like; mashing, this is where enzyme activity is required to produce wort, a lowering of pH in wort by increasing the level of calcium



compound. Calcium ions reacts with carbonate, phosphate and polypeptides to promote the release of protons and thus lowering of pH. For example,  $3Ca^{2+} + 2HPO_4^{2-} \rightarrow Ca_3(PO_4)_2 + 2H^+$ .

At whirl pooling, where there is addition of hops, pH decreases the solubility of bitter hops;  
Fermentation, upon addition of yeast cells all the way through the whole process of fermenting;  
Filtration, according to the requirement for the end product to ensure stability of shelf life.

- Dairy production

Also, attention can be directed towards the consumption of milk. When milk is opened, its pH starts to change immediately and it turns into lactic acid, which may be the reason why manufacturers on the package always write “once opened keep refrigerated and use within four days”, An example of pH application in the dairy industry is the production of cheese, yoghurt, madila, dairy juices, and UHT milk. The pH in dairy production is influenced by the presence of calcium ions and phosphate ions.

- Plant growth

The pH measurements are also of great importance to plants and garden soil. The pH in the soil is very important as it influences root development, microbial activity, fungi, symbiotic nitrogen fixation by legumes and the availability of nutrients. Cation and anion exchange is related to the soil’s ability to holding and supplying nutrients, and these cation and anion exchange capacities are influenced by soil pH [37]. These are examples of methods of pH amendments in soil;

$CaCO_3 + H_2O \rightarrow \text{calcium soil} + OH^-$ , OR,  $MgCO_3 + H_2O \rightarrow \text{magnesium soil} + OH^-$ ; ;  
lime neutralizes acidity and adds calcium to the soil.  
 $sulfur (S) + oxygen(O_2) + water(H_2O) \rightarrow \text{sulfuric acid}(H_2SO_4) + \text{soil calcium}(Ca)$ ;  
sulfur and sulfuric acid lower the pH

The control of pH is important in other fields like leather production, fertilizer production, detergents, prevention of corrosion, dyeing, glue manufacturing.

### 1.11 Application of pH measurements in titration

The estimate of uncertainty can be assigned to the equivalence point that is determined from a titration curve, but there are some difficulties in doing so. It is stated that there are well-known means to calculate the uncertainty of the pH – value of a titration curve like the use of the nonlinear regression method or using an electronic graphical display spreadsheet [38]. According to Marti *et al* [39], there are different purposes that titration can be used for such as, to determine the concentration of acid or base or to determine the physio-chemical parameters. Taking pH as a parameter of interest and potentiometric titration as the method of choice. Potentiometric titration is a volumetric method of measuring the potential between two electrodes as a function of added reagent volume, but, in this research, it was performed as a function of the added mass and the estimate of uncertainty calculations are formulated using the concentrations in units of w/w %. The main aim of potentiometric acid-base titration is to determine the equivalence point. The results are used to plot a curve. The shape of the titration curve is reliant on the acid and base concentrations; the s-shaped curve is a result of titration of a strong acid with a strong base or strong base with a strong acid [39].

### 1.12 Method validation of pH measurements

Method validation is also known as measurement assurance [40]. It is an essential component of the measures that a laboratory should implement to allow it to produce reliable analytical data [41] and it forms the first level of Quality Assurance. These validated methods are used by the analytical laboratory to study the overall performance of the measurement procedure [42], to show qualification and competency [43, 44] as well prove that analytical method is acceptable for its intended use [16, 49-51]. Validation is verification of requirements that are intended for a specific use [23, 46-47], and Stöckl *et al* [48], clarifies the definition to mean that the process should specify the intended use of a measurement procedure, define the analytical performance requirements, provide data from validation experiments, and interpret the validation data by use of a statistical test. For routine use in laboratories, experimental work is the foremost approval needed in order to validate the aptitude of use as well as the performances of these methods of analysis [49]. Validation is a mandatory tool which is used on daily practices to make decisions on analytical data [50]. According to EURACHEM working group [51], awareness of the importance of

validation, why it should be done, when it should be done, and exactly what needs to be done, seems to be poor amongst analytical chemists.

In method validation, the quantitative characteristics of interest relate to the accuracy of the result likely to be obtained. Therefore, it is generally true to say that method validation is tantamount to the task of estimating the uncertainty of measurement [41]. Method validation is carried out once, or at relatively frequent intervals during the working life of a method. Validation is associated with the assurance and confirmation that the product submits to the set requirements/ parameters as well as satisfying certain defined criteria [52]. The validation parameters are listed by Kuselman and Shenhar [53], e.g. repeatability, reproducibility, and accuracy. Method validation involves method scope, calibration-related parameters, method precision, and recovery [54]. It is acknowledged as one of the essential components in achieving Total Quality Management. The methods an analytical laboratory uses must be validated to be fit for purpose [55, 56], validation should be done before routine use in the laboratory to ensure that the instrument itself has satisfactory accuracy, precision and measuring range. All this will assist the laboratory in demonstrating that their tests/methods are fit for the intended use in samples.

### 1.13 Analytical quality assurance

There is a close relationship between validation, uncertainty and quality assurance, the relationship is recognized by Andersen, [26] describing quality to the analytical chemist to mean that the true uncertainty should be attached to the measurement. Quality assurance is an essential organizational infrastructure that underlies all reliable analytical measurements [10] and it consists of a set of activities that ensure production of excellent products to meet the customer and consumers demands [57], it is a program which provides confidence that the quality requirements will be fulfilled [58] and it reduces measurement errors to tolerable limits [59]. The International Guide to Quality in Analytical Chemistry, EURACHEM/ CITAC Guide [3, 19] express it as the procedures that are being used by the laboratory to ensure its operation are of high quality and it proves reliability in results [60, 50]. Quality assurance consists of a number of different activities that are put in place such as record keeping, staff training, equipment monitoring and maintenance, good laboratory conditions, calibration schedules, good storage conditions, [57]. There are quality

assurance certifications that help the companies and laboratories to ensure quality to their customers and other stakeholders, like the ISO 9000 which comprises of a family of standards in quality assurance and quality management that are established for companies to help in effective documentation of quality system elements that when implemented will maintain the efficiency of quality management system [61]. The system elements include among other statistical techniques, quality records, quality system, inspection measuring and testing equipment, document control, corrective actions.

The main aim of analytical quality assurance is to provide accurate and reliable data instead of poor data, according to Wenclawiak *et al* [62], quality assurance determines the reliability of the data generated and its estimate is an expression of respectable accurate and reliable data.

#### 1.14 Benefits of Quality Assurance

Quality assurance is very beneficial to the manufacturing industries at large as it is an identification tool of the weaknesses and inconsistencies of the company in its production stages. It reduces loss, waste of time and energy by keeping the main focus on the key issues which determine quality results, costs and timeliness and avoiding use of energies on less important issues, and it ensures greater use of testing equipment. However, laboratories and customers' need to realize and understand that Quality Assurance cannot guarantee that hundred percent of all of the individual results will be reliable recognized by Andersen [28], who declares that in a well-run laboratory, the incidence of mistakes will be minor, but not zero. Mistakes may consist of, for example, gross errors such as a comprehensive instrument failure, accidentally dropping or disposing of a vital sample [4].

#### 1.15 Requirements for Quality assurance

Analytical quality has its own requirements, these requirements are specified by Zogovic *et al* [18], to be the requirement for uncertainty and the requirement for dispersion or results. Uncertainty is required in quality assurance as it is a way which customers can use to compare the reference value and individual results by showing the possible maximum deviation between the

two. Dispersion of results simply means repeatability or reproducibility; these are the quality characteristics which can be measured by means of internal quality control.

Since uncertainty is a requirement in the analytical laboratory, there is need to evaluate the uncertainty of pH result as a guide to quality assurance. Based on the research done by Menong and Andersen [63], measurements of pH meter readings generally do not agree closely with the calculated pH values. It shows clear differences that people did not care much about and Schmitz [64], says that we can never hope to get the difference between calculated and measured values. Therefore, this research's work is a big part of method validation. This is a good opportunity to understand the strengths and weaknesses of a methodology used [50], and it will tell us what performance we can expect the method to provide in the future as well give the values of the uncertainty of unknowns.

The Basic 20 and Thermo scientific Orion pH meters will be used to make measurements, and then the uncertainty that exists about their result will be evaluated to define how much and how big it is. Uncertainty will be predicted using the QUAM method, and in addition we will be testing whether it is possible to not prepare the uncertainty budget according to QUAM but simply establish a correspondence between observed and predicted uncertainties. The uncertainty of food samples will be estimated, this is to find out if uncertainty predicted from standard solutions corresponds to those of sample solutions and that would mean that our pH measurements were in statistical control and scientific methodology was fulfilled. In the end, we will find out if it's possible that a simple sensor like the pH electrode can measure concentrations that are lower than any advanced apparatuses in the world and if possible, find out the uncertainty associated with the measurement result.

All the work was done at Botswana International University of Science and Technology (BIUST), in the main laboratory of the Department of Chemical and Forensic Sciences. The pH measurements were measured using two pH meters, one from the Department of Chemical and Forensic Sciences which is (Basic 20 pH meter), and the other one was from Department of Biology and Biotechnology (the Thermo scientific Orion star). Chemicals were taken from the department of Chemistry at BIUST and some at the University of Agriculture and Natural Resources.

## Chapter 2. Aims and Objectives

The purpose of this research is to complete a full method validation of pH measurements which has not been done earlier, test the validity of uncertainty calculations that are based on pooled data, and estimate the uncertainty of pH - values.

### Specific objectives

- To estimate uncertainty using pooled calibration
- To complete uncertainty estimation using Uncertainty budget tool (according to the guide to Quantifying Uncertainty in Analytical Measurement (QUAM))
- To complete uncertainty estimation by correspondence between calculated uncertainty and predicted uncertainty using derived equations, then compare with uncertainty from the budget table
- To provide adequate confidence that the pH meter will perform satisfactorily and confirm with laboratory requirements
- To decide by statistical arguments, which one of the two pH meters is the better choice for pH measurements

## Chapter 3. Experimental

### 3.1 Apparatus

- pH meters

Measurements were performed by a Basic 20 pH meter (Fig. 3a). The meter has a large graphic display with a resolution of 0.01 units in the pH measurement mode and 0.1 in temperature. It has a measuring uncertainty ( $\pm 1$  digit),  $\leq 0.01$  in pH measurement and  $\leq 0.2$  in temperature. The meter is capable of measuring pH ranges from -2 to 16 by two measuring modes: by stability or in continuous mode. The calibration is programmable with validity between 0 h and 7 days and the meter gives an automatic recalibration warning. Calibration involves automatic recognition of technical buffers pH 2.00, 4.01, 7.00, 9.21, 10.90 values at 25°C with 1, 2 or 3 buffers selectable inside the range. It has magnetic stirrer as the key to precise and repeatable measurements. This instrument has ambient conditions such as; working temperature 5 to 40 °C, storage temperature -15 to 55 °C and Relative humidity < 80% (not condensed).

Another meter that was used is the Thermo Scientific Orion star (Fig. 3b). The meter has a large, informative screen; it has parameter specifications such as operating temperature 0°C- 50°C, pH range -2 to 16, with resolution 0.1 or 0.01, accuracy (mv)  $\pm 0.2$  mv, accuracy (pH) being  $\pm 0.002$  pH and up to three calibration points with easy recall of calibration point and the slope. It has included an electrode arm and holder that makes it easier to maintain and place probes into samples.

Both the pH meters use a combined pH electrode, the glass electrodes are very sensitive to hydrogen ions [1], according to Bier [65], it consists of a reference that is inside the glass membrane (pH probe) and a reference that is in contact with the solution outside of the glass membrane and temperature sensor for automatic temperature compensation. The internal fill and the reference fill are usually a similar solution; they contain an electrolyte which normally is 3.5 M KCl. During a measurement, the pH-sensitive glass gets in contact with the solution being measured, it then develops the potential ( $E$ ) that is proportional to the pH of the solution.

$$E = E_0 + 2.303 \left( \frac{RT}{F} \right) \log a_{H^+} = E_0 + 2.303 \left( \frac{RT}{F} \right) pH \dots\dots\dots \text{equation (iii) by Zhongqi [66]}$$

where  $E$  is a measured potential (voltage),  $E_0$  is the standard electrode potential at  $a_{H^+} = 1 \text{ mol/L}$ , which is zero,  $R$  is the gas constant,  $T$  is the temperature in kelvin,  $F$  is the Faraday constant.

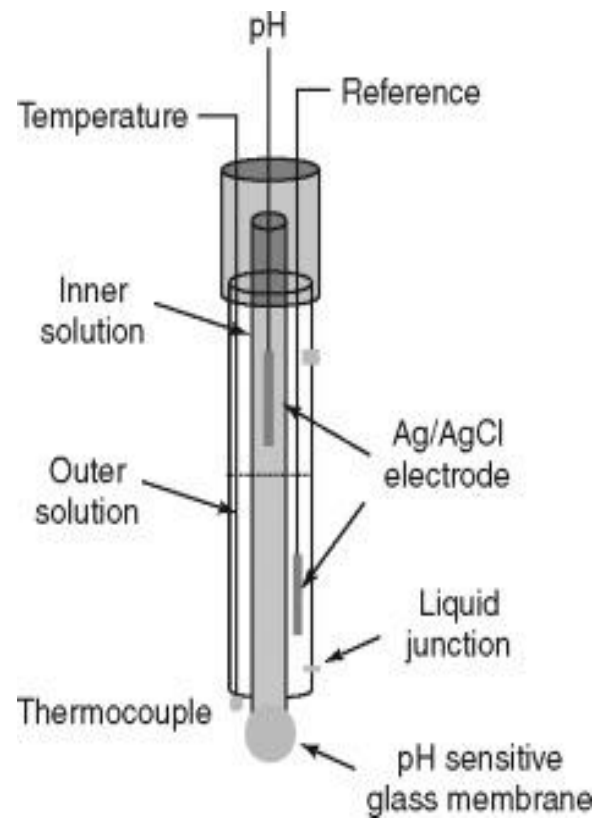


Figure 2: A typical example of a combined pH electrode, by Hulanicki [1]



Table 1; The uncertainty specifications of pH benchtop meters

Name of pH meter	Name of manufacturer	Accuracy (pH)	Accuracy (mv)	Accuracy (temp)	Reference
<i>Thermo Scientific Orion</i>	Fisher scientific	$\pm 0.002pH$	$\pm 0.2mv$	$\pm 0.1^{\circ}C$	[67]
<i>Basic 20</i>	Crison Instruments	$\leq 0.01pH$	$\leq 1$	$\leq 0.2$	[68]



Figure 3: pictures of (a) Basic 20 pH meter, (b)Thermo Scientific Orion Star pH meter



Figure 4: pictures of (a) Basic 20 pH meter probe, (b) A Thermo Scientific Orion pH meter probe

### 3.2 Materials

Water bath at 25 degrees Celsius, pH meter (Thermo Scientific Orion star A111, basic 20), analytical balance, 250 mL Erlenmeyer flask, 100 mL beaker, weighing boat, spatula, top pan balance, thermometer, were used throughout the study.

### 3.3 Chemicals

The following chemicals were used throughout the study;

- Ammonia solution ( $\text{NH}_4\text{OH}$ ), Rochelle chemicals, Assay min. 25%, 070515AM
- Ammonium chloride ( $\text{NH}_4\text{Cl}$ ), Merck (Pty) Ltd, Assay 99%, Uni lab SAAR1122700EM
- Acetic acid ( $\text{CH}_3\text{COOH}$ ), Rochelle chemicals, Assay 99.5%, 090215AA
- Sodium acetate ( $\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$ ), Merck (Pty) Ltd, Assay 99- 101.0 %
- Sodium sulfate ( $\text{NaSO}_4$ ), Rochelle chemicals, Assay 99.0%,
- Sodium hydrogen sulfate ( $\text{NaHSO}_4$ ) Rochelle chemicals, Assay 98%, batch2575
- Sodium hydrogen carbonate ( $\text{NaHCO}_3$ ), Rochelle chemicals, Assay 99%,
- Sodium hydroxide ( $\text{NaOH}$ ) Rochelle chemicals, Assay 97%, batch05121450
- Three standard buffer solutions (pH 4, 7 and 9)
- Electrode storage solution (3 M KCl)

### 3.4 Samples

- Robertsons Winery, EST 1941, chapel sweet red, 750 mL, Alc 7.5%, serve between 8-10°C, total acidity 6.86, pH 3.4, contain sulphites (allegens)
- Bonnita long life Milk- full cream, UHT process, the product of Parmalat, 500 mL
- Soil samples obtained in Botswana at North region (Pandamatenga), central district (Paje) and Kgatleng South (Tshele)
- Red bull, energy drink, made with sugar sourced from sugar beets, 13 kcal calories, 2g carbohydrates, per one can
- Spar orange juice, made with 100% orange juice, 185 KJ energy, 10g carbohydrates, 6.9g sugar, 3mg sodium, 0g fat, in a 100ml serving

- Diet coke, no added sugar, ingredients; Carbonated water, caramel color, aspartame, phosphoric acid, potassium benzoate, natural flavors, citric acid, caffeine

### 3.5 Preparation of the buffer solutions

In this experiment, the mole ratio was assigned and expected pH values calculated with Henderson-Haschelbach equation, and having the number of moles, the required mass was calculated using equation  $\left( moles = \frac{mass(g)}{molarmass(gmol)} \right)$  and buffer solution was then prepared. There were four sets of acid-base pairs that were used. These are:

- Acetic acid ( $\text{CH}_3\text{CO}_2\text{H}$ ,  $K_a = 1.8 \times 10^{-5}$ ) and sodium acetate ( $\text{CH}_3\text{COONa}$ )
- Ammonium chloride ( $\text{NH}_4\text{Cl}$ ,  $K_a$  for  $\text{NH}_4^+ = 5.6 \times 10^{-10}$ ) and ammonia solution ( $\text{NH}_3$ ).
- Sodium sulphate ( $\text{NaSO}_4$ ,  $K_a = 1.0 \times 10^{-2}$ ) and sodium hydrogen sulphate ( $\text{NaHSO}_4$ ).
- Sodium bicarbonate ( $\text{NaHCO}_3$ ,  $K_a = 4.7 \times 10^{-11}$ ) and sodium hydroxide ( $\text{NaOH}$ ).

### 3.6 Procedure

The buffers were prepared by taking the required mass ratio of acid to base that will produce the assigned pH, and then mixed the calculated amounts of the two compounds with enough deionized water to make 250 mL. The amount of the buffer component needed was measured accurately on an analytical balance (i.e.  $\text{NH}_4\text{Cl}$ ) and top pan balance (under a fume hood for  $\text{NH}_3$ ) and dissolved in a small quantity of water in the beaker. The solution was transferred quantitatively into a 250 mL volumetric flask and filled to the mark with distilled water. Then the solution was mixed by inverting a few times. The buffer solution was kept at a temperature of  $25.0 \pm 1.0$  degree Celsius.

The pH meters were set under a fume hood. Then standardized using three buffers (three-point calibration). The three standardization buffers used were pH 4.01 buffer, a pH 7.00 buffer, and a pH 9.00 buffer (at 25 °C) according to the manufacturer's instructions. About 25 mL of buffer solution was poured into a small beaker and the electrode inserted inside to measure the pH - value. At all times the electrode was rinsed off with deionized water and blot dried when transferring in

between different solutions. The pH-values of the solutions were not close to the expected value. The fume hood was used for ammonia buffer and acetic acid buffer, for all other buffer's measurements were done on the benchtop.

## Chapter 4. Results

### 4.1 Estimation of Uncertainty of (Standards) Buffer solutions

There are three principles of method validations; The ISO 17025/5725, EURACHEM/CITAC QUAM and Principle of pooled calibrations. The ISO method and the QUAM method are linked in a way that QUAM builds upon ISO but principle of pooled calibration is our own principle that is under study. The ISO and QUAM methods may be used to check traceability, as they predict uncertainty in terms of precision and recommends method validation according to a very few experiments. The principle of pooled calibration, on the other hand, does not include traceability but it fulfills scientific methodology, it focuses on making predicted uncertainty (standards) correspond to observed uncertainty (samples) and recommends extensive measurements and the apparatuses being switched on and off in between measurements. The ISO method and the QUAM methods recommends rejection of outliers, but with principle of pooled calibration all the data is utilized without rejection of outliers.

In this study, we follow the ISO and QUAM method to predict uncertainty and then we introduce repeatability conditions and engage the principle of pooled calibration. The statistical analysis of results was initially made on a short series of data obtained with a single experiment over a short period of time for each pH meter (Basic 20 and Thermo Scientific pH meter). The measurements were completed with three repetitions for each pH value. From the results, the uncertainty value for each pH meter of a single experiment that was performed over a short period of time according to the manufacturer's specification, provided results that did not correspond to the expected pH values. Hence introduction of repeatability conditions and pooled calibration. A higher number of repetitions of analysis was proposed, in order to further the experiments. The data from several experiments on several days was pooled for each pH meter and the results were analysed by statistics. Based on the statistical comparisons, decision was made to pool all data from each pH meter, accrue it and treat it as one pooled calibration. Then, the pooled calibration was used to provide better correspondence between uncertainties.

4.1.1 Statistical analysis of a short series of data obtained with a single experiment over a short period of time following the ISO/ QUAM method

A short series of data was obtained using two pH meters. Each meter's results were treated individually. The measurements were made within a short time frame and with using three repetitions. This procedure fulfills the ISO/ QUAM requirements. All analyses were done as per the manufacturers' instructions to each pH meter. All the expected pH values were calculated using the Henderson-Hasselbalch equation, and the calculations were used to prepare different buffers of sulfate, acetate, ammonia and carbonate. It was observed that uncertainty in the form of SD of both the meters were similar at all the expected pH's. The SD values were very low, and they corresponded very well with the manufacturer's specification. However, the measured pH values did not correspond to the calculated pH values, except at (at pH 9.25) on Table 2, that corresponded well with the observed pH values, so it may be argued that the uncertainty originated from the pH meter.

Table 2: Quantification of uncertainty using standard deviation at various calculated pH values of buffer solutions measured with Thermo Scientific Orion star and Basic 20 pH meter at  $25 \pm 0.1$  °C.

<i>Type of buffer</i>	<i>Calculated pH values (using Henderson-Hasselbalch equation)</i>	<i>(average + SD) measured by Thermo scientific</i>	<i>(average + SD) measured by Basic 20</i>
sulphate	-2.08	$-1.34 \pm 0.02$	$0.49 \pm 0.02$
sulphate	-1.08	$0.25 \pm 0.01$	$0.73 \pm 0.01$
Acetic	2.75	$2.51 \pm 0.01$	$2.27 \pm 0.01$
Acetic	3.75	$3.55 \pm 0.01$	$3.70 \pm 0.01$
Acetic	4.75	$4.64 \pm 0.01$	$4.71 \pm 0.01$
ammonia	6.75	$6.23 \pm 0.01$	$6.34 \pm 0.01$
ammonia	7.25	$7.02 \pm 0.01$	$6.88 \pm 0.01$
ammonia	8.25	$7.92 \pm 0.02$	$8.09 \pm 0.02$
ammonia	9.25	$9.25 \pm 0.04$	$9.28 \pm 0.04$
ammonia	10.25	$10.22 \pm 0.01$	$10.46 \pm 0.01$
ammonia	11.25	$11.06 \pm 0.01$	$11.23 \pm 0.01$
carbonate	12.03	$14.30 \pm 0.01$	$14.47 \pm 0.01$
carbonate	13.3	$14.14 \pm 0.02$	$14.51 \pm 0.02$

NB: uncertainty of temperature is estimated as type B uncertainty

Since the SD's of the two pH meters were equal at each calculated pH value (Table 2), it resulted in having similar Student's t test and Confidence Interval (CI) of the two pH meters. Therefore, the data is presented in a single table (Table 3). We use the Student's t test to determine the probability that these two set of measurement from these two pH meters are the same. With the probability greater than 0.05, there was no significant difference between the SD's. When the confidence interval was used to bound the mean in repeatability measurements, with a probability level of 0.05 or less, it may be stated with 95% level of confidence that the true mean lies within the CI. Thus, the uncertainty, as represented by CI's, were larger than the SD's of Table 2.

Table 3: Utilizing Student's t test and Confidence Interval as estimates of Experimental Uncertainty on single experiment with 3 repetitions (N = 3).

<b>Type of buffer</b>	<b>Calculated pH values</b>	<b>Student's t test</b>	<b>confidence Interval at 95%</b>
Sulphate	-2.08	4.30	0.05
Sulphate	-1.08	4.30	0.01
Acetate	2.75	4.30	0.01
Acetate	3.75	4.30	0.01
Acetate	4.75	4.30	0.02
Ammonium	6.75	4.30	0.03
Ammonium	7.25	4.30	0.01
Ammonium	8.25	4.30	0.04
Ammonium	9.25	4.30	0.09
Ammonium	10.25	4.30	0.01
Ammonium	11.25	4.30	0.01
Carbonate	12.03	4.30	0.01
Carbonate	13.3	4.30	0.05

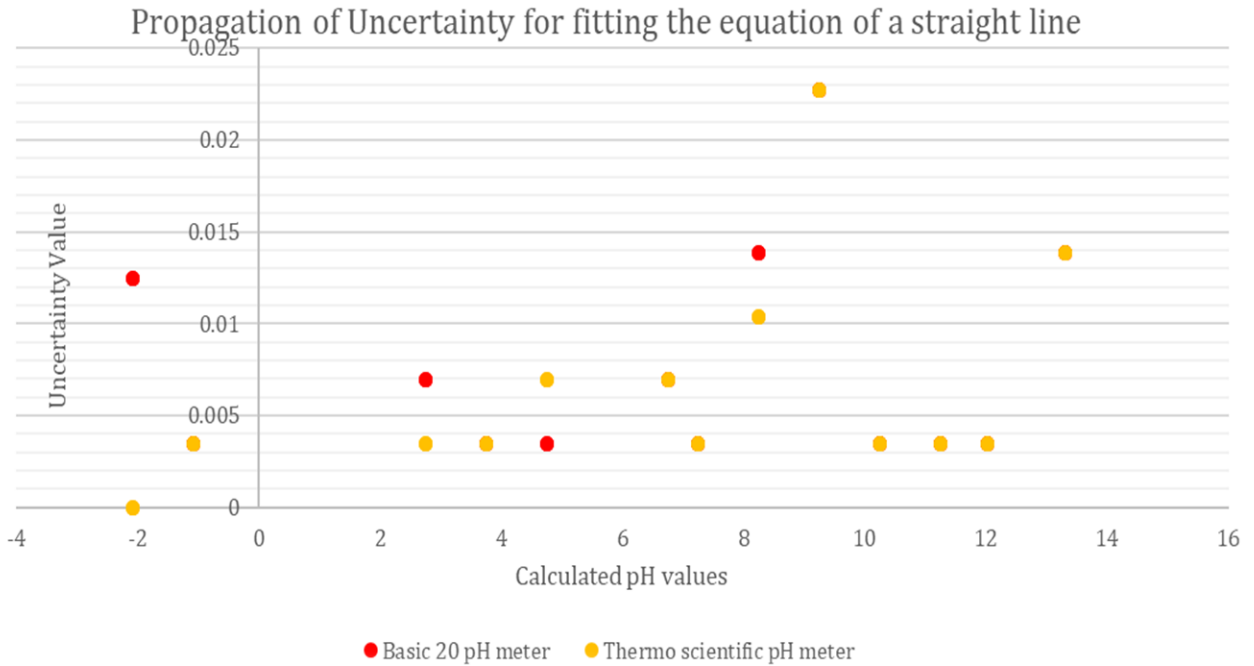


Figure 5: A graph showing uncertainty values as a function of calculated pH values.

At each calculated pH values, the uncertainty of measured pH was calculated using the law of propagation of uncertainty for fitting equation of a straight line. Using the following equation;

$$[Uncertainty\ in\ pH] = \frac{S_y}{|m|} \cdot \sqrt{\frac{1}{k} + \frac{1}{n} + \frac{(y-\bar{y})^2}{m^2 \sum(x-\bar{x})^2}} \dots \dots \dots \text{equation (iv)}$$

Where  $k$  is the number of replicate measurements,  $S_y$  is the standard deviation of measured pH values,  $|m|$  is the absolute value of the slope,  $n$  is the number of data points,  $m$  is the value of the slope,  $\bar{y}$  is mean the value of the measured pH ,  $\bar{x}$  is the mean value of the calculated pH,  $y$  is the measured pH value and  $x$  is the calculated pH value.

The measurements were obtained from single experiments with 3 repetitions of measurements made on buffers of sulfate, acetate, ammonia and carbonate. The data plotted fulfil the requirements of the ISO/ QUAM method. The results are supportive to Table 3, where it was found that the uncertainty of the two pH meters were significantly not different.



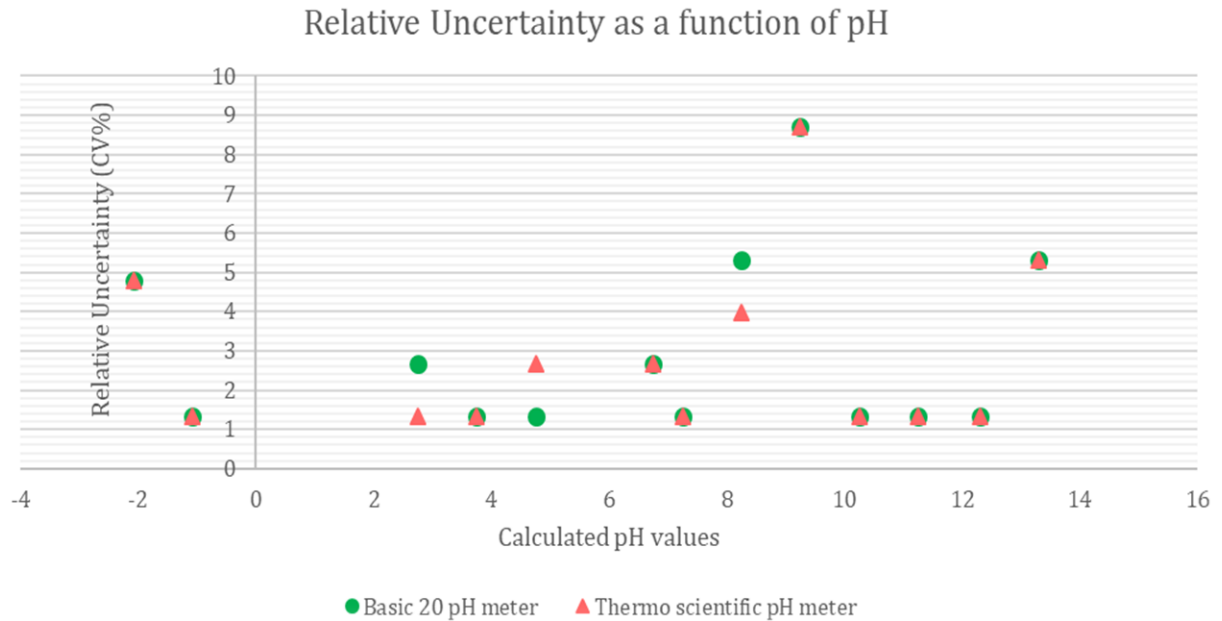


Figure 6: A graph showing relative uncertainty values of  $H^+$  as a function of calculated pH values.

The relative uncertainty of measured  $[H^+]$  was calculated at each calculated pH values using the following equation;

$$[S_{H^+}] = \frac{dH^+}{dpH} \cdot S_{pH} \dots \dots \dots \text{equation (v)}$$

Where  $SD$  is the standard deviation of measured pH - values.

The measurements were obtained from single experiments of both pH meters. Three repetitions of measurements were made on buffers of sulfate, acetate, ammonia and carbonate. These measurements were performed, in order to give an overview of uncertainty of the measured hydrogen ion concentration  $[H^+]$  in terms of the measured pH values. The shape is similar to uncertainty of the pH values, and its similar for both pH meters.

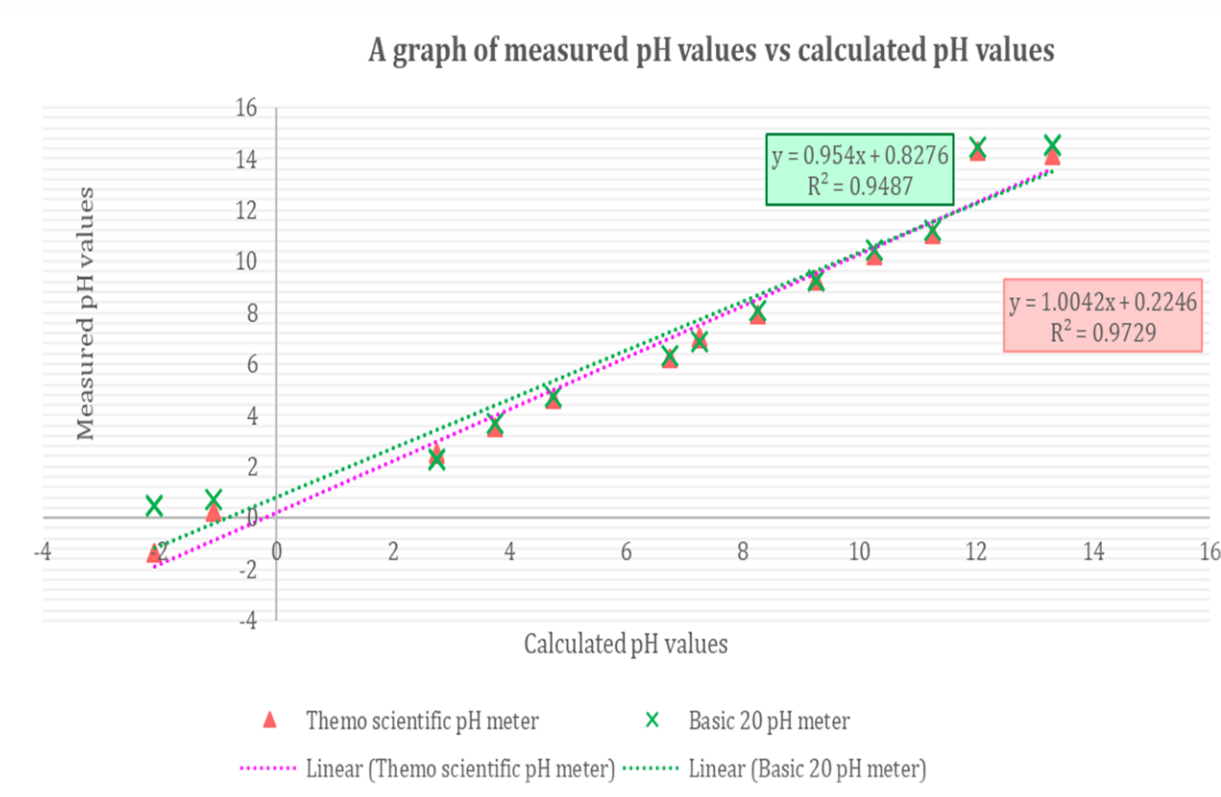


Figure 7: A graph showing calibration line of measured pH values vs calculated pH values.

The results are from a single experiment per individual meter. For the results of Thermo scientific pH meter, the uncertainty of slope is  $s_a = 0.028$  and the uncertainty of intercept  $s_b = 0.22$ . For basic 20 pH meter Uncertainty of slope is  $s_a = 0.037$  and uncertainty of intercept  $s_b = 0.30$ .

The calibration line shows the response of the analytical method to known quantities. The linear relation between the measured pH values and the calculated pH values is modeled by a straight line. We want to carefully evaluate characteristics of the calibration curve such as linear regression model, slope of the line, and correlation coefficient. The slope should be statistically different from 0, the intercept should not be statistically different from 0 and the regression coefficient should statistically be different from 1. In case of having a significant non-zero intercept, the accuracy of the method must be demonstrated.

#### 4.1.2 Statistical analysis of pooled data for each pH meter

The absence of correspondence between predicted pH values (standards) and observed pH values (samples) (Table 2), resulted in focusing on a method that can make the two correspond better. Repeatability conditions were introduced on the measurement technique. Measurements were performed at different days, but the conditions of measurements were kept the same throughout, this involved switching on and off of the measuring equipment in between the measurements. The measurements were performed using the two pH meters, all the data for each pH meter was pooled and analyzed as per individual pH meter. Each meter had two hundred and sixty repeated measurements. The results fulfil the requirements of pooled calibration, which is the method that is under scrutiny.

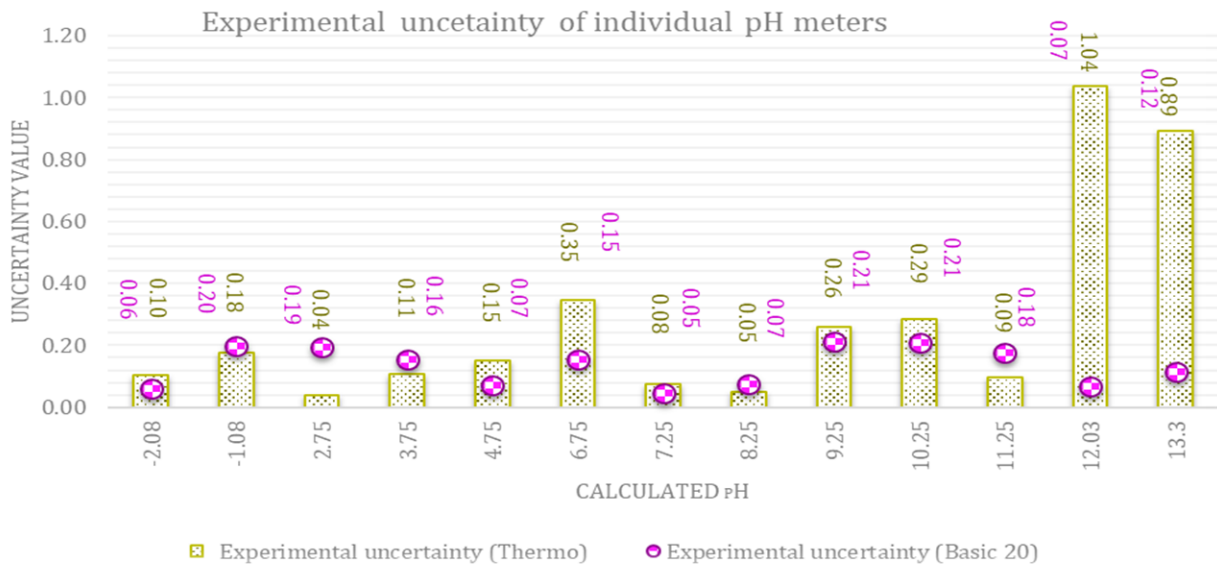


Figure 8: Using standard deviations as estimates of experimental uncertainty from pooled data of several days for individual pH meters.

The quantification of uncertainty was performed by using interval and confidence level at various calculated pH values of different buffer solutions (Table 4-7). The measurements were performed by using Thermo Scientific Orion star and Basic 20 pH meter in solutions at temperatures of  $25 \pm 0.1$  °C. Data of several days of measurements were pooled, 20 repetitions for each pH value, for each pH meter. For each pH value, the mean, SD, F test, t test and CI were calculated using the measured pH values. It was tested by statistics, if pooled data from each meter was different.

Table 4: Quantification of uncertainty using interval and confidence level at various calculated pH values of NaSO<sub>4</sub>/NaHSO<sub>4</sub> buffer.

<i>Parameter</i>	<i>Thermo Scientific Orion</i>		<i>Basic 20</i>	
At calculated pH	-2.08	-1.08	-2.08	-1.08
Mean of (measured pH)	-1.16	0.47	0.46	0.55
SD (measured pH)	0.10	0.18	0.06	0.20
F-value (measured pH)	3.06	1.27	3.06	1.27
Student's t-value (measured pH)	2.09	2.09	2.09	2.09
Confidence Interval at 95% (measured pH)	0.05	0.08	0.03	0.09

NB: uncertainty of temperature is estimated as type B uncertainty

Table 5: Quantification of uncertainty using interval and confidence level at various calculated pH values of CH<sub>3</sub>COOH/ NaC<sub>2</sub>H<sub>3</sub>O<sub>2</sub>. 3H<sub>2</sub>O buffer.

<i>Parameters</i>	<i>Thermo Scientific</i>			<i>Basic 20</i>		
At calculated pH	2.75	3.75	4.75	2.75	3.75	4.75
Mean of (measured pH)	2.47	3.59	4.64	2.59	3.61	4.67
SD (measured pH)	0.04	0.11	0.15	0.19	0.16	0.07
F- value (measured pH)	24.30	2.13	4.39	24.30	2.13	4.39
Student's t-value (measured pH)	2.09	2.09	2.09	2.09	2.09	2.09
Confidence Interval at 95% (measured pH)	0.02	0.05	0.07	0.09	0.07	0.03

NB: uncertainty of temperature is estimated as type B uncertainty

Table 6: Quantification of uncertainty using interval and confidence level at various calculated pH values of NH<sub>3</sub>/ NH<sub>4</sub>Cl buffer.

<i>Parameters</i>	<i>Thermo Scientific Orion pH meter</i>						<i>Basic 20 pH meter</i>					
At Calculated pH	6.75	7.25	8.25	9.25	10.25	11.25	6.75	7.25	8.25	9.25	10.25	11.25
Mean (measured pH)	6.39	6.98	7.90	9.09	10.07	10.98	6.16	6.87	7.96	9.15	10.24	11.37
SD (measured pH)	0.35	0.08	0.05	0.26	0.29	0.09	0.15	0.05	0.07	0.21	0.21	0.18
F-value (measured pH)	5.03	2.68	2.26	1.50	1.87	3.47	5.03	2.68	2.26	1.50	1.87	3.47
Student's t value (measured pH)	2.09	2.09	2.09	2.09	2.09	2.09	2.09	2.09	2.09	2.09	2.09	2.09
Confidence Interval (measured pH)	0.16	0.04	0.02	0.12	0.13	0.04	0.07	0.02	0.04	0.10	0.10	0.08

Table 7: Quantification of uncertainty using interval and confidence level at various calculated pH values of NaHCO<sub>3</sub> / NaOH / buffer.

<i>Parameter</i>	<i>Thermo Scientific Orion pH meter</i>		<i>Basic 20</i>	
At Calculated pH	12.03	13.30	12.03	13.30
Mean of (measured pH)	13.96	13.85	14.47	14.54
SD (measured pH)	1.04	0.89	0.07	0.12
F-value(measured pH)	244.21	59.00	244.21	59.00
Student's t-value(measured pH)	2.09	2.09	2.09	2.09
Confidence Interval (measured pH)	0.42	0.03	0.05	0.05

NB: uncertainty of temperature is estimated as type B uncertainty

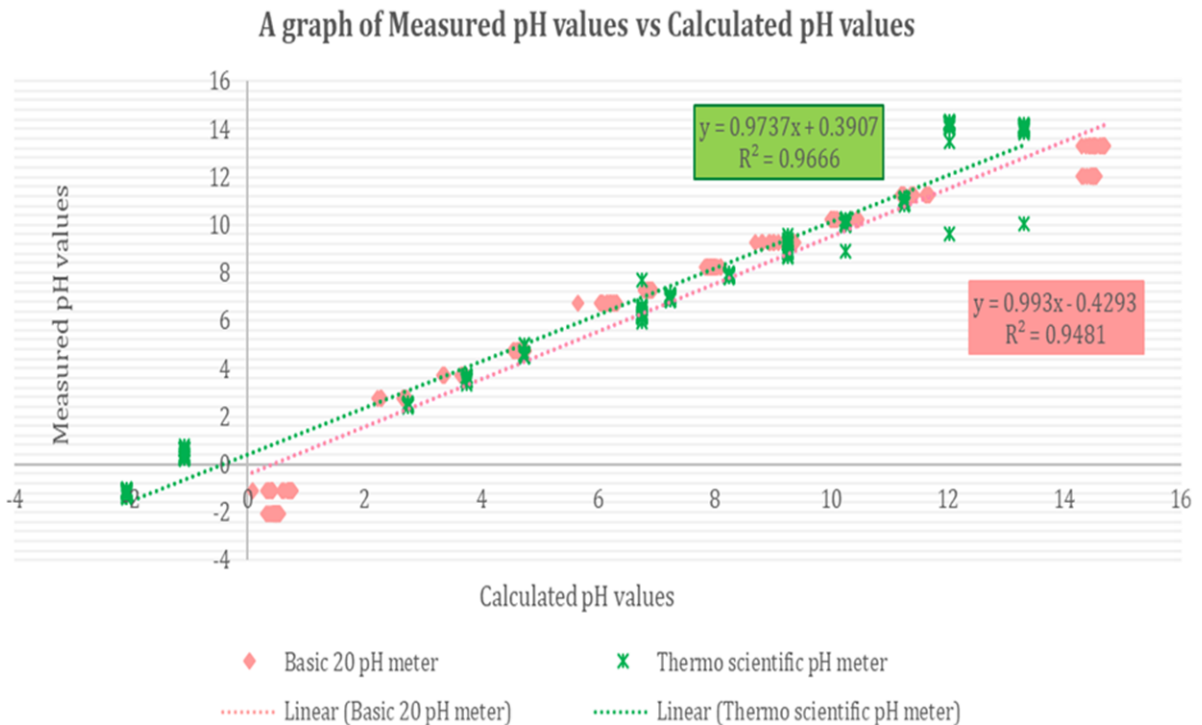


Figure 9: A graph showing the calibration line of measured pH values vs calculated pH values of pooled data from measurements of many days per individual meter.

Characteristics of the calibration curve was evaluated by pooled data, and the analysis was performed in the same manner, it was done to prepare the graph of Fig. 7. The uncertainty of slope and intercept were compared, in order to determine if it were possible to pool all the data of the two pH meters and treat it as a whole. If the uncertainty of slope and intercept were statistically similar, then the two collections of data were statistically indifferent; hence the option to pool all the data. For each measurement there were twenty repetitions made. The uncertainty of slope is  $s_a = 0.011$  and the uncertainty of intercept  $s_b = 0.095$ . For basic 20 pH meter which are similar for the thermo scientific Orion meter.

#### 4.1.3 Statistical analysis of pooled data from both pH meters using the Principle of Pooled Calibration

Data of several days obtained by the two pH meters were pooled. And the data was evaluated and treated as a single data set. The calibration curve and confidence level were used to determine outliers, and to determine if confidence level is an appropriate method for rejection of outliers in the method of pooled calibration. The results are presented in the following Fig. 10.

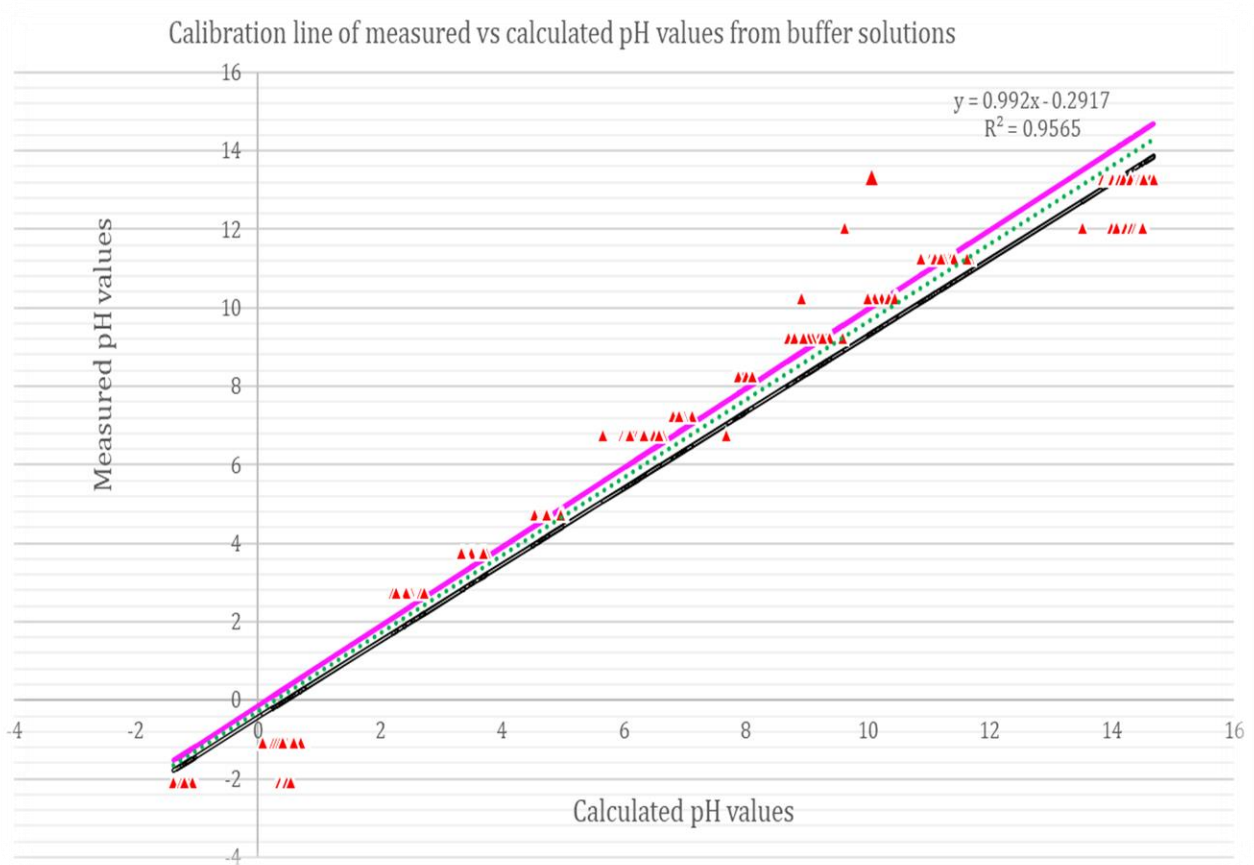


Figure 10: Calibration line of measured pH values vs calculated pH values of pooled data from both pH meters.

Data measured on several days gives a total of 520 repetitions ( $N = 520$ ). The purple line represents the upper confidence level and the black line represents is the lower confidence level. The uncertainty of slope is  $s_a = 0.0093$  and the uncertainty of intercept  $s_b = 0.078$ .



The statistical calculations were used to determine if using method of pooled calibration give better correspondence between measured pH values and calculated/expected pH values in comparison with the ISO/ QUAM method

Table 8: Quantification of uncertainty using standard deviation and confidence level on pooled data of several days.

<i>At calculated pH</i>	<i>MEAN</i>	<i>SD</i>	<i>Confidence Interval at 95% confidence level</i>
<b>-2.08</b>	-0.35	1.64	0.39
<b>-1.08</b>	0.51	0.38	0.09
<b>2.75</b>	2.53	0.30	0.07
<b>3.75</b>	3.6	0.26	0.06
<b>4.75</b>	4.66	0.24	0.06
<b>6.75</b>	6.28	0.58	0.14
<b>7.25</b>	6.92	0.16	0.04
<b>8.25</b>	7.93	0.14	0.03
<b>9.25</b>	9.12	0.48	0.11
<b>10.25</b>	10.15	0.52	0.12
<b>11.25</b>	11.18	0.48	0.11
<b>12.03</b>	14.22	1.54	0.36
<b>13.3</b>	14.19	1.44	0.34

NB: uncertainty of temperature is estimated as type B uncertainty

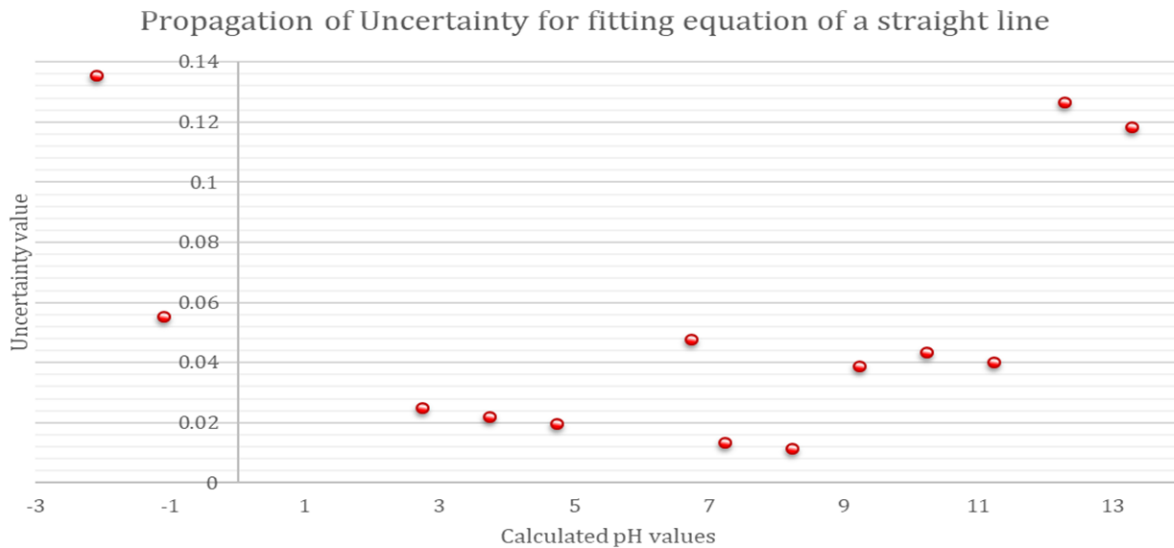


Figure 11: A graph showing Uncertainty values at various calculated pH values.

On the above Fig.11, the uncertainty of measured pH was calculated using the law of propagation of uncertainty for fitting equation of a straight line, using equation 4. The measurements were obtained from pooled data of both pH meters on several days with 40 repetitions per pH value.

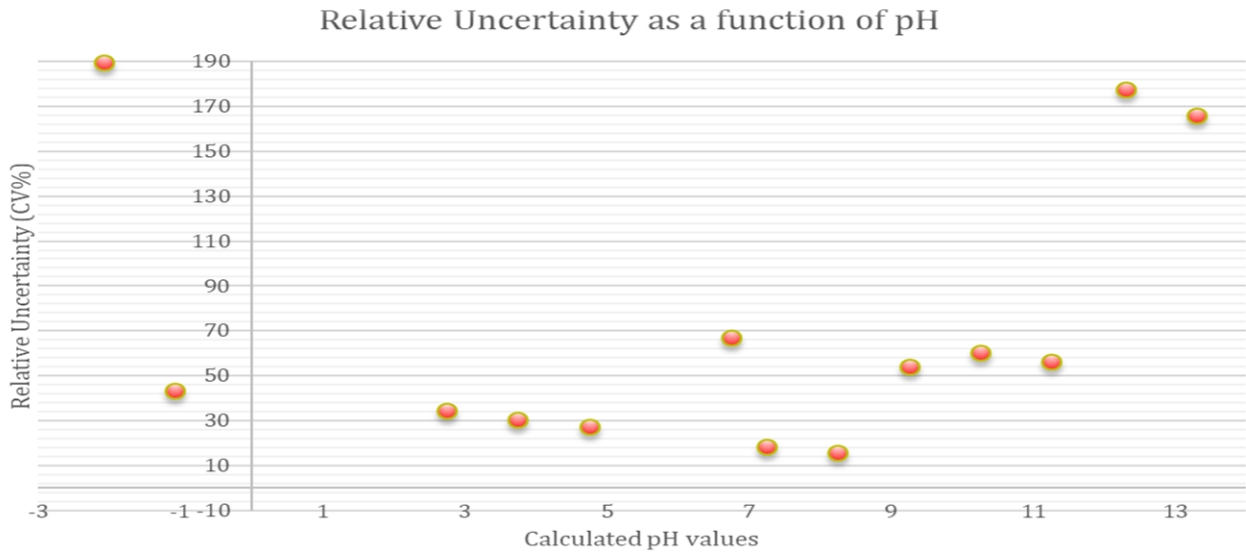


Figure 12: A graph showing relative uncertainty values of  $H^+$  as a function of calculated pH values.

The relative uncertainty of measured  $H^+$  was calculated at each calculated pH values using equation 5. The measurements were obtained from pooled data of both pH meter on several days, 520 repetitions. It was the aim to determine an overview of uncertainty of the pooled measured hydrogen ion concentration  $[H^+]$  in terms of the pooled measured pH values.

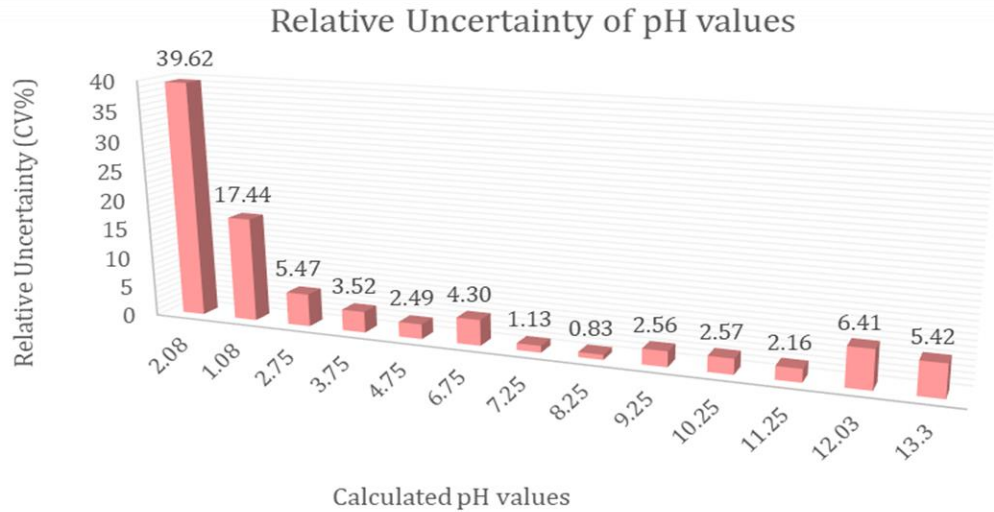


Figure 13: A graph showing Relative Uncertainty values of *pH* at various calculated *pH* values.

The relative uncertainty of measured *pH* was calculated at each calculated *pH* values using the following equation:

$$CV\% \text{ of } pH = \frac{SD}{pH} \cdot 100\% \dots \dots \dots \text{equation (vi)}$$

Where *SD* is the standard deviation and *pH* meaning the measured *pH* value.

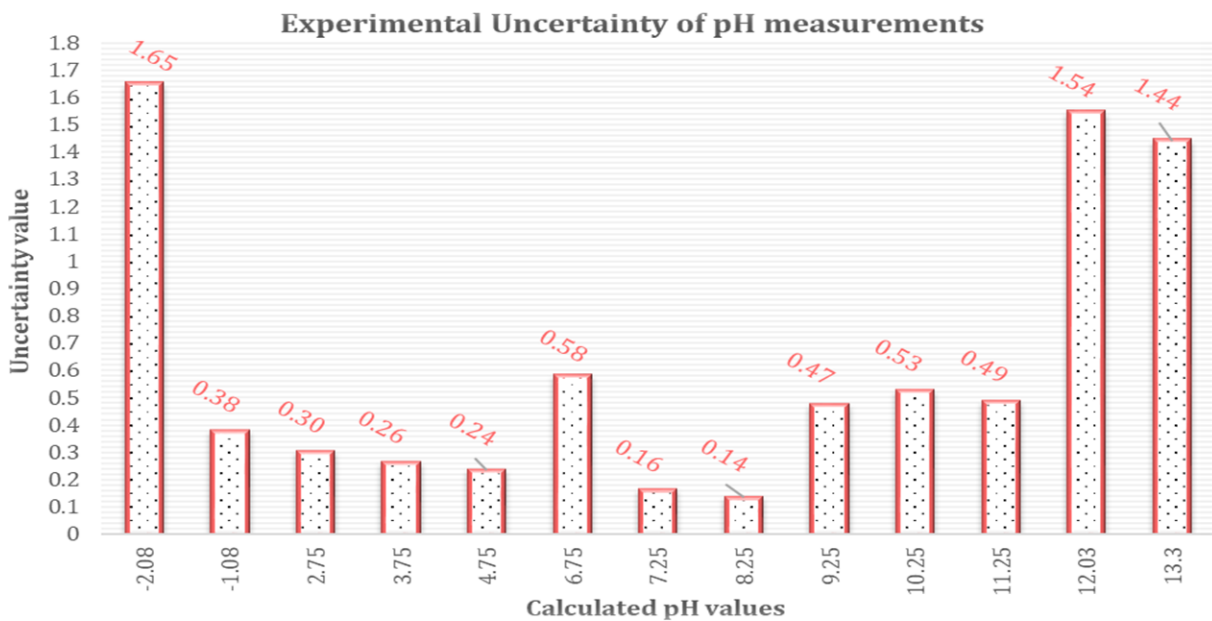


Figure 14: Using Standard Deviations as estimates of Experimental Uncertainty

On the above table, the uncertainty at each pH value was determined by following equation;

$$SD = 2 \cdot \sqrt{\frac{\sum (x - \bar{x})^2}{N-1}} \dots \dots \dots \text{equation (vii)}$$

Where  $x$  is the measured pH value,  $\bar{x}$  is the mean value of measured pH values and  $N$  is the number of repetitions and the factor of two is the coverage factor.

#### 4.1.4 Model equations for titration of strong acid and strong base

In this study measurement uncertainty was determined from prepared samples of different buffers, all these buffers were prepared using the H-H equation. This is the equation that is normally and most of the times used in chemistry. In the process of researching, reading and studying, it was found, that the equations have limitations, it can only calculate pH values of a buffer which is close to the  $pK_a$  value, it is appropriate in calculations of the pH of buffers that are made with acids whose  $pK_a$  falls within the range 5 to 9 and, the more the dissociation constant of the acid departs from  $10^{-7}$ , the more unreliable the equations become for calculating  $[H^+]$ . That calls for a need to derive some equations step by step to help improve or correct them.

During titration the initial analyte solution of mass  $m_A$  g of a monoprotic acid (0.01 M, HCl) was titrated with 0.01 M strong base (NaOH).  $m_B$  is mass of base,  $M_a$  is molar mass acid,  $M_b$  is molar mass base,  $P$  is purity of sample

- Before equivalence

Before equivalence point pH is determined by excess  $H^+$  in a solution,  $\Delta[H^+] = ((\text{moles acid}) - (\text{moles of base}))$

$$H^+ = (N_A) - (N_B) \dots \dots \dots \text{equation 1}$$

$$pH = -\log[H^+] \dots \dots \dots \text{equation 2}$$

$$[H^+] = \left[ \frac{(N_A) - (N_B)}{m} \right] \dots \dots \dots \text{equation 3}$$

$$N = \frac{m(g)}{M(g/mol)} \dots \dots \dots \text{equation 4}$$

$$\text{Substituting equation 4 into 3; } [H^+] = \left[ \frac{\left[ \frac{m_A}{M_a} \right] - \left[ \frac{m_B}{M_b} \right]}{[m]} \right] \dots \dots \dots \text{equation 5}$$

Substituting equation 5 into 2;

$$pH = -\log_{10} \left\{ \frac{\left( \frac{m_A}{M_a} \right) - \left( \frac{m_B}{M_b} \right)}{m} \right\} = \log_{10} \left\{ \frac{\left( \frac{m_A}{M_a} \right) - \left( \frac{m_B}{M_b} \right)}{m} \right\}^{-1} = \frac{\log_e \left\{ \frac{m}{\left( \frac{m_A}{M_a} \right) - \left( \frac{m_B}{M_b} \right)} \right\}}{\log_e 10} =$$

$$\left[ \frac{\log_e m - \log_e \left\{ \frac{\left( \frac{m_A}{M_a} \right) - \left( \frac{m_B}{M_b} \right)}{m} \right\}}{\log_e 10} \right] = \left[ \frac{\ln m - \ln \left\{ \frac{\left( \frac{m_A}{M_a} \right) - \left( \frac{m_B}{M_b} \right)}{m} \right\}}{\ln 10} \right] \dots \dots \dots \text{equation 6}$$

$$\text{NB: } \left( \frac{m_A}{M_a} - \frac{m_B}{M_b} \right) = \left( \frac{m_A M_b - m_B M_a}{M_a M_b} \right)$$

$$\frac{\ln(m)}{\ln 10} - \frac{\ln(m_A M_b - m_B M_a)}{\ln 10} - \frac{\ln(m_A M_b)}{\ln 10} \dots \dots \dots \text{equation 7}$$

Differentiating equation 7 with respect to  $m_A, m_B, M_a, M_b,$  and  $m$  we get;

$$\frac{dpH}{dm_A} = 0 - \left[ \left( \frac{1}{\ln 10} \right) \left( \frac{1}{(m_A M_b - m_B M_a)} \cdot M_b \right) \right] - \left( \frac{1}{\ln 10} \right) \left( \frac{1}{(m_A M_b)} \right) = - \left( \frac{M_b}{(\ln 10)(m_A M_b - m_B M_a)} \right) -$$

$$\left( \frac{1}{(\ln 10)(m_A M_b)} \right) \dots \dots \dots \text{equation 8}$$

$$\frac{dpH}{dm_B} = 0 - \left[ \left( \frac{1}{\ln 10} \right) \left( \frac{1}{(m_A M_b - m_B M_a)} \cdot M_a \right) \right] - 0 = - \left( \frac{M_a}{(\ln 10)(m_A M_b - m_B M_a)} \right) \dots \dots \dots \text{equation 9}$$

$$\frac{dpH}{dM_a} = 0 - \left[ \left( \frac{1}{\ln 10} \right) \left( \frac{1}{(m_A M_b - m_B M_a)} \cdot m_B \right) \right] - 0 = - \left( \frac{M_b}{(\ln 10)(m_A M_b - m_B M_a)} \right) \dots \dots \dots \text{equation 10}$$

$$\frac{dpH}{dM_b} = 0 - \left[ \left( \frac{1}{\ln 10} \right) \left( \frac{1}{(m_A M_b - m_B M_a)} \cdot m_A \right) \right] - \left( \frac{1}{\ln 10} \right) \left( \frac{1}{(m_A M_b)} \right) = - \left( \frac{m_A}{(\ln 10)(m_A M_b - m_B M_a)} \right) -$$

$$\left( \frac{1}{(\ln 10)(m_A M_b)} \right) \dots \dots \dots \text{equation 11}$$

$$\frac{dpH}{dm} = \left[ \left( \frac{1}{\ln 10} \right) \left( \frac{1}{m} \right) \right] - 0 - 0 = \left( \frac{1}{(\ln 10)(m)} \right) \dots \dots \dots \text{equation 12}$$

$$S^2 pH = \left(\frac{dpH}{dm_A} \cdot S_{m_A}\right)^2 + \left(\frac{dpH}{dm_B} \cdot S_{m_B}\right)^2 + \left(\frac{dpH}{dM_a} \cdot S_{M_a}\right)^2 + \left(\frac{dpH}{dM} \cdot S_{M_b}\right)^2 + \left(\frac{dpH}{dm} \cdot S_m\right)^2 + \left(\frac{dpH}{dP} \cdot S_P\right)^2 \dots \text{equation 13}$$

Substituting equation 8,9,10,11, and 12 into equation 13;

$$S^2 pH = \left(\left(-\frac{1}{m_A}\right) \cdot S_{m_A}\right)^2 + \left(-\frac{1}{m_B \ln 10} \cdot S_{m_B}\right)^2 + \left(\left(\frac{1}{M_a}\right) \cdot S_{M_a}\right)^2 + \left(\left(\frac{1}{M_b \ln 10}\right) \cdot S_{M_b}\right)^2 + \left(\frac{1}{m}\right) \cdot S_m\right)^2 + \left(\frac{du(P)}{dP} \cdot S_P\right)^2 \dots \text{equation 14}$$

NB:  $m$  is mass base minus excess mass everything divided by excess mass

- At equivalence point

At equivalence equation the moles of added acid are equal to added base  $\left(\frac{m_A}{M}\right) = \left(\frac{m_B}{m}\right)$

$$m_B = \left[\frac{\left(\frac{m_A}{M_a}\right)m}{M_b}\right] \dots \text{equation 15}$$

Substituting equation 15 into equation 1

$$pH = -\log \left[\frac{\left(\frac{m_A}{M_a}\right)m}{M_b}\right] = \log_{10} \left[\frac{M_b}{\left(\frac{m_A}{M_a}\right)m}\right] = \frac{\ln M_b - \ln \left[\frac{\left(m_A\right)m}{M_a}\right]}{\ln 10} = \frac{\ln M_b}{\ln 10} - \frac{\ln \left(\frac{m_A}{M_a}\right)m}{\ln 10} = \frac{\ln M_b}{\ln 10} - \frac{\ln \left(\frac{m_A}{M_a}\right)}{\ln 10} + \frac{\ln(m)}{\ln 10} \dots \text{equation 16}$$

Differentiating equation 16 with respect to  $m_A, M_a, M_b,$  and  $m$  we get;

$$\frac{dpH}{dm_A} = 0 - \left[\left(\frac{1}{\ln 10}\right) \left(\frac{1}{\left(\frac{m_A}{M_a}\right)}\right)\right] + 0 = -\left(\frac{1}{\ln 10}\right) \left(\frac{1}{\left(\frac{m_A}{M_a}\right)}\right) = -\left(\frac{1}{(\ln 10)\left(\frac{m_A}{M_a}\right)}\right) \dots \text{equation 17}$$

$$\frac{dpH}{dM_a} = 0 - \left[\left(\frac{1}{\ln 10}\right) \left(\frac{1}{\left(\frac{m_A}{M_a}\right)}\right)\right] + 0 = -\left(\frac{1}{(\ln 10)\left(\frac{m_A}{M_a}\right)}\right) \dots \text{equation 18}$$

$$\frac{dpH}{dM_b} = \left(\frac{1}{\ln 10}\right) \left(\frac{1}{M_b}\right) - 0 + 0 = \left(\frac{1}{(\ln 10)(M_b)}\right) \dots \text{equation 19}$$

$$\frac{dpH}{dm} = 0 - 0 + \left( \left( \frac{1}{\ln 10} \right) \left( \frac{1}{m} \right) \right) = \left( \frac{1}{(\ln 10)(m)} \right) \dots \text{equation 20}$$

Substituting equation 17,18, 19 and 20 into equation 13;

$$S^2 pH = \left( -\frac{1}{(\ln 10) \left( \frac{m_A}{M_a} \right)} \cdot S_{m_A} \right)^2 + \left( \left[ -\left( \frac{1}{(\ln 10) \left( \frac{m_A}{M_a} \right)} \right) \right] \cdot S_{M_a} \right)^2 + \left( \left( \frac{1}{(\ln 10)(M_b)} \right) \cdot S_{M_b} \right)^2 + \left( \left( \frac{1}{(\ln 10)(m)} \right) \cdot S_m \right)^2 + \left( \frac{du(P)}{dP} \cdot S_P \right)^2 \dots \text{equation 21}$$

○ After equivalence point

pH is determined by excess OH<sup>-</sup> in a solution, [OH<sup>-</sup>] = ((moles base) – (moles of acid))

$$pH = 14 - [-\log(OH^-)] \dots \text{equation 22}$$

$$OH^- = \left\{ \frac{\left( \frac{m_B}{M_{eq}} \right) - \left( \frac{m_A}{M} \right)}{\frac{k_w}{(m)}} \right\} \dots \text{equation 23}$$

$$\text{Substituting equation 13 into } 1 = 14 - \left[ -\log_{10} \left\{ \frac{\left( \frac{m_B}{M_b} \right) - \left( \frac{m_A}{M_a} \right)}{\left( \frac{k_w}{m} \right)} \right\} \right] = 14 - \left[ \log_{10} \left\{ \frac{\left( \frac{m_B}{M_b} \right) - \left( \frac{m_A}{M_a} \right)}{\left( \frac{k_w}{m} \right)} \right\}^{-1} \right] =$$

$$14 - \left[ \log_{10} \left\{ \frac{\left( \frac{k_w}{m} \right)}{\left( \frac{m_B}{M_b} \right) - \left( \frac{m_A}{M_a} \right)} \right\} \right] = 14 - \left[ \frac{\log_e \left\{ \frac{\left( \frac{k_w}{m} \right)}{\left( \frac{m_B}{M_b} \right) - \left( \frac{m_A}{M_a} \right)} \right\}}{\log_e} \right] = \left[ \frac{\log_e \left( \frac{k_w}{m} \right) - \log_e \left\{ \left( \frac{m_B}{M_b} \right) - \left( \frac{m_A}{M_a} \right) \right\}}{\log_e} \right] = 14 -$$

$$\left[ \frac{\ln \left( \frac{k_w}{m} \right) - \ln \left\{ \left( \frac{m_B}{M_b} \right) - \left( \frac{m_A}{M_a} \right) \right\}}{\ln 10} \right] = \frac{\ln \left( \frac{k_w}{m} \right)}{\ln 10} - \frac{\ln \left( \frac{m_B}{M_b} \right)}{\ln 10} - \frac{\ln \left( \frac{m_A}{M_a} \right)}{\ln 10} \dots \text{equation 24}$$

$$NB: \left( \frac{m_B}{M_b} - \frac{m_A}{M_a} \right) = \left( \frac{m_B M_a - m_A M_b}{M_b M_a} \right)$$

$$\frac{\ln \left( \frac{k_w}{m} \right)}{\ln 10} - \frac{\ln \left( \frac{m_B}{M_b} \right)}{\ln 10} - \frac{\ln \left( \frac{m_A}{M_a} \right)}{\ln 10} = \left[ \frac{\ln \left( \frac{k_w}{m} \right)}{\ln 10} - \left( \frac{\ln(m_B M_a - m_A M_b)}{\ln 10} \right) + \left( \frac{\ln M_b M_a}{\ln 10} \right) \right] \dots \text{equation 25}$$

Differentiating equation 25 with respect to  $m_A, m_B, M_a, M_b,$  and  $m$  we get;

$$\frac{dpH}{dm_A} = 0 - \left[ \left( \frac{1}{\ln 10} \right) \left( \frac{1}{m_B M_a - m_A M_b} \cdot -M_b \right) \right] + 0 = \left( \frac{M_b}{(\ln 10)(m_B M_a - m_A M_b)} \right) \dots\dots\dots \text{equation 26}$$

$$\frac{dpH}{dm_B} = 0 - \left[ \left( \frac{1}{\ln 10} \right) \left( \frac{1}{m_B M_a - m_A M_b} \cdot M_a \right) \right] + 0 = \left( - \frac{M_a}{(\ln 10)(m_B M_a - m_A M_b)} \right) \dots\dots\dots \text{equation 27}$$

$$\frac{dpH}{dM_a} = 0 - \left[ \left( \frac{1}{\ln 10} \right) \left( \frac{1}{m_B M_a - m_A M_b} \cdot m_B \right) \right] + \left[ \left( \frac{1}{\ln 10} \right) \left( \frac{1}{M_a M_b} \right) \right] = \left( - \frac{m_B}{(\ln 10)(m_B M_a - m_A M_b)} \right) + \left( \frac{1}{(\ln 10)(M_a M_b)} \right) \dots\dots\dots \text{equation 28}$$

$$\frac{dpH}{dM_b} = 0 - \left[ \left( \frac{1}{\ln 10} \right) \left( \frac{1}{m_B M_a - m_A M_b} \cdot -m_A \right) \right] + \left[ \left( \frac{1}{\ln 10} \right) \left( \frac{1}{M_a M_b} \right) \right] = \left( \frac{m_A}{(\ln 10)(m_B M_a - m_A M_b)} \right) + \left( \frac{1}{(\ln 10)(M_a M_b)} \right) \dots\dots\dots \text{equation 29}$$

$$\frac{dpH}{dm} = \left[ \left( \frac{1}{\ln 10} \right) \left( \frac{1}{\frac{k_w}{m}} \right) \right] - 0 + 0 = \left( \left( \frac{1}{\ln 10} \right) \frac{1}{\left( \frac{k_w}{m} \right)} \right) \dots\dots\dots \text{equation 30}$$

$$S^2 pH = \left\{ \left( \frac{dpH}{dm_A} \cdot S_{m_A} \right)^2 + \left( \frac{dpH}{dm_B} \cdot S_{m_B} \right)^2 + \left( \frac{dpH}{dM_a} \cdot S_{M_a} \right)^2 + \left( \frac{dpH}{dM_b} \cdot S_{M_b} \right)^2 + \left( \frac{dpH}{dm} \cdot S_m \right)^2 + \left( \frac{du(P)}{dP} \cdot S_P \right)^2 \right\} \dots\dots\dots \text{equation 31}$$

NB:  $m$  is addition of mass of base and excess mass, divided by excess mass

#### 4.1.5 Model equations for calculations of pH values of weak acids, bases and salts

- Before equivalence

	$BH^+ < \text{-----} > B + H^+$	
<i>initial</i>	$C_0 = \frac{n_0}{v_0}$	0      0
<i>change</i>	$\frac{n_0 - x}{v_0}$	$\frac{x}{v_0}$ $\frac{x}{v_0}$
<i>Base addition</i>	$\frac{n_0 - x}{v_0 + v_1}$	$\frac{x + n_1}{v_0 + v_1}$ $\frac{x}{v_0 + v_1}$



$$\text{Equilibrium} \quad \frac{n_0-x+y}{v_0+v_1} \qquad \frac{x+n_1-y}{v_0+v_1} \qquad \frac{x-y}{v_0+v_1}$$

$$\frac{n_0-z}{v_0+v_1} \qquad \frac{n_1+z}{v_0+v_1} \qquad \frac{z}{v_0+v_1}$$

NB;  $z = x - y$ ;  $z < n_0$

$$k_a = \left( \frac{B \cdot H^+}{BH^+} \right) = \left( \frac{\left( \frac{n_1+z}{v_0+v_1} \right) \left( \frac{z}{v_0+v_1} \right)}{\left( \frac{n_0-z}{v_0+v_1} \right)} \right) = \left( \frac{z(n_1+z)}{(n_0-z)(v_0+v_1)} \right) = [H^+] \frac{n_1+z}{n_0-z} = [H^+] \frac{c_1 v_1 + z}{c_0 v_0 - z}$$

$$\Rightarrow k_a = (n_0 - z)(v_0 + v_1) = z(n_1 + z) = zn_1 + z^2$$

$$\Rightarrow z^2 + zn_1 = k_a n_0 (v_0 + v_1) - k_a (v_0 + v_1) \cdot z$$

$$\Rightarrow z^2 + [n_1 + k_a(v_0 + v_1)]z - k_a n_0 (v_0 + v_1) = 0$$

$$\Rightarrow z = \frac{[-n_1 + k_a(v_0 + v_1)] \pm \sqrt{[n_1 + k_a(v_0 + v_1)]^2 + 4k_a n_0 (v_0 + v_1)}}{2}$$

$$\Rightarrow z = \frac{[-c_1 v_1 + k_a(v_0 + v_1)] \pm \sqrt{[c_1 v_1 + k_a(v_0 + v_1)]^2 + 4k_a c_0 v_0 (v_0 + v_1)}}{2}$$

Henderson Hasselbalch equation;  $[H^+] = k_a \cdot \frac{c_1 v_1}{c_0 v_0}$ , Adjusting this value by the z value we get;

$$[H^+] = k_a \cdot \left[ \frac{c_1 v_1 + z}{c_0 v_0 - z} \right] \dots \dots \dots \text{equation 32}$$

Substituting equation 32 into 1;

$$pH = -\log \left( k_a \cdot \left[ \frac{c_1 v_1 + z}{c_0 v_0 - z} \right] \right)$$

$$= -\log_{10} \left( k_a \cdot \left[ \frac{c_1 v_1 + z}{c_0 v_0 - z} \right] \right)^{-1} = \log_{10} \left( k_a \cdot \left[ \frac{c_0 v_0 - z}{c_1 v_1 + z} \right] \right) \dots \dots \dots \text{equation 33}$$

Differentiating equation 33 with respect to  $k_a, c_1, c_0, v_1$  and  $v_0$  we get;

$$pH = \frac{1}{u} \frac{\partial u}{\partial k_a}; \text{ let } u = \left( k_a \cdot \left[ \frac{c_0 v_0 - z}{c_1 v_1 + z} \right] \right)$$

$$u' = 1 \cdot \left( \frac{c_0 v_0 - z}{c_1 v_1 + z} \right) + k_a \left( \frac{0 - \frac{1}{2} \left[ -0 + (v_0 + v_1) \right] \pm \left( [c_1 v_1 + k_a (v_0 + v_1)]^2 + 4k_a c_0 v_0 (v_0 + v_1) \right)^{-\frac{1}{2}} \cdot [2(c_1 v_1 + k_a (v_0 + v_1)) * (v_0 + v_1) 4c_0 v_0 (v_0 + v_1)]}{0 + \frac{1}{2} \left[ -0 + (v_0 + v_1) \right] \pm \left( [c_1 v_1 + k_a (v_0 + v_1)]^2 + 4k_a c_0 v_0 (v_0 + v_1) \right)^{-\frac{1}{2}} \cdot [2(c_1 v_1 + k_a (v_0 + v_1)) * (v_0 + v_1) 4c_0 v_0 (v_0 + v_1)]} \right)$$

$$\frac{\partial pH}{\partial k_a} = \frac{\left( \frac{c_0 v_0 - z}{c_1 v_1 + z} \right) + k_a}{k_a \left( \frac{c_0 v_0 - z}{c_1 v_1 + z} \right)} \dots \dots \dots \text{equation 34}$$

$$pH = \frac{1}{u} \frac{\partial u}{\partial c_1};$$

$$u' = k_a \left( \frac{0 - \frac{1}{2} \left[ -v_1 + k_a \pm \left( [c_1 v_1 + k_a (v_0 + v_1)]^2 + 4k_a c_0 v_0 (v_0 + v_1) \right)^{-\frac{1}{2}} \cdot [2(c_1 v_1 + k_a (v_0 + v_1)) * v_1 + 0] \right]}{v_1 + \frac{1}{2} \left[ v_1 + 0 \pm \left( [c_1 v_1 + k_a (v_0 + v_1)]^2 + 4k_a c_0 v_0 (v_0 + v_1) \right)^{-\frac{1}{2}} \cdot [2(c_1 v_1 + k_a (v_0 + v_1)) * v_1] \right]} \right)$$

$$\frac{\partial pH}{\partial c_1} = \frac{k_a \left( -\frac{1}{2} \left[ -v_1 k_a \pm \left( \frac{c_1 v_1 + k_a (v_0 + v_1) v_1}{[c_1 v_1 + k_a (v_0 + v_1)]^2 + 4k_a c_0 v_0 (v_0 + v_1)} \right) \right] \div \left( v_1 + \frac{1}{2} \left[ v_1 \pm \left( \frac{c_1 v_1 + k_a (v_0 + v_1) v_1}{[c_1 v_1 + k_a (v_0 + v_1)]^2 + 4k_a c_0 v_0 (v_0 + v_1)} \right) \right] \right)}{k_a \left( \frac{c_0 v_0 - z}{c_1 v_1 + z} \right)}$$

.....equation 35

$$pH = \frac{1}{u} \frac{\partial u}{\partial c_0}$$

$$u' = k_a \left( \frac{v_0 - \frac{1}{2} \left[ \frac{-0 + 0 \pm \frac{1}{2} \left( [c_1 v_1 + k_a (v_0 + v_1)]^2 + 4k_a c_0 v_0 (v_0 + v_1) \right)^{-\frac{1}{2}} * (0 + 4k_a v_0 (v_0 + v_1))}{2} \right]}{0 + \frac{1}{2} \left[ \frac{\pm \frac{1}{2} \left( [c_1 v_1 + k_a (v_0 + v_1)]^2 + 4k_a c_0 v_0 (v_0 + v_1) \right)^{-\frac{1}{2}} * (0 + 4k_a v_0 (v_0 + v_1))}{2} \right]} \right)$$

$$\frac{\partial pH}{\partial c_0} = \frac{k_a \left( v_0 - \frac{1}{2} \left[ \pm \left( \frac{4k_a v_0 (v_0 + v_1)}{[c_1 v_1 + k_a (v_0 + v_1)]^2 + 4k_a c_0 v_0 (v_0 + v_1)} \right) \right] \div \left( \frac{1}{2} \left[ \pm \left( \frac{4k_a v_0 (v_0 + v_1)}{[c_1 v_1 + k_a (v_0 + v_1)]^2 + 4k_a c_0 v_0 (v_0 + v_1)} \right) \right] \right)}{k_a \left( \frac{c_0 v_0 - z}{c_1 v_1 + z} \right)} \dots \dots \dots$$

.....equation 36

$$pH = \frac{1}{u} \frac{\partial u}{\partial v_1};$$

$$u' = k_a \left( \frac{0 - \frac{1}{2} \left[ -c_1 \pm \left( [c_1 v_1 + k_a(v_0 + v_1)]^2 + 4k_a c_0 v_0 (v_0 + v_1) \right)^{\frac{1}{2}} \cdot 2(c_1 v_1 + k_a(v_0 + v_1) \cdot c_1) \right]}{c_1 + \frac{1}{2} \left[ c_1 + 0 \pm \left( [c_1 v_1 + k_a(v_0 + v_1)]^2 + 4k_a c_0 v_0 (v_0 + v_1) \right)^{\frac{1}{2}} \cdot 2(c_1 v_1 + k_a(v_0 + v_1) \cdot c_1) \right]} \right)$$

$$\frac{\partial pH}{\partial v_1} = \frac{k_a \cdot \left( -\frac{1}{2} \left[ \pm \frac{(c_1 v_1 + k_a(v_0 + v_1)) c_1}{\sqrt{[c_1 v_1 + k_a(v_0 + v_1)]^2 + 4k_a c_0 v_0 (v_0 + v_1)}} \right] + c_1 + \frac{1}{2} \left[ c_1 \pm \frac{(c_1 v_1 + k_a(v_0 + v_1)) c_1}{\sqrt{[c_1 v_1 + k_a(v_0 + v_1)]^2 + 4k_a c_0 v_0 (v_0 + v_1)}} \right]}{k_a \left( \frac{c_0 v_0 - z}{c_1 v_1 + z} \right)} \right) \dots \text{equation 37}$$

$$pH = \frac{1}{u} \frac{\partial u}{\partial v_0};$$

$$u' =$$

$$k_a \left( \frac{c_0 - \frac{1}{2} \left[ -0 + k_a \pm \left( [c_1 v_1 + k_a(v_0 + v_1)]^2 + 4k_a c_0 v_0 (v_0 + v_1) \right)^{\frac{1}{2}} \cdot 2(c_1 v_1 + k_a(v_0 + v_1)(0 + k_a)) + 4k_a c_0 (v_0 + v_1) + 4k_a c_0 v_0 \right]}{0 + \frac{1}{2} \left[ -0 + k_a \pm \left( [c_1 v_1 + k_a(v_0 + v_1)]^2 + 4k_a c_0 v_0 (v_0 + v_1) \right)^{\frac{1}{2}} \cdot 2(c_1 v_1 + k_a(v_0 + v_1)(0 + k_a)) + 4k_a c_0 (v_0 + v_1) + 4k_a c_0 v_0 \right]} \right)$$

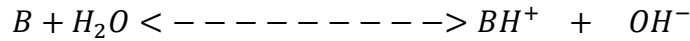
$$\frac{\partial pH}{\partial v_0} = \frac{k_a \left( \frac{c_0 - \frac{1}{2} \left( (k_a \pm 2(c_1 v_1 + k_a(v_0 + v_1)(k_a)) + 4k_a c_0 (v_0 + v_1) + 4k_a c_0 v_0) \div \sqrt{[c_1 v_1 + k_a(v_0 + v_1)]^2 + 4k_a c_0 v_0 (v_0 + v_1)} \right)}{\left[ \frac{1}{2} \left( (k_a \pm 2(c_1 v_1 + k_a(v_0 + v_1)(k_a)) + 4k_a c_0 (v_0 + v_1) + 4k_a c_0 v_0) \div \sqrt{[c_1 v_1 + k_a(v_0 + v_1)]^2 + 4k_a c_0 v_0 (v_0 + v_1)} \right) \right]} \right)}{k_a \left( \frac{c_0 v_0 - z}{c_1 v_1 + z} \right)}$$

..... equation 38

Substituting equation 35,36,37,38 and 39 into equation 13;

$$S^2 pH = \left\{ \left( \frac{\partial pH}{\partial k_a} \cdot S_{k_a} \right)^2 + \left( \frac{\partial pH}{\partial c_1} \cdot S_{c_1} \right)^2 + \left( \frac{\partial pH}{\partial c_0} \cdot S_{c_0} \right)^2 + \left( \frac{\partial pH}{\partial v_1} \cdot S_{v_1} \right)^2 + \left( \frac{\partial pH}{\partial v_0} \cdot S_{v_0} \right)^2 + \left( \frac{dpH}{dr} \cdot S_r \right)^2 \right\} \dots \dots \dots \text{equation 39}$$

○ After equivalence point



<i>initial</i>	$C_0$	0	0	
<i>Change</i>	$\frac{n_1-x}{v_1}$	$x$	$x$	
<i>Base addition</i>	$\frac{n_1-x}{v_0+v_1}$	$\frac{x+n_1}{v_0+v_1}$	$\frac{x}{v_0+v_1}$	
<i>Equilibrium</i>	$\frac{n_1-x+y}{v_0+v_1}$	$\frac{x+n_0-y}{v_0+v_1}$	$\frac{x-y}{v_0+v_1}$	<i>NB; Z = x - y</i>
	$\frac{n_1-z}{v_0+v_1}$	$\frac{n_0+z}{v_0+v_1}$	$\frac{z}{v_0+v_1}$	

$$k_b = \frac{(BH^+)(OH^-)}{B} = \frac{\left(\frac{n_0+z}{v_0+v_1}\right)\left(\frac{z}{v_0+v_1}\right)}{\frac{n_1-z}{v_0+v_1}} = \frac{(n_0+z)z}{(n_1-z)(v_0+v_1)} = [H^+] \frac{n_0+z}{n_1-z} = [H^+] \frac{c_0v_0+z}{c_1v_1-z}$$

$$\Rightarrow Z = \frac{[-n_0+k_b(v_0+v_1)] \pm \sqrt{[n_0+k_b(v_0+v_1)]^2 + 4k_b n_1(v_0+v_1)}}{2}$$

$$\Rightarrow Z = \frac{[-c_0v_0+k_b(v_0+v_1)] \pm \sqrt{[c_0v_0+k_b(v_0+v_1)]^2 + 4k_b c_1 v_1(v_0+v_1)}}{2}$$

$$\Rightarrow [OH^-] = k_b \cdot \frac{c_0v_0+z}{c_1v_1-z} \dots \dots \dots \text{equation 40}$$

Substituting equation 40 into 22;

$$pH = 14 - \left[ -\log \left( k_b \cdot \left[ \frac{c_0v_0+z}{c_1v_1-z} \right] \right) \right] = 14 - \left[ \log_{10} \left\{ k_b \cdot \left[ \frac{c_0v_0+z}{c_1v_1-z} \right] \right\}^{-1} \right] = 14 - \left[ \log_{10} \left\{ k_b \cdot \left[ \frac{c_1v_1-z}{c_0v_0+z} \right] \right\} \right] \dots \dots \dots \text{equation 41}$$

Differentiating equation 41 with respect to  $k_b, c_1, c_0, v_1$  and  $v_0$  we get;

$$pH = \frac{1}{u} \frac{\partial u}{\partial k_b}; \text{ let } u = \left( k_b \cdot \left[ \frac{c_1v_1-z}{c_0v_0+z} \right] \right)$$

$$u' = 1 \cdot \left( \frac{c_1 v_1 - z}{c_0 v_0 + z} \right) + k_b \cdot$$

$$\left( \frac{0 - \frac{1}{2} \left[ -0 + (v_0 + v_1) \right] \pm \left( [c_0 v_0 + k_b (v_0 + v_1)]^2 + 4k_b c_0 v_0 (v_0 + v_1) \right)^{-\frac{1}{2}} \cdot 2[c_0 v_0 + k_b (v_0 + v_1)] \cdot (v_0 + v_1) + 4c_0 v_0 (v_0 + v_1)}{0 + \frac{1}{2} \left[ -0 + (v_0 + v_1) \right] \pm \left( [c_0 v_0 + k_b (v_0 + v_1)]^2 + 4k_b c_0 v_0 (v_0 + v_1) \right)^{-\frac{1}{2}} \cdot 2[c_0 v_0 + k_b (v_0 + v_1)] \cdot (v_0 + v_1) + 4c_0 v_0 (v_0 + v_1)} \right)$$

$$\frac{\partial pH}{\partial k_b} = \left\{ \frac{\left( \frac{c_1 v_1 - z}{c_0 v_0 + z} \right) + k_b}{k_b \cdot \left[ \frac{c_1 v_1 - z}{c_0 v_0 + z} \right]} \right\} \dots \text{equation 42}$$

$$pH = \frac{1}{u} \frac{\partial u}{\partial c_1};$$

$$u' = k_b \left( \frac{v_1 - \frac{1}{2} \left[ -0 + 0 \right] \pm \left( [c_0 v_0 + k_b (v_0 + v_1)]^2 + 4k_b c_0 v_0 (v_0 + v_1) \right)^{-\frac{1}{2}} \cdot [2(c_0 v_0 + k_b (v_0 + v_1)) * 0 + 0]}{0 + \frac{1}{2} \left[ -0 + 0 \right] \pm \left( [c_0 v_0 + k_b (v_0 + v_1)]^2 + 4k_b c_0 v_0 (v_0 + v_1) \right)^{-\frac{1}{2}} \cdot [2(c_0 v_0 + k_b (v_0 + v_1)) * 0 + 0]} \right)$$

$$\frac{\partial pH}{\partial c_1} = \left\{ k_b (v_1) \div \left( k_b \cdot \left[ \frac{c_1 v_1 - z}{c_0 v_0 + z} \right] \right) \right\} \dots \text{equation 43}$$

$$pH = \frac{1}{u} \frac{\partial u}{\partial c_0};$$

$$u' = k_b \left( \frac{0 - \frac{1}{2} \left[ -v_0 + 0 \right] \pm \left( [c_0 v_0 + k_b (v_0 + v_1)]^2 + 4k_b c_0 v_0 (v_0 + v_1) \right)^{-\frac{1}{2}} \cdot 2[c_0 v_0 + k_b (v_0 + v_1)] \cdot 2(v_0 + 0) + 4k_b v_0 (v_0 + v_1)}{v_0 + \frac{1}{2} \left[ -v_0 + 0 \right] \pm \left( [c_0 v_0 + k_b (v_0 + v_1)]^2 + 4k_b c_0 v_0 (v_0 + v_1) \right)^{-\frac{1}{2}} \cdot 2[c_0 v_0 + k_b (v_0 + v_1)] \cdot 2(v_0 + 0) + 4k_b v_0 (v_0 + v_1)} \right)$$

$$\frac{\partial pH}{\partial c_0} =$$

$$\left\{ \frac{k_b \cdot \left( \frac{1}{2} \left[ v_0 \pm \left( \frac{2[c_0 v_0 + k_b (v_0 + v_1)] \cdot 2(v_0) + 4k_b v_0 (v_0 + v_1)}{\sqrt{[c_0 v_0 + k_b (v_0 + v_1)]^2 + 4k_b c_0 v_0 (v_0 + v_1)}} \right) \right] \right) \div \left( v_0 + \frac{1}{2} \left[ -v_0 \pm \left( \frac{2[c_0 v_0 + k_b (v_0 + v_1)] \cdot 2(v_0) + 4k_b v_0 (v_0 + v_1)}{\sqrt{[c_0 v_0 + k_b (v_0 + v_1)]^2 + 4k_b c_0 v_0 (v_0 + v_1)}} \right) \right] \right)}{k_b \cdot \left[ \frac{c_1 v_1 - z}{c_0 v_0 + z} \right]} \right\} \dots$$

$$\dots \text{equation 44}$$

$$pH = \frac{1}{u} \frac{\partial u}{\partial v_1};$$

$$u' = k_b \left( \frac{c_1 - \frac{1}{2} \left[ -0 + kb \pm ([c_0 v_0 + k_b(v_0 + v_1)]^2 + 4k_b c_0 v_0(v_0 + v_1))^{-\frac{1}{2}} \cdot 2(c_0 v_0 + k_b(v_0 + v_1)) kb + 4k_b c_0 v_0 \right]}{0 + \frac{1}{2} \left[ -0 + kb \pm ([c_0 v_0 + k_b(v_0 + v_1)]^2 + 4k_b c_0 v_0(v_0 + v_1))^{-\frac{1}{2}} \cdot 2(c_0 v_0 + k_b(v_0 + v_1)) kb + 4k_b c_0 v_0 \right]} \right)$$

$$\frac{\partial pH}{\partial v_1} = \left\{ \frac{k_b \cdot \left( c_1 - \frac{1}{2} \left[ kb \pm \left( \frac{2(c_0 v_0 + k_b(v_0 + v_1)) kb + 4k_b c_0 v_0}{\sqrt{[c_0 v_0 + k_b(v_0 + v_1)]^2 + 4k_b c_0 v_0(v_0 + v_1)}} \right) \right] \right)}{k_b \cdot \left[ \frac{c_1 v_1 - z}{c_0 v_0 + z} \right]} \right\}$$

..... equation 45

$$pH = \frac{1}{u} \frac{\partial u}{\partial v_0};$$

$$u' =$$

$$k_b \left( \frac{0 - \frac{1}{2} \left[ -c_0 + kb \pm ([c_0 v_0 + k_b(v_0 + v_1)]^2 + 4k_b c_0 v_0(v_0 + v_1))^{-\frac{1}{2}} \cdot 2(c_0 v_0 + k_b(v_0 + v_1))(c_0 + kb) + 4k_b c_0(v_0 + v_1) + 4k_b c_0 v_0 \right]}{c_0 + \frac{1}{2} \left[ -c_0 + kb \pm ([c_0 v_0 + k_b(v_0 + v_1)]^2 + 4k_b c_0 v_0(v_0 + v_1))^{-\frac{1}{2}} \cdot 2(c_0 v_0 + k_b(v_0 + v_1))(c_0 + kb) + 4k_b c_0(v_0 + v_1) + 4k_b c_0 v_0 \right]} \right)$$

$$\frac{\partial pH}{\partial v_0} = k_b \left( \frac{\frac{1}{2} \left[ \frac{c_0 + kb \pm [2(c_0 v_0 + k_b(v_0 + v_1))(c_0 + kb) + 4k_b c_0(v_0 + v_1) + 4k_b c_0 v_0]}{\sqrt{[c_0 v_0 + k_b(v_0 + v_1)]^2 + 4k_b c_0 v_0(v_0 + v_1)}} \right]}{c_0 + \frac{1}{2} \left[ \frac{-c_0 + kb \pm [2(c_0 v_0 + k_b(v_0 + v_1))(c_0 + kb) + 4k_b c_0(v_0 + v_1) + 4k_b c_0 v_0]}{\sqrt{[c_0 v_0 + k_b(v_0 + v_1)]^2 + 4k_b c_0 v_0(v_0 + v_1)}} \right]} \right) \div k_b \left[ \frac{c_1 v_1 - z}{c_0 v_0 + z} \right] \dots \text{equation 46}$$

Substituting equation 43,44,45,46 and 47 into equation 13;

$$S^2 pH = \left( \frac{\partial pH}{\partial k_b} \cdot S_{k_b} \right)^2 + \left( \frac{\partial pH}{\partial c_1} \cdot S_{c_1} \right)^2 + \left( \frac{\partial pH}{\partial c_0} \cdot S_{c_0} \right)^2 + \left( \frac{\partial pH}{\partial v_1} \cdot S_{v_1} \right)^2 + \left( \frac{\partial pH}{\partial v_0} \cdot S_{v_0} \right)^2 + \left( \frac{dpH}{dr} \cdot S_r \right)^2 \dots \text{equation 47}$$

**NB;** For all the results, all calculations, tables and figures, all of the raw data (from Fig. 22) was utilized without rejecting outliers.  $c_1$  is the concentration of base,  $c_0$  is the concentration of acid,  $v_1$  is volume of base,  $v_0$  is volume of acid,  $k_a$  is acid dissociation constant,  $k_b$  is base dissociation constant,  $n_0$  is moles of acid,  $n_1$  is moles of base,  $k_w$  is the ionic strength of water.

#### 4.1.6 Applications of the derived equations

The equations derived before equivalence point, at equivalence point, and after equivalence point from titrations of strong acid and strong base were used to draw a titration curve. These equations were derived with the knowledge that the H-H equation cannot be used to draw a titration curve according to Po and Senozan [69]. The derived equations were used to calculate pH values and the values were compared with the measured pH values, and the pH values calculated using equation 1. Equation 1 is the one that is normally used to draw a titration curve.

For the derived equations, before the equilibrium point pH values were calculated using equation 7, at equilibrium equation 16 was used and after equilibrium calculation were completed using equation 22. The results plotted on the following figure, a dotted peach line is a curve drawn using the derived equations.

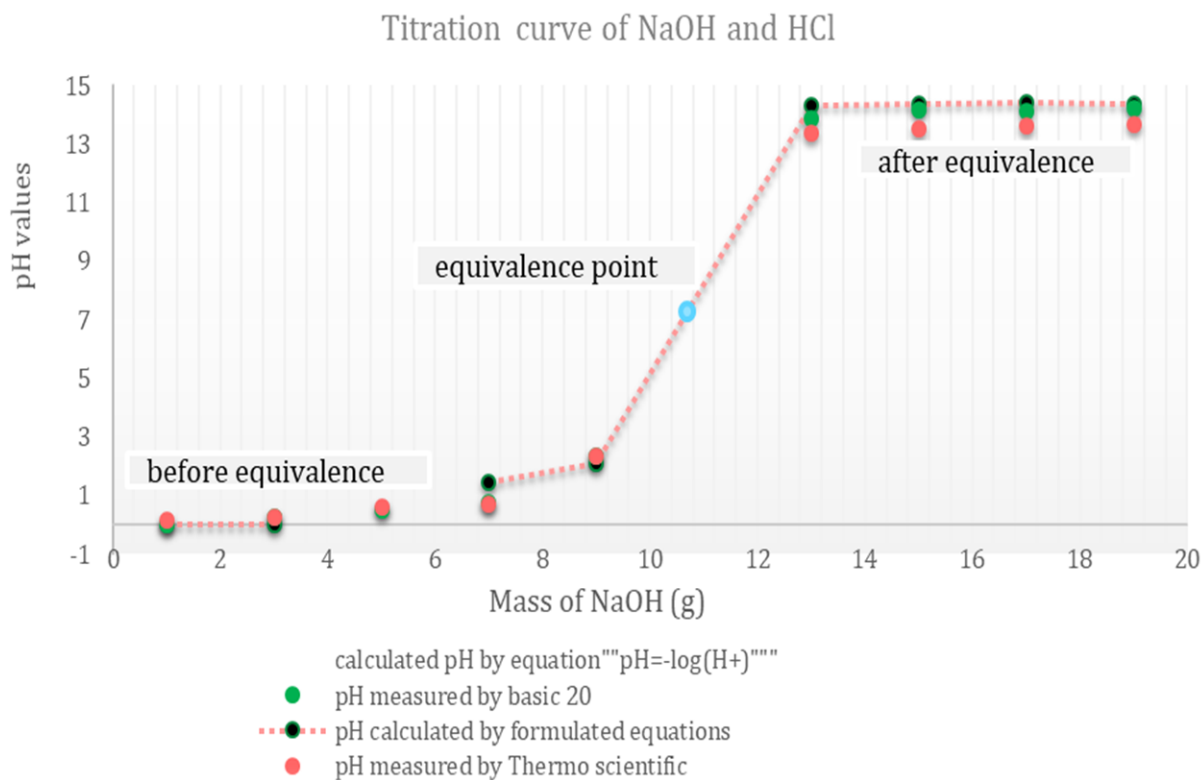


Figure 15: Graph of a titration curve of a strong acid and strong base prepared by weighing

Uncertainty was predicted from titration of weak acid, weak bases and salts at pH -2.08 and 13.3. Firstly, uncertainty was predicted using the uncertainty budget table. This is a tool that is used by QUAM to predict uncertainty. Then, the uncertainty was calculated using the derived equations for comparison. For calculations on an acid medium, the uncertainty budget table (Table 9) was used. The table predicted uncertainty on (Fig. 16) which was compared to uncertainty value calculated by derived equations (Fig. 17). For calculations on a basic medium, the uncertainty budget table (Table 10) was used. The table predicted uncertainty on (Fig. 18) which was compared to uncertainty value calculated by derived equations (Fig. 19).

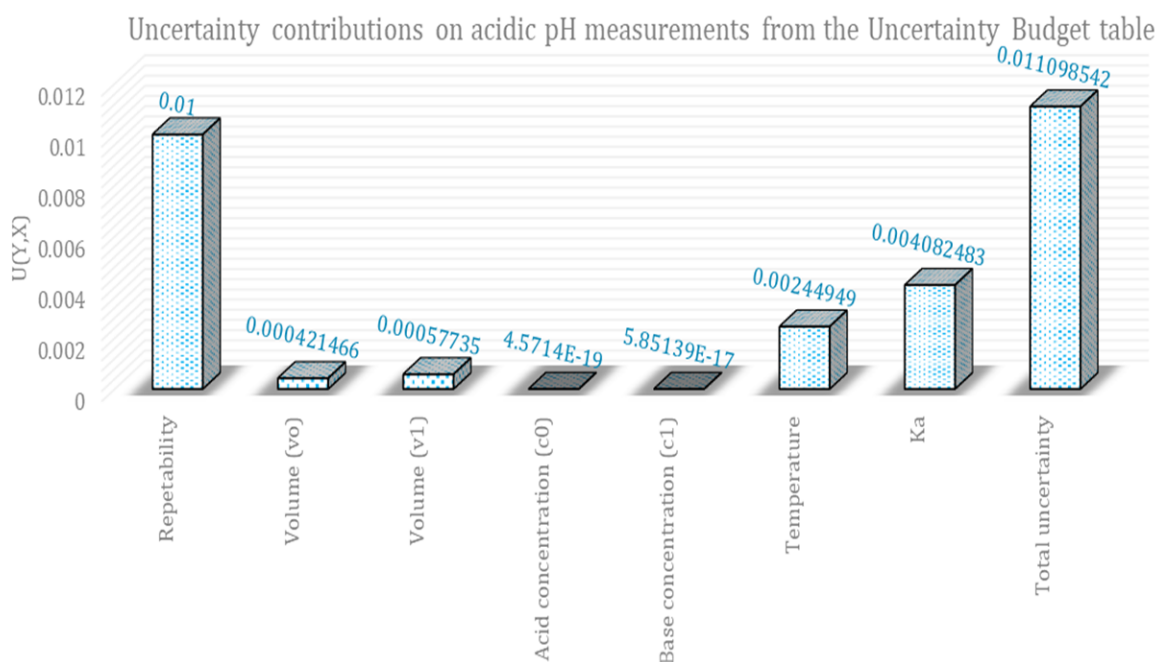


Figure 16: A graph showing total uncertainty and the sources of uncertainty on measurements of acidic pH values that were obtained from the Uncertainty budget table.

The above figure shows the calculations performed using the uncertainty budget table (Table 9). Repeatability, Concentration of acid and base are type A sources of uncertainty obtained from short series of data, the rest are type B uncertainty sources. The total uncertainty at pH -2.08 is 0.01.



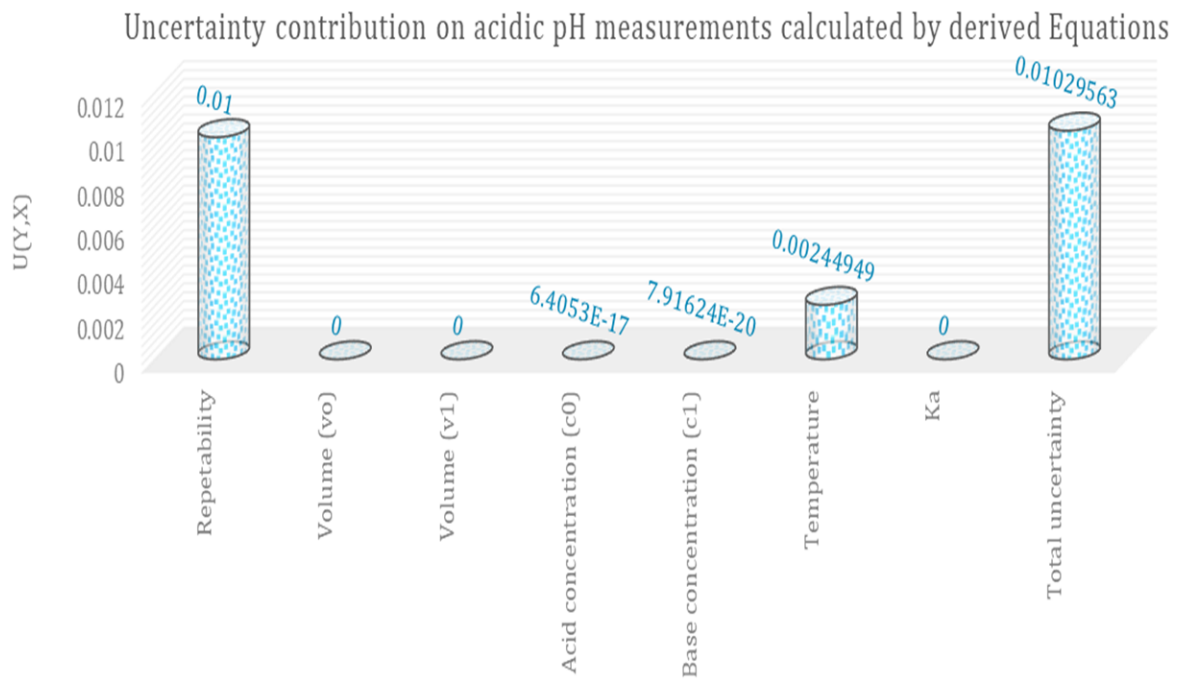


Figure 17: A graph showing total uncertainty and the sources of uncertainty on measurements of acidic pH values that were obtained from calculations by equations 39.

The above figure shows the calculations performed using a derived equation 39. Temperature is type B, the rest are type A uncertainty sources obtained from pooled data. The total uncertainty at pH -2.08 is 0.01.

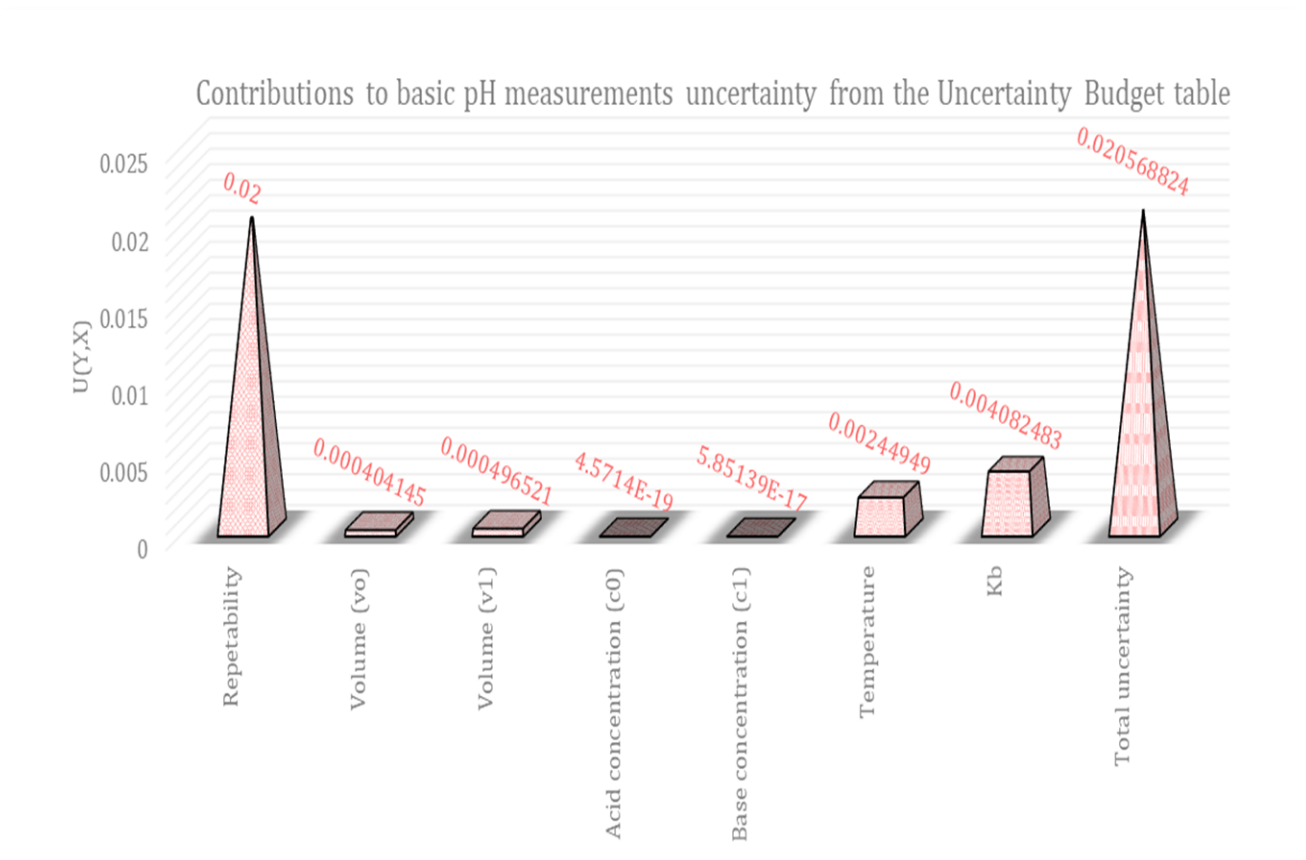


Figure 18: A graph showing total uncertainty and the sources of uncertainty on measurements of basic pH values that were obtained from the Uncertainty budget table.

The above figure shows the calculations performed using the uncertainty budget table (Table 10). Repeatability, Concentration of acid and base are type A sources of uncertainty obtained from short series of data, the rest are type B uncertainty sources. The total uncertainty at pH 13.3 is 0.02.

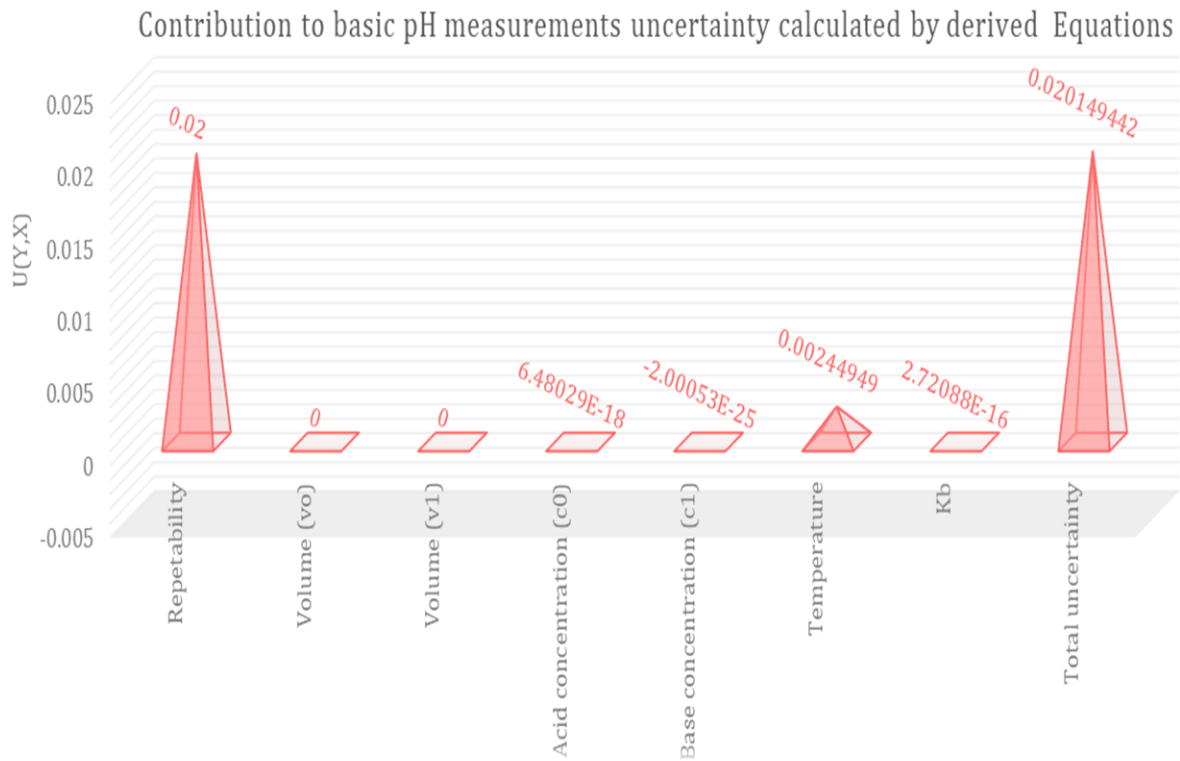


Figure 19: A graph showing total uncertainty and the sources of uncertainty on measurements of basic pH values that were obtained from calculations by equations 47.

The above figure shows the calculations performed using a derived equation 47. Temperature is type B, the rest are type A uncertainty sources obtained from pooled data. The total uncertainty at pH 13.3 is 0.02.

Table 9: Uncertainty budget table of single cross-sectional data for acidic pH measurements

A full uncertainty budget for measurement of acidic pH values using Basic 20 pH meter and Thermo scientific Orion star pH meter										
Basic 20 pH meter (relative accuracy of ±1digit) and Thermo scientific Orion star (relative accuracy of ± 0.01)										
pH range -2 to 16										
Uncertainty analysis										
	sensitivity coefficient	Uncertainty value	Unit of measure	Type	source of the information	probability distribution	divisor	std uncertainty	degrees of freedom	significance check
Temperature	1	0.00244949	°C	B	guides	triangular	2.4494897	0.001	1	0.73%
Repeatability	1	0.02		A	calculations	rectangular	1.7320508	0.011547005	1	97.18%
Volume (v0)	1	0.00042147	L	B	guides	triangular	2.4494897	0.000172063	1	0.02%
Added volume (v1)	1	0.00057735	L	B	guides	triangular	2.4494897	0.000235702	1	0.04%
Acid dissociation (Ka)	1	0.00408248		B	guides	triangular	2.4494897	0.001666667	1	2.02%
Acid concentration (c0)	1	4.5714E-19	M	A	calculations	rectangular	1.7320508	2.6393E-19	1	0.00%
Base concentration (c1)	1	5.8514E-17	M	A	calculations	rectangular	1.7320508	3.3783E-17	1	0.00%
Combined uncertainty $U_c(y)$								0.011713081	$\nu_{eff}$ 1.058264	100.00%
Expansion coefficient								1.96		
Expanded uncertainty (k=2, 95% confidence level)								0.022957639		
notes										
The sources of uncertainty were identified by analysing anything that had occurred in the laboratory										
Uncertainties that was collected by self from testing and experimenting is treated as Type A										
Uncertainties that was collected from other sources is treated as Type B										
Calculated pH values to prepare buffer solution provided type A data										
The reference materials were obtained from repots, guides and books										
Reapetability is the standard deviation of reapeted measurements										
divisor is what we use to devide the rectangular/triangular uncertainty to convert to standard uncertainty										

Table 10: Uncertainty budget table of single cross-sectional data for basic pH measurements

A full uncertainty budget for measurement of basic pH values using Basic 20 pH meter and Thermo scientific Orion star pH meter																	
Basic 20 pH meter (relative accuracy of $\pm 1$ digit) and Thermo scientific Orion star (relative accuracy of $\pm 0.01$ )																	
pH range - 2 to 16																	
Uncertainty analysis																	
	sensitivity coefficient	Uncertainty value	Unit of measure	Type	source of the information	probability distribution	divisor	std uncertainty	degrees of freedom	significance check							
Temperature	1	0.00244949	°C	B	publications	triangular	2.4495	0.001	1	2.69%							
Repeatability	1	0.01		A	calculations	rectangular	1.7321	0.0057735	1	89.61%							
Volume (v0)	1	0.00042147	L	B	calculations	triangular	2.4495	0.00017206	1	0.08%							
Added volume (v1)	1	0.00057735	L	B	calculations	triangular	2.4495	0.0002357	1	0.15%							
Base dissociation (Kb)	1	0.00408248		B	guides	triangular	2.4495	0.00166667	1	7.47%							
Acid concentration (c0)	1	4.5714E-19	M	A	calculations	rectangular	1.7321	2.6393E-19	1	0.00%							
Base concentration (c1)	1	5.8514E-17	M	A	calculations	rectangular	1.7321	3.3783E-17	1	0.00%							
Combined uncertainty $U_c(y)$																	
Expansion coefficient																	
Expanded uncertainty (k=2, 95% confidence level)																	
notes																	
The sources of uncertainty were identified by analysing anything that had occurred in the laboratory																	
Uncertainties that was collected by self from testing and experimenting is treated as Type A																	
Uncertainties that was collected from other sources is treated as Type B																	
Calculated pH values to prepare buffer solution provided type A data																	
The reference materials were obtained from reports, guides and books																	
Repeatability is the standard deviation of repeated measurements																	
divisor is what we use to divide the rectangular/triangular uncertainty to convert to standard uncertainty																	
<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%;"></td> <td style="width: 50%; text-align: right;"><math>v_{eff}</math></td> </tr> <tr> <td>Combined uncertainty <math>U_c(y)</math></td> <td style="text-align: right;">0.00609887</td> </tr> <tr> <td>Expansion coefficient</td> <td style="text-align: right;">1.96</td> </tr> <tr> <td>Expanded uncertainty (k=2, 95% confidence level)</td> <td style="text-align: right;">0.01195379</td> </tr> </table>											$v_{eff}$	Combined uncertainty $U_c(y)$	0.00609887	Expansion coefficient	1.96	Expanded uncertainty (k=2, 95% confidence level)	0.01195379
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Expanded uncertainty (k=2, 95% confidence level)	0.01195379																

## The headings of the uncertainty budget columns

Sensitivity coefficient; it is evaluated as  $c_i = \Delta y / \Delta x_i$ , it describes how the value of  $y$  varies with changes in  $x_i$ ,

Uncertainty value; it varies with the types of uncertainty sources. For type A sources uncertainty values were calculated, for type B sources the value was taken from literature and adjusted to uncertainty value by dividing with the divisor.

Type; type A evaluation which is uncertainty estimate using statistics, or type B evaluation which is uncertainty estimate from any other source other than statistical evaluation.

Source of information; it is the origin of the uncertainty value, where it was taken from

Distribution; it is the probability distribution function of the value. It is simply the shape of the errors which take different forms such as triangular, rectangular and normal distributions.

Divisor; these are factors for adjusting the distributions. The triangular distribution is adjusted using  $\sqrt{3}$  and rectangular distribution is adjusted using  $\sqrt{6}$

Standard uncertainty; it is uncertainty given at standard deviation level, calculated by  $\left( \text{sensitivity coefficient} \cdot \frac{\text{uncertainty value}}{\text{divisor}} \right)$ .

Degrees of freedom; it is the number of repetitions minus one ( $n - 1$ ).

Significance check; the square of standard uncertainty of an input quantity over the sum of squares which is taken over all standard uncertainties of input quantities, expressed as

percentages  $\left( \frac{U_{std(1)}^2}{\sum (U_{std(1)}^2 + U_{std(n)}^2)} \right)$ .

#### 4.2 Estimation of Uncertainty of food samples

Uncertainty of food samples was determined from short series of data, over a short period of time. Ten measurement repetitions (N=10), were performed for each food sample. The data fulfills the ISO/ QUAM method, and the motive is to evaluate if the pH measurements were in statistical control. Predicted uncertainty was obtained by use of uncertainty budget table, while pooled calibration was obtained by combining results of individual meters.

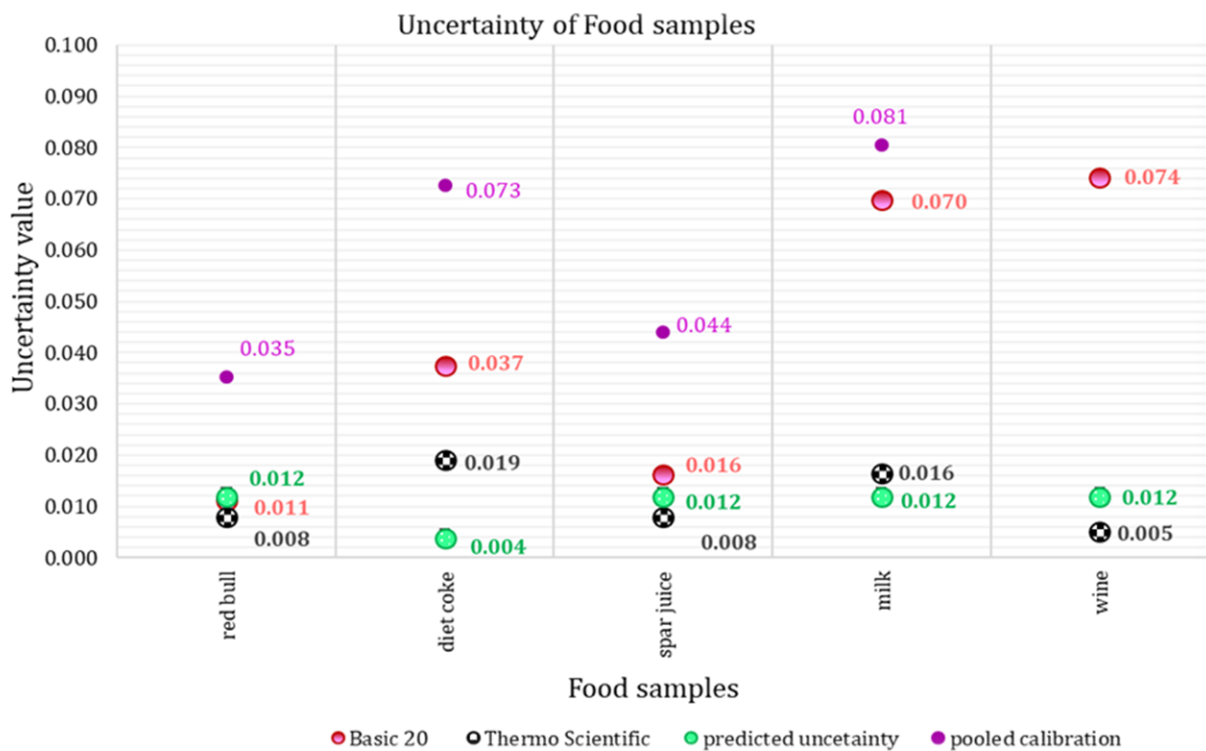


Figure 20: A graph showing Uncertainty estimate in the form of standard deviation of food samples measured by pH meters.

Table 11: Comparison of Relative Standard Deviation of measured pH values with the Relative Standard Deviation predicted using HorRat value.

<b>At pH measurement</b>	<b>-2.08</b>	<b>13.3</b>
Proton concentration (g/g)	0.049	4.97E-05
Relative Standard Deviation (CV%) (of pooled data of several days from pH meters)	234.3	5.07
Predicted Relative Standard Deviation (CV%) (by Horwitz equation)	3.15	8.89
Horrat comparison (RSD measured /RSD predicted)	74.43	0.57

On the above table, measurement of pH -2.07 was made on sulphate buffer, 13.3 on carbonate buffer. The data used was pooled from both meters over several days. The aim is to evaluate method performance at acidic and basic pH values.



## Chapter 5. Discussion

The experiment completed well, the pH measurements were measured following the manufacturer's calibration and operational instructions and produced statistical data. Using a short series of data, obtained with a single experiment over a short period of time, the results are displayed (Table 2). Both the pH meters used (Thermo Scientific Orion and Basic 20) did not have the potential to measure results that corresponds to the calculated pH values. It is shown that uncertainties of both pH meters expressed in the form of standard deviations of pH values in the range -2.08 to 13.03 are low, identical and they correspond with the manufacturer's specification. This confirms that the manufacturer's measurement method is practicable, applicable and reliable, confirming with Horwitz [27] saying at all times method evaluation is based on three physiognomies being practicability, applicability and reliability. Even though the uncertainties in the form of standard deviation (Table 2) correspond with manufacturer's specification, they do not correspond with the calculated/ expected pH values. There is only one calculated pH value that is covered in the range within which the measured pH values should lie which is pH 9.25. The differences between the calculated pH values and the measured values were extremely high at low pH values, this may be due to increased hydrogen ion concentration to measure which result in unstable fluctuations.

Student's t test and the Confidence Interval at 95% confidence level were used as estimates of experimental uncertainty on a short series of data with three repetitions. From the results displayed (Table 3), there is 95% confidence that the true mean lies within the confidence interval. This simply means that on three recurrence of the measurements to compute the mean and standard deviation, the 95% confidence interval would include the true population mean in 95% of the sets of measurements. Table 3 also shows the results of student's t-test, which is used to test if repeated measurements reproduced the known pH value. The known pH values fall within 95% confidence Interval, giving the conclusion that the method gives the "same" result from the known result. The measured uncertainties correspond with the manufacturer's specification but not with the calculated/ expected pH values.

We used the method of least squares to draw the best straight line between the experimental data obtained from the single experiment for each pH meter. The best line is drawn such that some of the data lie above and some below the line in order to relate the measured pH values to a set of

calculated pH values of known concentration, (Fig. 7). Some of the measured pH values were distributed about the straight line but some of the pH values were measured some distance from the line. The least squares equations are shown on the graphs with the least square slopes and intercept, the uncertainty of the slope and the intercept were estimated. For measurements of pH by Thermo Scientific pH meter, the uncertainty of the slope was,  $s_a = 0.028$  and uncertainty of the intercept,  $s_b = 0.22$ , for Basic 20 pH measured values uncertainty of the slope was,  $s_a = 0.037$  and uncertainty of the intercept,  $s_b = 0.30$ . The graph serves as an opportunity to see if the calibration points falls within a straight line. These graphs are not used as an opportunity to reject outliers but it is an incentive to increase repetitions of measurements. With more repeated data, a conclusion can be drawn that straight line is or is not an appropriate tool for rejection of outliers using the lower and upper level of confidence.

A higher number of repetitions of analysis was proposed further in the experiment. This was because the repeatability technique was needed to fulfil the conditions of the central limit theorem [26] [70]. All data was utilized without removal of outliers as their impact on the end result can be eliminated by an increased number of repetitions [26]. Uncertainty of the pH measurements hinge on deviations of experimental details [71], but to disregard this proposition, the measurements were performed numerous times under similar temperature conditions, on the same fresh buffer solutions, done by the same operator, using the same equipment, and the results were not the same as the calculated values. The results of pooled data from several experiments on several days for each pH meter are displayed (Table 4 to 7). The standard deviation of pooled data for each pH meter are greater than that of single experiment (Table 2), this shows that the results produced by the two-set apparatus using the two different methods (QUAM/ ISO and principle of pooled calibration) were statistically different. Higher repetitions of measurements resulted in more inconsistency, as a result the confidence band narrows as a function of the inverse square. The greater standard deviations show the greater spread of measurement values from the average; therefore, practically it is anticipated that uncertainty is dependent on the pH meter itself. This is agreeing with Leito *et al* [71], saying uncertainty of pH is reliant on pH value itself, and Schmitz [64] who said calculated values and measured values must be expectedly similar in common practices.

Most of the measured pH values and the calculated pH values were not close to each other, the issue was with measuring equipment but there were no issues with the buffers. Pooled data resulted in several calculated pH values falling in the range within which the measured pH values were expected to lie, when using standard deviation as an estimate of uncertainty. The results (Table 4 to 7), of Basic 20 pH meter showed that measured uncertainty of pH 3.75, 2.75, 9.25, 10.25, 11.25 corresponds with the predicted uncertainty. Also, the results for Thermo Scientific Orion showed that the measured uncertainty at pH 4.75, 9.25, 10.25, corresponds with the predicted uncertainty. The standard deviations as estimates of experimental uncertainty were graphically displayed (Fig. 8), they demonstrate the general performance of the two pH meters. Uncertainties indicate that the meters 's performance is mostly parallel. There are two unusual uncertainty values for the Basic 20 pH meter in Fig. 8 i.e. pH 12.03 and 13.3. The values are extremely high. They differ to that extent from the other values maybe because at high pH values there is decrease in hydrogen ion concentrations and the pH meter could not sense and stabilize the reading resulting in poor output.

The statistics from each pH meter were pooled into a graph to relate the measured pH values to a set of calculated pH values of known concentration and displayed their linear relationship (Fig. 9). The uncertainty of slopes ( $s_a$ ), and intercepts ( $s_b$ ), for the graphs on this figure were exactly the same, making the pooled data technique a possibility. The data of each pH meter were pooled together into one graph (Fig. 10). Our R – squared, described as a goodness of fit [72], is close to one, it shows that there is a strong relationship between the calculated pH variable results and the measured pH values.. The purple line (Fig. 10), signifies upper confidence level (UCL) and the black line the lower confidence level (LCL). The LCL of measured pH values was expressed in terms of the lower SD on the slope and on the intercept at 95% confidence level. The UCL of measured pH values was expressed in terms of the upper SD on the slope and on the intercept at 95% confidence level. There are numerous pH measurements that fall outside the confidence of levels, almost half of the data. Therefore, a conclusion was drawn that a straight line is not an appropriate tool for rejection of outliers using the lower and upper confidence level, on the method of pooled calibration for the reason that most of our measurements would be neglected.

Statistical assessment of the pooled data was achieved and signified in Table 8, using standard deviation as estimate of uncertainty on pooled data of the two meters. The results show that uncertainty of the measured pH values corresponds with the predicted uncertainty at almost all the

pH values, except pH -2.08, -1.08, and 7.25. Comparing the correspondence of the measured uncertainty (in form of SD) and the predicted uncertainty of a short series of data with pooled calibration, we realise that correspondence is discovered mostly on pooled calibration. The results of confidence interval as an estimate of experimental uncertainty can be safely neglected since most of the data fall outside the range within which they are asserted to lie.

The law of propagation of uncertainty with a calibration curve was used to evaluate uncertainty of the measured pH values on pooled calibration. Propagation of uncertainties for fitting equation of a straight line yielded some results (Fig. 11). The insert displays the distribution of the data points in a U-shaped arrangement. The general trend is suggestive on pooled calibrations in which uncertainty approaches zero at neutral pH values and increases at more acidic and more basic values. The shape of the distribution (Fig. 11) is comparable to the shape of the distribution (Fig.14), which shows standard deviations as estimate of the experimental uncertainty for pooled calibration. Also, the shape of distribution is similar to the figure of CV% of pooled calibration (Fig. 12). Comparing pooled calibration to single experiment, the shape of the distribution of single experiment is random (Fig.5), it is impossible to categorically tell how pH effects uncertainty. But with pooled calibration a clear judgement can safely be notable. High uncertainty on the acid phase may be due to too much variation on pH values from the two meters at the lowest pH. Utmost uncertainty on the basic phase may be due to less proton concentration of a solution resulting in inconsistency in measuring.

An overview of expected uncertainties was created using the relative uncertainty percentage. According to Andersen [73], the approximate CV can be utilized to generate an overview of uncertainty even before any measurements are made on samples. The CV% of  $H^+$  ions (Fig. 6), only three pH values were more than 50%. Based on the results, we conclude that Basic 20 and Thermo scientific pH meters were eligible to measure  $H^+$  ions on solutions of pH values in the range -2.08 to 13.3 (except pH 8.25, 9.25 and 13.3) to produce short series of data over a short period of time following the ISO/ QUAM method. As higher number of repetitions of analysis was proposed using the method of pooled calibration (Fig. 12), some of the CV% lie above 50% and some lie below the 50%, we reject the results if the CV is larger than  $\frac{1}{2}$ . Results of pooled calibration show that the pH meters were eligible to measure  $H^+$  ions on solutions of pH values -1.08, 2.75, 3.75, 4.75, 7.25 and 8.25. The solutions with pH values -2.08, 6.75, 9.25, 10.25, 11.25,

12.03, 13.3 had greater than 50 CV% of  $H^+$  ions associated with, therefore we reject the result, this observation might be for the reason that at high pH values the  $H^+$  ions are few for the pH meters to be able to measure and also there are other salts within the solution that might be tempering with the reading. And with repeatability of measurements there are more deviations which may describe these observations.

The CV% of pH values (Fig. 13), shows that all the pH values were suitably measured due to acceptable CV. But, pH -2.08, 12.03 and 13.3 were unsuitable to measure in absolute terms due to the higher standard deviations associated with from (Table 8) and (Fig. 14). Then again, there are pH values that were eligible to measure also looking at their lower values of standard deviations (from Table 8 and Fig. 14), these are pH values -1.08, 2.75, 3.75, 4.75, 6.75, 7.25, 8.25, 9.25, 10.25, and 11.25.

There are two methods that have been of greater use on estimating uncertainty: the EURACHEM/CITAC guide method and the IUPAC method. The latter is represented in ISO standards, ISO 5725, ISO 17025 and others [74]. IUPAC method mentions that outliers be rejected. The rejection of outliers is not a good practice in the process of possessing significant statistics about the performance of the equipment. This is in agreement with Horwitz [27] saying that outliers should be tolerated. The IUPAC mentioned methods, some of their procedures and concepts are outmoded [74]. The EURACHEM/CITAC guide method uses the uncertainty budget tool and expanded uncertainty which encompasses a large fraction, 95 % of the distribution of values [74]. According to Andersen [28], none of the various published reviews of QA focused on correspondence between predicted and observed uncertainty. In this study, we established new equations which can evade going through all the phases of making a budget table and predict uncertainty by simply establishing correspondence between observed and predicted uncertainties on each measurement value. According to Leito *et al* [71], it is a necessity to calculate uncertainty values separately for each measurement.

For comparison purposes between the budget table results and the new equations, firstly an uncertainty budget table was established according to methodologies described by the BIPM *Guide to the expression of uncertainty in measurement*' (GUM). For the results of unknown uncertainty which were needed as part of the uncertainty budget, they were evaluated by triangular distribution

resulting in adjustments made by a factor of  $\sqrt{6}$ , then the square distribution where adjustments were made by a factor of  $\sqrt{3}$ . The budget was constructed for the acidic solution (Table 9) and the basic solution (Table 10). The predicted uncertainty of the acidic solution was  $\pm 0.02$  and for the basic solution was  $\pm 0.01$ .

The new equations for evaluating uncertainty involves the Henderson-Hasselbalch equation. This is the equation that was used in calculations of expected pH values. It is a very important equation which teaches much about the acid-base equilibrium, and it is very vital in the buffer problems and titration curves to mention a few [69]. According to Menong and Andersen [63], in agreement with Po and Senozan [69], the equation was found to have limitation associated with. It can only calculate pH values of a buffer which is close to the  $pK_a$  value, it is highly appropriate in calculations of the pH of buffers that are made with acids whose  $pK_a$  falls within the range 5 to 9, and the more the dissociation constant of the acid departs from  $10^{-7}$  by more than two orders of magnitude, the more unreliable the equations become for calculating pH [69]. As a result, the effort was necessary to formulate an equation for pH calculations as shown by equation 1 to 47.

The equations were derived by taking the Henderson-Hasselbalch equation and adjusting it with a  $z$  value. Since the Henderson-Hasselbalch equation can calculate pH values that are close to the  $pK_a$  value, the  $z$  value is introduced to widen the range within which the pH can be calculated. The model equations for calculations of pH values of weak acids, bases and salts were derived as shown by (Eq. 32 to 47). Before equilibrium point, pH is calculated by (Eq. 33) and its associated uncertainty is calculated by (Eq. 39). After the equilibrium point, pH is calculated by (Eq. 41) and its associated uncertainty is calculated by (Eq. 47).

Fig 16 and Fig. 17 illustrates the uncertainty contribution on the acidic pH measurements, the former shows contributions compiled by the uncertainty budget table, and it indicates that repeatability of measurement contributed a lot to the total uncertainty value and the concentration of acid and base are the least contributors. Fig 17 shows contributions compiled by the derived equations, it confirms as well that contributions of uncertainty are high from repeatability of measurement and other sources are least contributors. Both the uncertainty budget table and the derived equations predicted uncertainty on acidic solution to be  $\pm 0.01$ . This proves a relation that is amongst the equations and the budget table, it verifies that these derived equations can predict

uncertainty truly well. The same finding is displayed by (Fig. 18& 19) in the basic medium, both the uncertainty budget table and the derived equations predicted uncertainty to be  $\pm 0.02$ . According to literature, Wiora [24] used Monte Carlo (MC) simulations implemented in a program and in a spreadsheet and predict the expanded uncertainty of pH to be 0.042. As well, Damasceno *et al* [75] used the ISO-GUM approach and predicted standard uncertainty to be 0.0083. The former has a higher uncertainty and the latter has nearly equal uncertainty as of this study.

The model equations were derived for to the titration of strong acid and strong base. We consider three kinds of calculations which are key players in the approximate derivation of pH during a titration. There is, before equivalence point, when moles of acid is in excess,  $[H_e^+] = ((moles\ acid) - (moles\ of\ base))$ , At the equivalence point, when the moles of the added base is in equals amount as the number of moles of acid  $((moles\ acid) = (moles\ of\ base))$ , and After equivalence point when the moles of the base are in excess  $[OH^-] = ((moles\ base) - (moles\ of\ acid))$ . To construct a titration curve we need calculations that can be used but, according to Po and Senozan [69] the Henderson-Hasselbalch equation is inappropriate on deriving titration curves because of its limitations. Therefore, we used pH as the decimal logarithm of the reciprocal of the molar concentration of protons [66], equation 1, throughout all the calculations.

The new pH equation for titration of strong acids and strong bases were derived using the mole value. Then mole value was set into the decimal logarithm of the reciprocal of the molar concentration of protons equation, and differentiations were made. At before equilibrium point, pH is calculated by (Eq. 6), at equilibrium point, pH is calculated by (Eq. 16) and after equilibrium point, pH is calculated by (Eq. 24). These equations were inserted on excel spreadsheet and used to make the titration curve (Fig. 15). The pH values calculated by derived equations are represented by black dots. These equations have the capability to predict pH that are above 14 on a titration curve. For comparability purposes, also the titration curve was made from measurements made by each pH meter. All the pH values measured by each meter, pH calculated by equation 1, and pH of the derived equations correspond well to each other. The associated uncertainty is calculated by (Eq. 14) before equilibrium, (Eq. 21) at equilibrium and (Eq. 31) after equilibrium.

The Uncertainty budget table was used to calculate the predicted uncertainties of food samples. These pH values of the food samples were a result of single experiment over a short period of time for each pH meter. The standard deviation of the measured pH values of the food samples was used as an estimate of sample uncertainty. The results from each pH meter were accumulated and used as pooled calibration over a short period of time. The predicted uncertainties were compared with the sample uncertainty (Fig. 20), to evaluate if the pH measurements were in statistical control. It is expected that the sample uncertainty will correspond better to predicted uncertainty for the reason that, the budget uncertainty compares better to results of a single experiment over a short period of time. The budget uncertainty does not correspond better to pooled calibration over a short period of time. We suggest that maybe the predicted uncertainty would have corresponded better to pooled calibrations of several experiment over several days. The pH measurements of red bull, wine and spar juice were in statistical control, and that means scientific methodology was fulfilled. The opposite goes for milk and diet coke as the predicted uncertainty is lesser than standard deviation of the sample pH.

According to the manufacturer's specifications, the pH meters that were used for the experiments can measure pH values as lowest as -2 and as highest as 16. We introduce the coefficient of variation ( $CV\%$ ) as a tool to measure the relative variability at these lowest and highest pH values. The values from the Horwitz equation constituted a judgement, (Table 11). Under repeatability conditions, accepted values were between 0.3 and 1.3. at pH -2.08, the HorRat value was  $\gg 1$ , this means the method was certainly performing poorly, there is a significant difference between the coefficients of variation, so variances were not homogenous. At pH 13.3, the HorRat value was between 0.3 and 1.3, the value explains that the measured pH values were within the limits of acceptance, there was no significant difference between the coefficients of variation, so variances are homogenous. The Horwitz ratio shows that pH meters performed poorly at pH -2.08, the results correspond to the  $CV\%$  of  $H^+$  and  $CV\%$  of pH values.



## Chapter 6. Conclusion

The pH meters that were used was Basic 20 and Thermo Scientific Orion. The statistical results showed that they do not have great potential in the measurement of calculated pH - values. They cannot reproduce the expected/ calculated pH values. The uncertainty of measured pH in the form of standard deviation confirm with the manufacturer's specification, but they did not correspond with the predicted uncertainty. Therefore, pooled calibration was introduced in the study. Uncertainty analysis was performed on multiple experimental data. Numerous measurements were made, five hundred and 20 pH measurements, and all data were used without rejection of outliers. The pooled calibration created a satisfactory correspondence between predicted uncertainties and those observed by experiment except single experiment.

Correspondence of the measured pH values with manufacturer's specification (Table 2), is a strong indication that the manufacturer's calibration procedure and measurement procedures that were used were valid to predict uncertainty in terms of precision following the ISO/ QUAM guidelines, but they were not valid to predict uncertainty in terms of accuracy. All of the pH values are suitable for measuring due to the acceptable CV% of pH values (Fig. 13), and lower values of standard deviations. But, at pH -2.08, 12.03 and 13.3 the measurements had higher standard deviations that they were associated with.

The measurements were performed numerous times under the similar temperature conditions, on the same fresh buffer solutions, done by the same operator and using the same equipment. This was performed in order to take care of needless errors and disregard the proposition that uncertainty of the pH measurements originates from deviations of experimental details. The measured pH values results were not the same as the calculated or expected pH values, but the calculated values and measured values should be similar in common practices. Therefore, it was anticipated that the uncertainty was dependent on the pH meter itself.

The performance of pH meters was evaluated using the Horwitz equation. Using pooled calibration, the HorRat value indicated that the method is performing poorly at pH value -2.08.

The results correspond to the CV% of  $H^+$  and CV% of pH values as well. HorRat ratio shows that there was a significant difference between the coefficients of variation and variances were not homogenous at pH -2.08. At pH 13.3, there was no significant difference between the coefficients of variation and variances were homogenous, the measured pH values were within the limits of acceptance.

Measurement result obtained from these meters were used in predicting pH and uncertainty values of acidic and basic solutions. For prediction of pH values on weak acid, bases and salts, a number of equations were derived. At before equilibrium point, the pH – value was calculated by (Eq. 33) and after equilibrium point, the pH – value was also calculated by (Eq. 41). For titration of strong acids and bases, the pH values were calculated using equation 6 before equilibrium point, equation 16 at equilibrium point and equation 24 after equilibrium point. The uncertainty was firstly estimated using the uncertainty budget tool, following the EURACHEM/ CITAC guide method. Then secondly, correspondence between observed and predicted uncertainties was established and some equations were derived which were used to predict uncertainty using the law of propagation of errors. On the weak acids, bases and salts, before the equilibrium point the uncertainty was calculated by (Eq. 39), and after the equilibrium point the uncertainty was also calculated by (Eq. 47). Equation 14, 21 and 31 predict the uncertainty of acidic, neutral and basic solution respectively from titration of strong acid and strong bases. All the equations present a clear picture of what occurs in a solution.

The predicted uncertainty varies at differing pH values. In this study, the uncertainty was predicted from titration of weak acid, weak bases and salts at pH -2.08 and 13.3 and it was 0.01 and 0.02 respectively by both approaches. This was sufficient confirmation that the derived calculation would predict the uncertainty well, thus, we present them as a new technique that can be applied on pH calculations. The technique is not restricted by the  $pK_a$  values; it is a simple, easy correspondence to use. At calculated pH 3.75, for titration of weak acids bases and salts, the derived equation predicted pH to be 3.85, at calculated pH 12.03, the predicted pH by derived equation was 11.94

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## Appendices

Table 13: A figure showing table of pH measurements that were measured by individual meters on fresh buffers

Expected Calculated pH	Name of pH meter	buffer 1	buffer 2	buffer 3	buffer 4	buffer 5	buffer 6	buffer 7	buffer 8	buffer 9	buffer 10	buffer 11	buffer 12	buffer 13	buffer 14	buffer 15	buffer 16	buffer 17	buffer 18	buffer 19	buffer 20
-2.08	Thermo	-1.38	-1.35	-1.34	-1.13	-1.06	-1.13	-1.15	-1.15	-1.06	-1.07	-1.09	-1.26	-1.03	-1.17	-1.12	-1.07	-1.2	-1.06	-1.08	-1.2
	Basic 20	0.47	0.48	0.51	0.32	0.35	0.49	0.48	0.42	0.46	0.49	0.51	0.45	0.48	0.54	0.46	0.39	0.46	0.43	0.55	0.54
-1.08	Thermo	0.25	0.26	0.25	0.29	0.35	0.24	0.34	0.48	0.37	0.39	0.66	0.76	0.6	0.4	0.66	0.56	0.64	0.63	0.66	0.66
	Basic 20	0.73	0.73	0.72	0.77	0.68	0.75	0.75	0.7	0.62	0.71	0.43	0.59	0.36	0.08	0.43	0.31	0.39	0.35	0.41	0.4
2.75	Thermo	2.51	2.51	2.5	2.48	2.55	2.45	2.44	2.44	2.5	2.47	2.45	2.51	2.48	2.43	2.39	2.43	2.47	2.52	2.47	2.43
	Basic 20	2.27	2.27	2.29	2.27	2.22	2.72	2.73	2.73	2.66	2.71	2.69	2.66	2.67	2.73	2.68	2.73	2.68	2.67	2.67	2.72
3.75	Thermo	3.55	3.54	3.55	3.55	3.33	3.49	3.5	3.49	3.5	3.52	3.5	3.69	3.69	3.73	3.69	3.68	3.68	3.67	3.69	3.68
	Basic 20	3.69	3.7	3.7	3.38	3.36	3.71	3.68	3.64	3.68	3.7	3.71	3.71	3.37	3.72	3.71	3.72	3.68	3.74	3.33	3.33
4.75	Thermo	4.64	4.64	4.62	4.64	4.65	4.61	4.62	5	4.97	4.96	4.64	4.6	4.58	4.57	4.55	4.5	4.51	4.52	4.51	4.55
	Basic 20	4.76	4.76	4.75	4.74	4.71	4.64	4.65	4.61	4.56	4.64	4.6	4.56	4.63	4.54	4.71	4.71	4.76	4.67	4.7	4.74
6.75	Thermo	6.33	6.33	6.35	6.27	6.15	6.32	6.64	7.67	6.49	6.5	6.2	6.59	6.2	6.21	6.23	6.5	6.15	6.19	6.58	5.98
	Basic 20	6.22	6.22	6.24	6.32	6.15	6.05	6.05	5.66	6.06	6.16	6.22	6.27	6.18	6.08	6.33	6.29	6.03	6.28	6.33	6.1
7.25	Thermo	7.02	7.02	7.01	6.98	6.87	6.97	6.87	6.95	6.97	6.88	7.01	7.1	6.89	6.97	6.95	6.95	7.1	6.88	7.11	6.98
	Basic 20	6.88	6.88	6.87	6.83	6.87	6.92	6.9	6.84	6.79	6.93	6.8	6.9	6.79	6.9	6.89	6.87	6.91	6.8	6.93	6.91
8.25	Thermo	7.94	7.91	7.91	7.89	7.96	7.91	7.83	7.96	7.86	7.94	7.81	7.94	7.89	7.84	7.89	7.91	7.96	7.91	7.98	7.82
	Basic 20	8.1	8.1	8.06	7.84	7.95	7.96	7.91	7.93	7.98	7.89	7.98	7.88	7.93	7.95	7.88	8.01	7.88	7.88	8	8.02
9.25	Thermo	9.23	9.22	9.29	9.59	8.68	8.94	8.71	8.83	8.89	8.96	9.1	8.83	8.82	9.2	8.82	9.37	9.37	9.28	9.27	9.31
	Basic 20	9.25	9.26	9.32	8.93	8.8	9.19	9.09	9.02	8.95	9.33	9.25	9.01	8.7	8.83	9.28	9.38	9.33	9.37	9.32	9.32
10.25	Thermo	10.21	10.22	10.22	10.22	10.22	10.21	10.22	10.2	10.21	10.22	8.91	10.05	10.05	10	10.05	10.05	10.03	10.01	10.04	10.03
	Basic 20	10.46	10.46	10.45	10.47	10.46	10.45	10.44	10.3	10.45	10.44	10.06	10.05	10.05	10.06	10.02	10.03	10	10.11	10	10.01
11.25	Thermo	11.06	11.07	11.06	11.07	11.06	11.01	11.06	11.1	11.12	11.1	10.86	10.91	10.93	10.92	10.9	10.87	10.89	10.89	10.89	10.87
	Basic 20	11.23	11.23	11.24	11.37	11.39	11.68	11.67	11.6	11.61	11.63	11.21	11.21	11.26	11.23	11.21	11.42	11.21	11.42	11.41	11.23
12.03	Thermo	14.29	14.29	14.31	14.13	14.28	14.26	14.23	14.3	14.29	13.51	14	9.62	14.1	14.26	14.31	14.07	14.24	14.24	14.23	14.23
	Basic 20	14.47	14.47	14.48	14.44	14.45	14.3	14.33	14.4	14.49	14.5	14.48	14.49	14.5	14.51	14.51	14.53	14.53	14.54	14.54	14.54
13.3	Thermo	14.15	14.15	14.11	14	14.04	14.07	14.06	14	13.99	13.98	14.01	10.07	14.12	14.15	14.13	14.13	14.13	13.85	13.96	13.91
	Basic 20	14.51	14.51	14.55	14.69	14.51	14.65	14.68	14.6	14.37	14.69	14.27	14.42	14.43	14.46	14.51	14.51	14.51	14.67	14.61	14.65

Table 13: The masses of chemicals that were used in preparation of Ammonia buffer.

<i>Buffer</i>	<i>moles of ammonia</i>	<i>moles of ammonium chloride</i>	<i>mass ammonia</i>	<i>mass ammonium chloride</i>	<i>Calculated pH</i>
<i>1</i>	<i>1.00</i>	<i>0.10</i>	<i>35.05</i>	<i>0.5349</i>	<i>11.25</i>
<i>2</i>	<i>1.00</i>	<i>0.01</i>	<i>35.05</i>	<i>5.349</i>	<i>10.25</i>
<i>3</i>	<i>0.01</i>	<i>0.01</i>	<i>0.3505</i>	<i>0.5349</i>	<i>9.25</i>
<i>4</i>	<i>0.01</i>	<i>1.00</i>	<i>3.505</i>	<i>53.49</i>	<i>8.25</i>
<i>5</i>	<i>0.10</i>	<i>1.00</i>	<i>0.3505</i>	<i>53.49</i>	<i>7.25</i>
<i>6</i>	<i>0.001</i>	<i>0.32</i>	<i>0.03505</i>	<i>16.9028</i>	<i>6.75</i>

Table 14: The masses of chemicals that were used in preparation of Acetate buffer.

<i>Buffer</i>	<i>moles of acetic acid</i>	<i>moles of sodium acetate</i>	<i>mass acetic acid</i>	<i>mass sodium acetate</i>	<i>Calculated pH</i>
<i>1</i>	<i>1.00</i>	<i>0.10</i>	<i>60.05</i>	<i>1.3608</i>	<i>2.75</i>
<i>2</i>	<i>1.00</i>	<i>0.01</i>	<i>60.05</i>	<i>13.608</i>	<i>3.75</i>
<i>3</i>	<i>0.01</i>	<i>0.01</i>	<i>0.6005</i>	<i>1.3608</i>	<i>4.75</i>

Table 15: The masses of chemicals that were used in preparation of Sulphate buffer.

<i>Buffer</i>	<i>moles of sodium sulphate</i>	<i>moles of sodium hydrogen sulphate</i>	<i>mass sodium sulphate</i>	<i>mass of sodium hydrogen sulphate</i>	<i>Calculated pH</i>
<i>1</i>	<i>0.00001</i>	<i>0.10</i>	<i>0.00142</i>	<i>12.0662</i>	<i>-2.08</i>
<i>2</i>	<i>0.0001</i>	<i>0.10</i>	<i>0.0142</i>	<i>12.066</i>	<i>-1.08</i>

Table 13: The masses of chemicals that were used in preparation of Carbonate buffer.

<b>Buffer</b>	<b>moles of sodium bicarbonate</b>	<b>moles of sodium hydroxide</b>	<b>mass sodium bicarbonate</b>	<b>mass of sodium hydroxide</b>	<b>Calculated pH</b>
<b>1</b>	<i>0.001</i>	<i>0.10</i>	<i>0.08401</i>	<i>4</i>	<i>12.3</i>
<b>2</b>	<i>0.0001</i>	<i>0.10</i>	<i>0.008401</i>	<i>4</i>	<i>13.3</i>