TÜBİTAK

ULUSAL METROLOJİ ENSTİTÜSÜ

Certification Report

Certified pH Buffers
UME CRM 1401
UME CRM 1402
UME CRM 1403

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ABBREVIATIONS

Ag	Silver
AgCl	Silver Choride
α	Significance Level
ANOVA	Analysis of Variance
ISO	International Organization for Standardization
NaCl	Sodium Choride
KCl	Potasium Choride
k	Coverage Factor
$MS_{between}$	Mean of Square Between Bottles (ANOVA)
MS_{within}	Mean of Square Within Bottles (ANOVA)
NIST	National Institute of Standards and Technology (USA)
n	Number of Replicates for Each Unit
pH	Pouvoir Hydrogen
Pd	Palladium
Pt	Platinum
PTB	Physikalisch-Technische Bundesanstalt (Germany)
RSD	Relative Standard Deviation
s	Standard Deviation
s_{bb}	Between Units Standard Deviation (ANOVA)
$s_{bb.rel}$	Between Units Relative Standard Deviation
SGT	Single Grubbs Test
SI	International Units of Measurement
CRM	Certified Reference Material
s_w	Within-unit Standard Deviation
s_{wb}	Within-bottle Standard Deviation
$s_{wb.rel}$	Within-bottle Relative Standard Deviation of the Method
$\bar{t}$	Mean of Whole Time Points
t_α	Critical t value (Two-tailed)
t_i	Time Point for Each Parallel
TRaNS	Random Stratified Sample Selection

u_{bb}	Standard Uncertainty Related to Possible Between Bottle Homogeneity
$u_{bb.rel}$	Relative Standard Uncertainty Related to Possible Between-bottle Homogeneity
u_{char}	Standard Uncertainty Related to Characterization
$u_{char.rel}$	Relative Standard Uncertainty Related to Characterization
u_{CRM}	Standard Uncertainty of the Certified Value
$u_{CRM.rel}$	Relative Standard Uncertainty of the Certified Value
U_{CRM}	Expanded Uncertainty of the Certified Value
$U_{CRM.rel}$	Relative Expanded Uncertainty of the Certified Value
u_{lts}	Standard Uncertainty Related to Long Term Stability
$u_{lts.rel}$	Relative Standard Uncertainty Related to Long Term Stability
u_{sts}	Standard Uncertainty Related to Short Term Stability
$u_{sts.rel}$	Relative Standard Uncertainty Related to Short Term Stability
$v_{MSwithin}$	Degrees of Freedom of MS_{within}

ABSTRACT

The measurement and control of pH is essential both in industrial processes and research studies in the fields of food, chemical and environmental sciences and in ensuring compliance of a product with the legal requirements.

In all of the above-mentioned fields pH is measured by pH-meters or pH measuring systems. In Turkey, all laboratories (state, private or university laboratories) that are carrying out pH measurements use buffer solutions to calibrate their equipments and currently these solutions are imported from abroad. Therefore, production and certification of the required buffer solutions is planned. Local supply of the buffer solutions will ensure measurement traceability chain through TÜBİTAK UME and the exchange spent for these solutions will be retained in the country.

INTRODUCTION

Buffer solutions with pH values of 4, 7 and 10 which are most frequently used in pH measurements were produced using the infrastructure of TÜBİTAK UME and certified according to requirements of ISO Guide 34 [1] and ISO Guide 35 [2]. The following studies had been planned before the production:

- Definition of the methods to be used in this study.
- Carry out material processing, homogeneity, stability and characterization studies.
- Preparation of a certificate and a certification report.

pH is the negative logarithm of hydronium ion activity in a solution, indicating the acidity or alkalinity of solution. The mathematical function of pH defined by Sorensen and Lindstrom-Lang is given below:

$$pH = - \log \alpha H^{+} = - \log mH^{+} \gamma H^{+}$$

Where αH^{+} is the activity of H_3O^{+} ion, mH^{+} is molality of H_3O^{+} and γ_{H+} is activity coefficient of H_3O^{+}.

Because of γ_{H+} is negligable in a diluted solutions, pH equation can be given as follow:

$$pH = -\log [H_3O^{+}]$$

Due to logarithmic function, a pH 4 solution has 10 times more hydronium ions than pH 5 solution.

The measurement and control of pH is important both in industrial processes and research studies in the fields of food, chemical and environmental sciences and in ensuring compliance of a product with legal and technical requirements.

A pH measurement system consists of two main component; a pH-meter (high impedans voltmeter) and pH measuring probe. A pH measuring probe is a combined electrode which combines both the glass and reference electrodes into one body. The main components of a combined electrode are a sensing sphere part made from a specific glass, internal and external reference electrodes usually silver-silver chloride electrode, internal and external solutions, usually 0.1 mol/L KCl and saturated KCl solutions, porous ceramic diaphragm for liquid junction, body of electrode made from non-conductive glass or plastics. During the measurement, measured solution contacts with the reference solution through the diaphragm and this completes the circuit and allows measurement. This contact point is named as liquid junction. Any junction between two electrolyte solutions of different composition results in a potential difference called the liquid junction potential. Since the value of liquid junction potential differs with different solutions it can not be measured exactly and increases the value of measurement uncertainty.

Since the stability of glass electrode is very short, it should be calibrated frequently. These solutions have known pH values (4, 7 and 10) and these pH values do not change with the addition of small amounts of water, acid or base.

pH Buffer solutions used in Turkey are procured from abroad. A considerable amount of foreign currency is spent for pH measurements in a wide variety of applications and procurement takes 4 to

6 weeks and sometimes up to 8 weeks. In addition to these, we still depend upon other NMIs in different countries for traceability.

For these reasons local production and certification of pH buffer solutions has been planned. In order to achieve this goal, the primary level pH measurement system has been establihed. The primary level pH measurement method does not require any measuring standard solution to produce a result.

The electrochemical cell used in this measurement system does not have neither liquid junction nor liquid junction potential. This cell is named as Harned cell uses two electrodes to function. Ag/AgCl electrode is used as working electrode and standard hydrogen electrode as reference. Both electrodes are prepared in the laboratory in order to reduce the measurement uncertainty. Ag/AgCl electrode is prepared using a short Pt wire made in spiral shape and covered with silver paste, heated to 400°C for reduction to Ag and after the surface is chlorinated. Procedure of the preparation of hydrogen electrode varies according to the type of the buffer solution to be measured. For pH 7 (phosphate) and pH 10 (carbonate) buffers hydrogen electrodes are prepared by covering a 1 cm^2 Pt sheet with platinum and passing hydrogen gas at 1 atmosphere pressure in the cell. For pH 4 (phthalate) buffer hydrogen electrodes are prepared by covering a 1 cm^2 Pt sheet with palladium and passing hydrogen gas at 1 atmosphere pressure in the cell. Standard potential of this electrode is independent of the solution and accepted as 0 volt. Thus if Ag/AgCl electrode and standard hydrogen electrode are together in a Harned cell, the measured voltage can be recorded as the standard potential value of Ag/AgCl electrode. pH value of a buffer solution is defined in two steps in this system. In the first step, the standard potential of the Ag/AgCl electrode is determined using 0.01 molal HCl solution whose exact concentration is determined with coulometric titration.

In the second step, the acidity function value (p_a^0) ($p_a^0 = -\log(a_{H+}\ \gamma_{Cl-})$ of the sample solution is determined. In order to do this, the cells are filled with sample buffer solution containing increasing concentrations of 0.01 0.03 and 0.05 molal NaCl (or KCl) solutions and acidity function (p_a) of the buffer solutions are determined. The intercept of the acidity function versus Cl^- molality graph gives the acidity function value (p_a^0) of the sample buffer.

The aim of reference material production project is preparation and certification of buffer solutions with pH values of 4, 7 and 10 and conveys them to users.

PARTICIPANTS

UME CRM 1401, UME CRM 1402 ve UME CRM 1403 coded certified reference materials are produced by TÜBİTAK UME.

MATERIAL PROCESSING

Chemical materials required for the production of buffer solutions of given pH values are represented in Table 1. Deionized water required for the production of buffer solutions was obtained from water purification system present at TÜBİTAK UME laboratories. Buffer solutions were prepared according to the instructions given at “IUPAC Recommendation 2002 Measurement of pH – Definition, Standards and Procedures” [4]. According to this document, the chemical materials were dissolved in 150 L deionized water and homogenized for 8 hours using our homogenizing equipment and pH 4, 7 and 10 candidate buffer solutions are prepared (300 units each).

The prepared solutions were labeled according to filling order and stored at +4 ºC.

Table 1. Used Chemicals

pH Value	Used Chemicals
4	Potassium Hydrogen Phthalate
7	Potassium dihydrogen Phosphate + Disodium Hydrogen Phosphate
10	Sodium Hydrogen Carbonate + Sodium Carbonate

HOMOGENEITY

For homogeneity studies 10 units were selected. Selection of these units was realized randomly using TRaNS software to represent 300 bottles. The measurements for homogeneity study were performed by a pH-meter system employing a combined glass electrode with a thermostat for adjusting and controlling the temperature. Merck Certipur® 4.00, 7.00 and 10.00 standard buffer solutions, traceable to NIST and PTB certified reference materials, were used for calibration and intermediate controls.

Results of measurements were evaluated using one-way variance (ANOVA) analysis. Data were evaluated statistically for the presence of any trend and/or inconsistent value. No trend was observed according to filling and measurement order.

Within bottle standard deviation (s_{wb}) and between units standard deviation (s_{bb}) values were calculated according to ANOVA method using following equations:

$$s_{wb} = \sqrt{MS_{within}}$$

$$s_{bb} = \sqrt{\frac{MS_{between} - MS_{within}}{n}}$$

s_{wb} : Within-bottle Standard Deviation

MS_{within} : Mean of Square Within Bottle

s_{bb} : Between Units Standard Deviation

MS_{between} : Mean of Square Between Bottles

n : Number of Replicates for Each Unit

When $MS_{between}$ is smaller than MS_{within}, s_{bb} cannot be calculated. In this case u^*_{bb} standard uncertainty of the maximum heterogeneity which may be hidden by method repeatability is calculated by the formula given below:

$$u^*_{bb} = \frac{s_{wb}}{\sqrt{n}} \sqrt[4]{\frac{2}{\nu_{MSwithin}}}$$

$\nu_{MSwithin}$: Degrees of freedom of MS_{within}

Standard uncertainty values related to a possible between bottle heterogeneities are in Table 2. The plots of the measurement results for homogeneity study are given in Annex 1.

Table 2. The homogeneity study test results

CRM	$s_{wb.rel}$ (%)	$s_{bb.rel}$ (%)	$u^*_{bb.rel}$ (%)	$u_{bb.rel}$ (%)
UME CRM 1401	0.096	0.145	0.046	0.145
UME CRM 1402	0.045	0.051	0.021	0.051
UME CRM 1403	0.022	0.058	0.011	0.058

STABILITY

Stability studies were carried out by simulating environmental conditions for transportation of certified reference material to user (short term stability) and for storage conditions (long term stability). Stability test measurements were performed by the same pH-meter system that was used for the homogeneity study measurements. Units for short term stability and long term stability tests were selected by stratified random sample selection method using TRaNS software.

Temperatures for the short term stability were specified as 18 °C ve 50 °C with the time periods of 1, 2, 3 and 4 weeks. For each test period two units of the each candidate CRM (UME CRM 1401, UME CRM 1402 ve UME CRM 1403) were placed at each test temperature (18°C and 50 °C). Two units of each batch were put at +4 °C for the reference point. After each test period, two units from each test temperature were transferred to +4°C, which was the reference temperature. At the end of four weeks testing period, all units were transferred to reference temperature and measured together with the reference samples.

For the long term stability study, test samples were stored at 18°C for 3, 6 and 9 months. At the end of each test period, the samples were transferred to +4 °C and at the end of 9 months measurements of these units were conducted.

Short Term Stability Study Results:

Evaluations for 18 °C and 50 °C are realized separately.

The results were screened for single outliers by applying the Grubbs' test at confidence levels of 95% and 99%. The measured pH values were plotted against time and the regression lines were calculated to check for significant trends indicating possible changes in the pH values by time. The calculated slope values were tested for significance using a t-test, with $t_{\alpha.df}$ being the critical t-value (two-tailed) for a significance level α = 0.05 (95% confidence level). The graphs are given in Annex 2.

The data points were plotted against storage time at the test temperature and the regression line was calculated. In all cases the slope of the regression line was not found to be significantly different from zero. The data evaluation results for the short-term stability at +18 °C and +50 °C are summarised in Table 3.

Table 3. The short term stability test results

Parameter		UME CRM 1401	UME CRM 1402	UME CRM 1403
Slope significantly different from zero at a level of 95% and 99% confidence?	**18 °C**	No	No	No
	50 °C	No	No	No
Outlier*		-	-	-

* SGT: Single Grubbs Test

The evaluation of the results show that the certified reference materials are stable for 4 week at 18 °C and 50 °C. Thus, the samples can be dispatched without any additional cooling elements under conditions where the temperatures do not exceed 50 °C for up to 4 weeks.

Uncertainty values obtained from short term stability studies are given in Table 4.

Table 4. Uncertainty values of the short term stability

Uncertainty values of the short term stability		UME CRM 1401	UME CRM 1402	UME CRM 1403
$u_{sts.rel}$ **(%)**	**18 °C**	0.053	0.020	0.007
	50 °C	0.055	0.019	0.008

Long Term Stability Study Results:

The shelf life of the CRM has been determined through long term stability measurements. These measurements were performed with UME CRM 1401, UME CRM 1402 and UME CRM 1403. Two samples of each batch (1 main, 1 spare, 18 in total) for 3, 6 and 9 months which were stored at 18 °C and pH values were determined at the end of the periods.

The graphs were plotted against time and the regression lines were calculated. The long term stability uncertainty, u_{lts}, of the material was then calculated for the required shelf life as:

$$u_{lts} = \frac{RSD}{\sqrt{\Sigma(t_i - \bar{t})^2}} \times t$$

Where;

RSD : Relative standard deviation of all results of the stability study.

t_i : Time point for each replicate

$\bar{t}$: Mean of all time points

t : Shelf life proposed for 18 °C

The uncertainty contribution, u_{lts} was established for 12 months (t) at 18 °C. This uncertainty was one of the four parameters of the overall uncertainty budget of the certified values. The results are given in Table 5. The Graphs for long term stability study are given in Annex 3.

Table 5. Results of the long-term stability tests of 12 months

SRM	UME CRM 1401	UME CRM 1402	UME CRM 1403
Slope significantly different from zero at 18 °C*	No	No	No
$u_{lts.rel}$ (%) for shelf-life of 12 months at 18 °C	0	0.066	0.033

* Data are evaluated at a confidence level of 95 %.

According to the results obtained, the storage temperature for the material was set to 18°C. In addition to ensure stability beyond the initial shelf life. It will be re-evaluated in certain periods based on the results of regular post-certification monitoring.

CHARACTERIZATION

According to ISO Guide 34, the characterization and the value assignment can be carried out in different ways. In this study, a single laboratory and primary method approach was selected. The characterization of the materials was performed using primary level pH measurement system which is a primary method.

The uncertainty arising from the characterization study, u_{char}, was also taken into account for the calculation of uncertainty on the property value. The characterization uncertainty, u_{char}, was calculated using measurement results obtained for 2 different temperatures and 3 units for each CRM were measured at 3 different days.

PROPERTY OF VALUE AND ASSIGNMENT OF UNCERTAINTY

The uncertainty of the certified values include characterization uncertainty (u_{char}), homogeneity uncertainty (u_{bb}), short term stability uncertainty (u_{sts}) and long term stability uncertainty (u_{lts}).

These different parameters were combined to obtain the uncertainty of CRM:

$$U_{CRM} = k \cdot \sqrt{u_{char}^2 + u_{bb}^2 + us_{sts}^2 + u_{lts}^2}$$

The expanded uncertainty of the certified value is calculated with a coverage factor of k=2 representing a confidence level of approximately 95%. The certified values and uncertainties are summarised in Table 6 and Table 7. The uncertainty contribution percentages are given in Table 8 and Table 9.

Table 6. Certified values and their uncertainties at 20°C

CRM	**pH**	U_{CRM}	$u_{bb.rel}$ **(%)**	$U_{sts.rel}$ **(%)**	$U_{lts.rel}$ **(%)**	$u_{char.rel}$ **(%)**	$U_{CRM.rel}$ **(%)**
UME CRM 1401	4.034	0.014	0.145	0.055	0	0.066	0.337
UME CRM 1402	7.051	0.014	0.051	0.019	0.066	0.035	0.185
UME CRM 1403	10.021	0.015	0.058	0.008	0.033	0.024	0.143

Table 7. Certified values and their uncertainties at 25°C

CRM	**pH**	U_{CRM}	$u_{bb.rel}$ **(%)**	$U_{sts.rel}$ **(%)**	$U_{lts.rel}$ **(%)**	$u_{char.rel}$ **(%)**	$U_{CRM.rel}$ **(%)**
UME CRM 1401	4.041	0.014	0.145	0.055	0	0.064	0.335
UME CRM 1402	7.043	0.014	0.051	0.019	0.066	0.036	0.185
UME CRM 1403	9.991	0.015	0.058	0.008	0.033	0.026	0.144

Table 8. **T**he percent contribution of each parameter which constitutes the U_{CRM} at 20°C

CRM	$u_{bb.rel}$ (%)	$u_{sts.rel}$ (%)	$u_{lts.rel}$ (%)	$u_{char.rel}$ (%)
UME CRM 1401	54.4	20.6	0	24.9
UME CRM 1402	29.8	11.1	38.5	20.7
UME CRM 1403	47.3	6.5	26.9	19.3

Table 9. **T**he percent contribution of each parameter which constitutes the U_{CRM} at 25°C

CRM	$u_{bb.rel}$ (%)	$u_{sts.rel}$ (%)	$u_{lts.rel}$ (%)	$u_{char.rel}$ (%)
UME CRM 1401	55.0	20.9	0	24.1
UME CRM 1402	29.7	11.1	38.5	20.7
UME CRM 1403	46.5	6.4	26.5	20.6

TRACEABILITY

Metrological traceability of the assigned values of the produced certified reference materials is provided by using the primary level pH measurement system. All of the solutions used in the characterization studies were prepared gravimetrically. Balances used for weighing were controlled before each use with calibration mass set traceable to TÜBİTAK UME. Temperature measurements were carried out using PT-100 sensor (with ± 0.03°C uncertainty) which was calibrated at TÜBİTAK UME and temperature were monitored continuously. The measurement uncertainty of the characterization measurements were calculated using the data obtained from primary level pH measurement system.

INSTRUCTIONS FOR USE

Storage Conditions

The material should be stored at (+18 ± 5) °C. The bottle should be shaken before opening the cap in order to prevent the effect of condensation on the upper surfaces of the bottle. During analysis electrode or any other material should not be immersed into the bottle. Bottles should not be left open. Analysis should be performed as taking the sufficient amount of sample to another clean beaker or container. TÜBITAK UME will not be responsible for the reference material – especially open bottles – stored in unsuitable conditions at the customer facility.

Minimum Sample Intake

Minimum sample amount that should be taken is the amount that allows the salt bridge of pH electrode used in the measurement to be inside the solution. This amount can be changed depending on the type of the electrode but it is approximately 25 mL.

Safety Precautions

The usual laboratory safety measures apply. The material should be used and disposed according to given safety rules. Please read SDS before using the material.

ACKNOWLEDGMENT

We would like to thank to Assoc. Prof. Dr. Nilgün TOKMAN for her support throughout the project and to Zehra ÇAKILBAHÇE for her support at the homogenisation step of the project.

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[2] ISO Guide 35. Reference materials – General and statistical principles for certification. ISO. Geneva (2006).

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REVISION HISTORY

Date	Description
11.12.2015	First Publication
18.05.2016	The uncertainty value is revised and the certificate is updated.

Annex 1. Results of the Homogeneity Studies

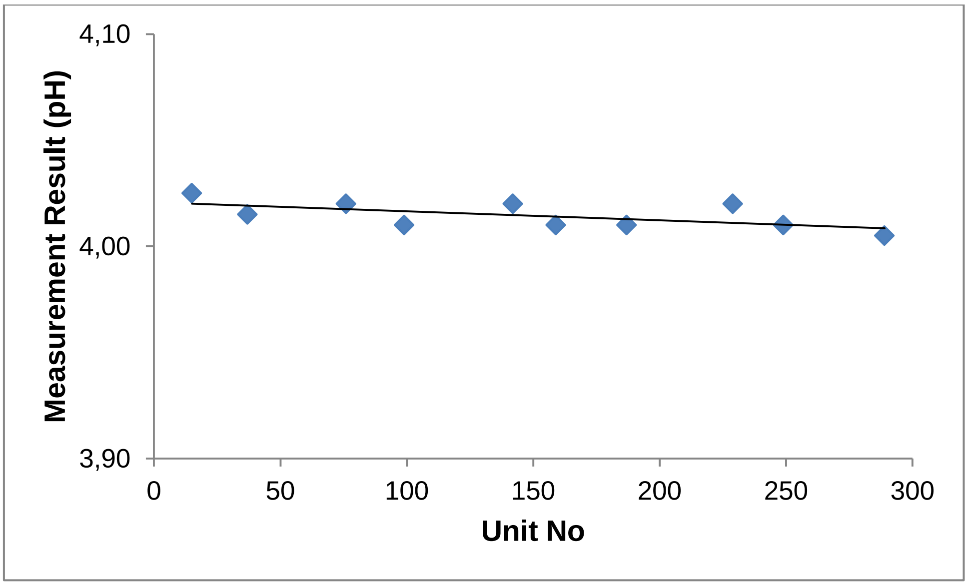

Figure 1. UME CRM 1401 Homogeneity

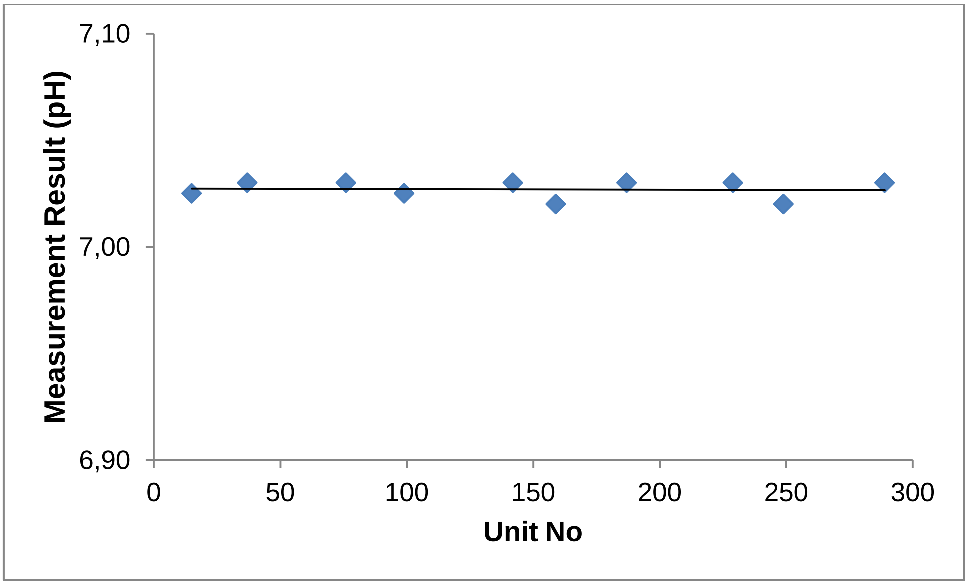

Figure 2. UME CRM 1402 Homogeneity

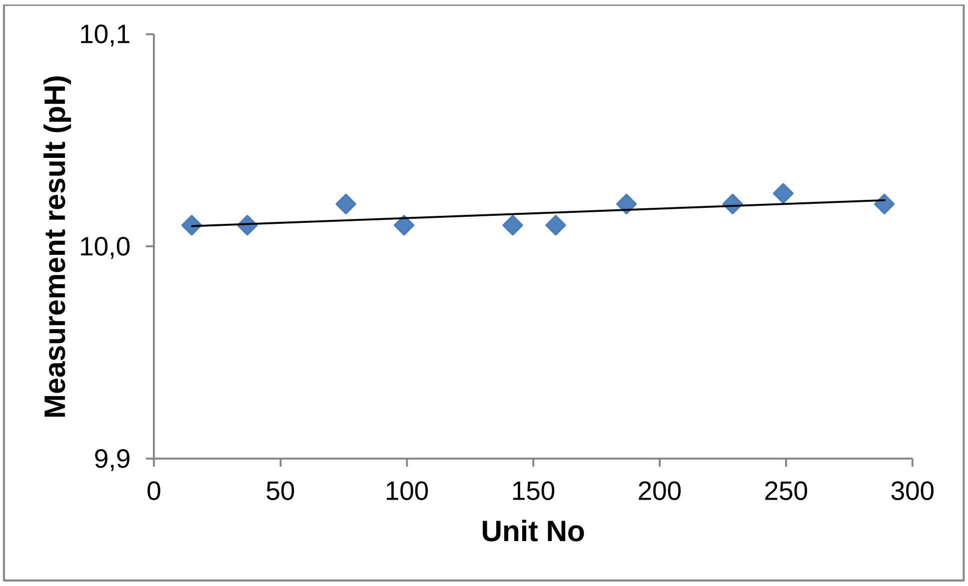

Figure 3. UME CRM 1403 Homogeneity

Annex 2. Results of the Short Term Stability Studies

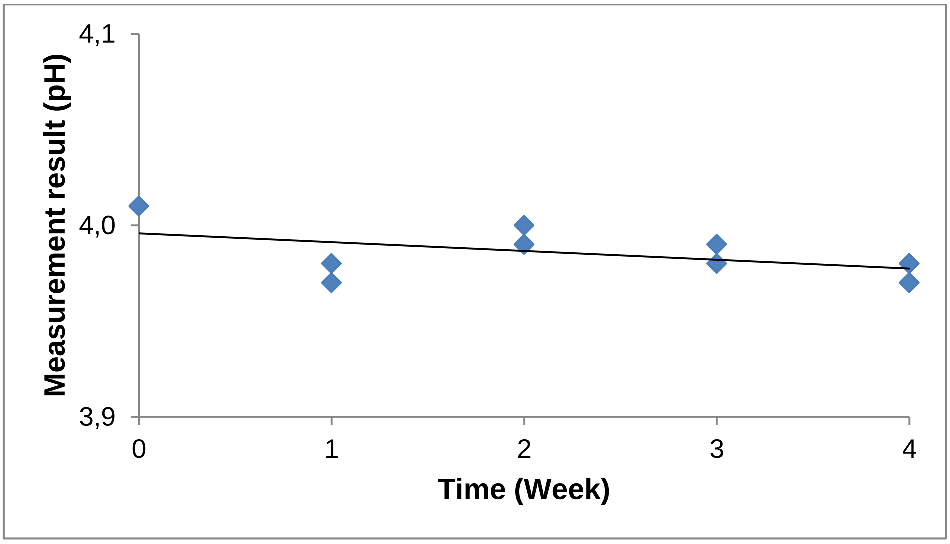

Figure 4. UME CRM 1401 Short Term Stability at 18°C

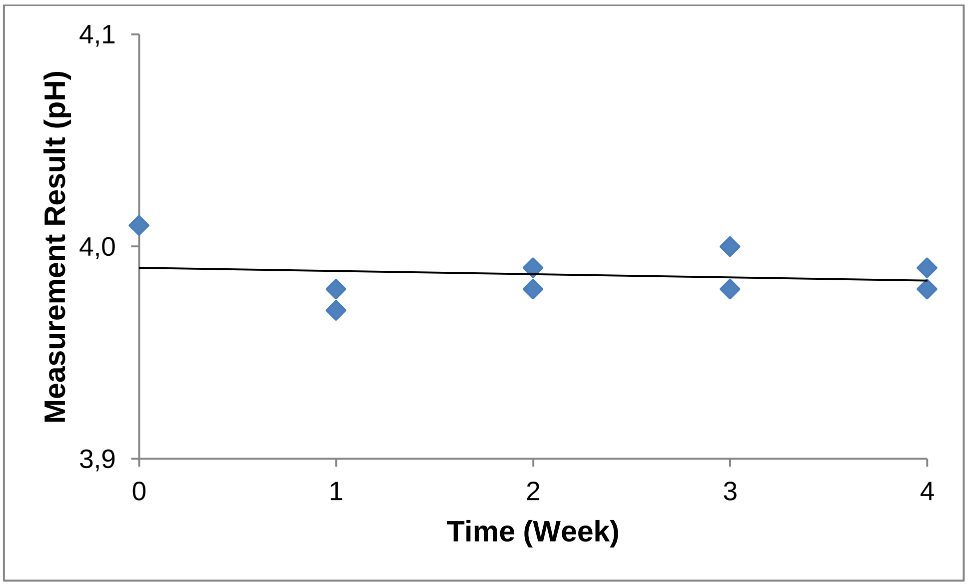

Figure 5. UME CRM 1401 Short Term Stability at 50°C

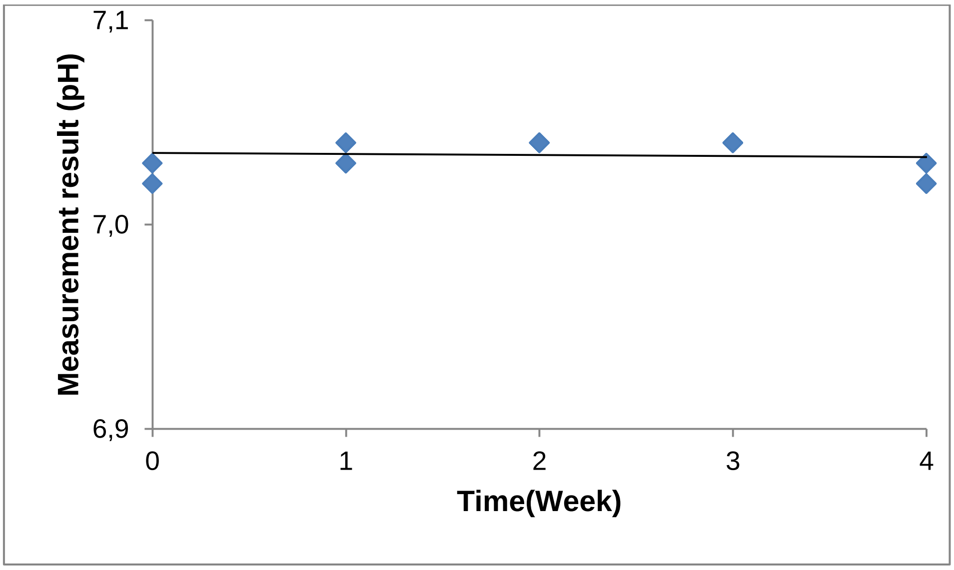

Figure 6. UME CRM 1402 Short Term Stability at 18°C

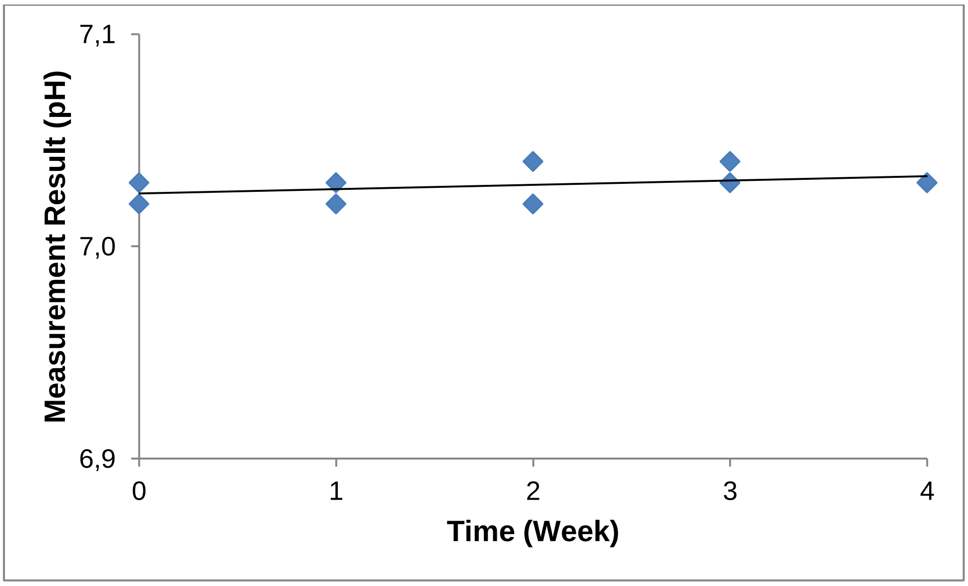

Figure 7. UME CRM 1402 Short Term Stability at 50°C

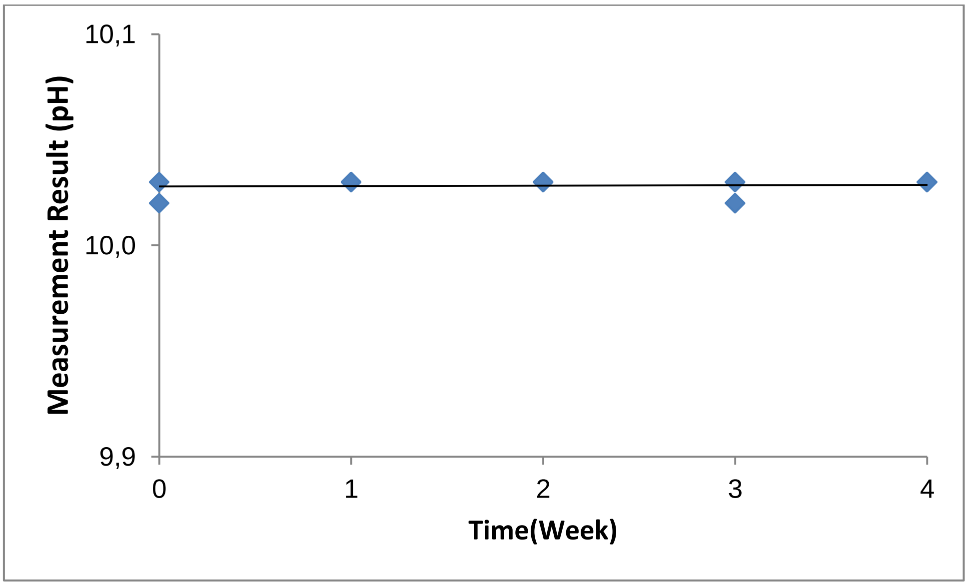

Figure 8. UME CRM 1403 Short Term Stability at 18°C

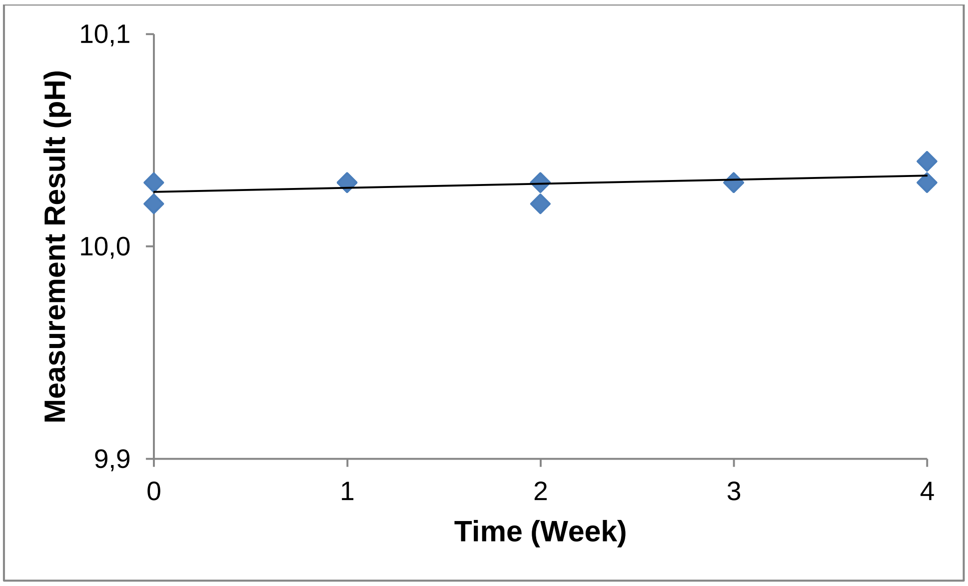

Figure 9. UME CRM 1403 Short Term Stability at 50°C

Annex 3. Results of the Long Term Stability Studies

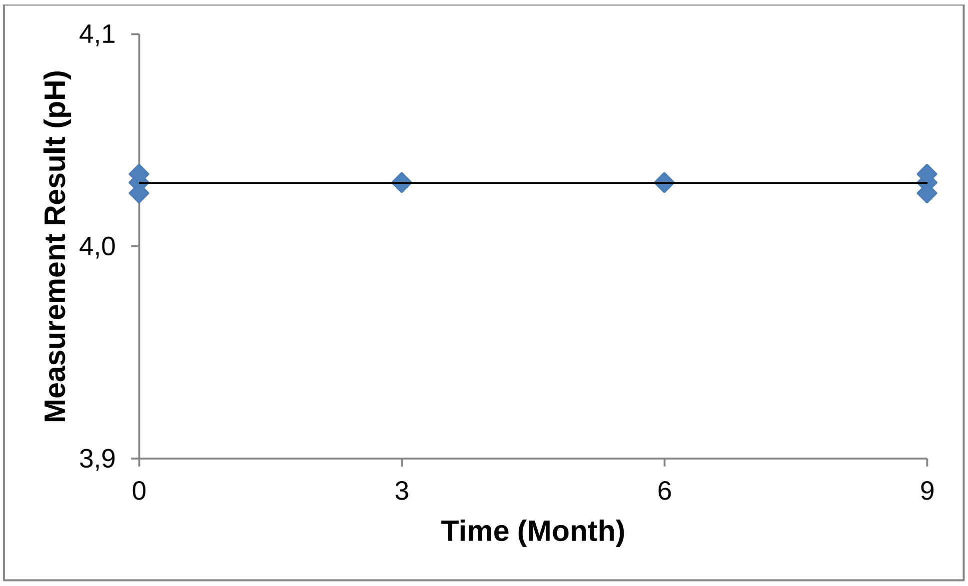

Figure 10. UME CRM 1401 Long Term Stability at 18°C

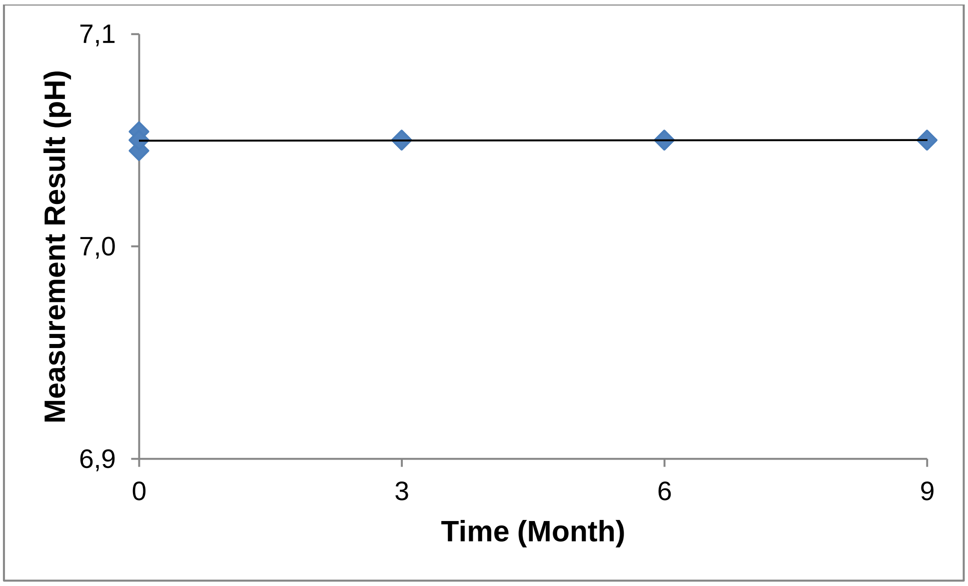

Figure 11. UME CRM 1402 Long Term Stability at 18°C

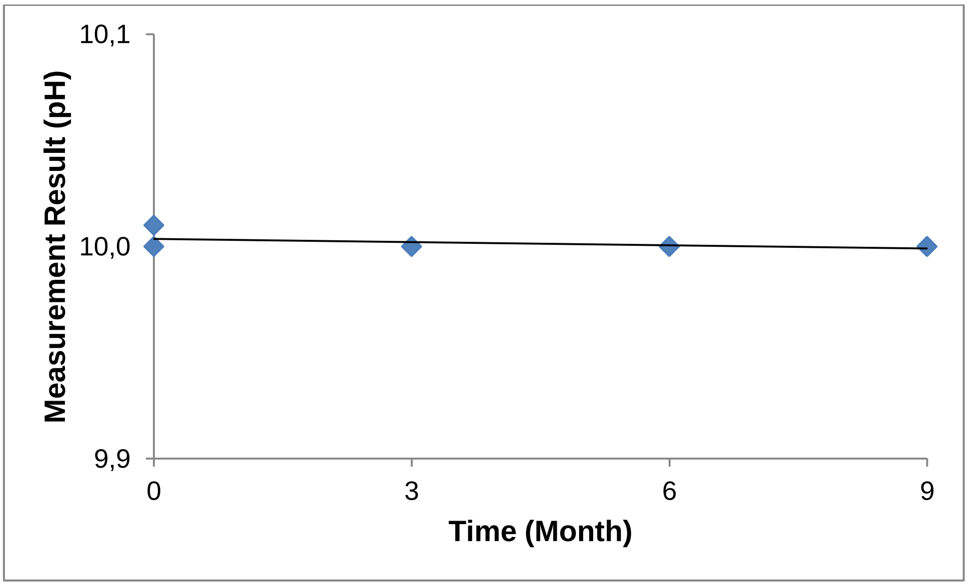

Figure 12. UME CRM 1403 Long Term Stability at 18°C