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Statistical Analysis of Ambient Conditions and Water Temperature on Standard Achievement of Volume Calibration of Small Laboratory Glassware

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Abstract

Volumetric apparatus calibration is a very sensitive matter in metrological institutions. Identification and evaluation of the uncertainty factors affecting volume calibration of volumetric apparatus such as small laboratory glassware is a critical issue to investigate in order to increase accuracy in calibration. This study investigates the contributions of ambient conditions and water temperature in volume calibration of small laboratory glassware. The study used existing empirical data from the Tanzania Bureau of Standards. The multiple linear regression model was used to establish better relationship between explanatory variables and response variable. The model analyzed three predictor variables namely ambient temperature, pressure and relative humidity. Water temperature was dropped due to high multicollinearity with ambient temperature. The results from this study revealed that the variations in calibration of small volumetric laboratory glassware have strong association with ambient temperature, pressure and their interaction and weak one with ambient relative humidity. It is therefore recommended to have appropriate settings of these ambient conditions in volume calibration of small laboratory glassware to ensure that the glassware used for analysis and other practices are accurately calibrated for betterment of practical or test results.

Keywords: Calibration, ambient conditions, small laboratory glassware, uncertainty factors

Introduction

Calibration is the process of configuring an instrument to provide a result for a sample within an acceptable range of accuracy. This practice is performed by special organs such as accredited laboratories and institutes of standards. The present study focused on uncertainty factors associated with calibration of volumetric glassware, especially small laboratory glassware such as micropipette or piston pipettes, graduated tubes and other volumetric vessels (Almeida et al. 2013, Rahman et al. 2015, de Groot 2018). Small laboratory glassware are important equipment which find use in different fields. The measurement of small amounts of liquids is very important in fields like research, health, chemistry, microbiology and genetics (Almeida et al. 2013, Rahman et al. 2015). So, for accurate results from volumetric glassware, accurate and precise calibration is important for best results in production, investigation or research.

Small laboratory glassware is calibrated by the use of gravimetric method, using a liquid of known specific density (generally pure water e.g., distilled water) at a reference temperature of 20 °C and an analytical balance (Almeida et al. 2013, de Groot 2018). This practice is based upon determination of the volume of water either contained in or delivered by the vessel under specified ambient conditions (specified room temperature, pressure and relative humidity) (Rahman et al. 2015). This method is not a straight forward approach (de Groot 2018). The method involves a lot of processes and measurements like weighing the weight of water contained or delivered by a vessel and then convert it to volume using special formula at a reference temperature (normally 20 °C) (Sutton and Reid 2017, Almeida et al. 2013, de Groot 2018). The formula used for conversion of liquid weight to volume depends on accuracy measurements of water temperature, air temperature and pressure (Almeida et al. 2013, de Groot 2018). Also, appropriate setting of ambient relative humidity helps in accuracy weight measurements of water if evaporation is a concern (Faison and Brickenkamp 2004, Sutton and Reid 2018). Normally, ambient conditions of the calibration laboratory are controlled by weather control system with installed equipment like barometer, thermometer and hygrometer to monitor and control the ambient conditions (Ogu et al. 2016, de Groot 2018).

When ambient conditions and water temperature measurement are not well monitored and controlled, they will result in wrong or poor calibration (Rahman et al. 2015, de Groot 2018). To manage degree of uncertainty caused by irregularities of ambient conditions and water temperature during calibration, several studies have been done to ascertain the degree of uncertainty for ambient conditions and water temperature (Faison and Brickenkamp 2004, Sutton and Reid 2017). The findings have shown that calibration within uncertainty range of relative air humidity between 40% and 60% with an error of ± 10% and temperature between 20 °C and 23 °C at local constant of ± 1 °C produces an accurate calibration result (Faison and Brickenkamp 2004). Malengo et al. (2018) in their report on ambient conditions for gravimetric volume calibration, the setting of ambient pressure between 600 hPa and 1100 hPa, ambient temperature between 15 °C and 27 °C and relative humidity between 20% and 80% have shown better calibration results. Also, to overcome calibration errors due to water temperature, the findings have shown accurate calibration when the test water was allowed to stay in the working room for a sufficient time (1 h to 2 h) to reach equilibrium with the room conditions (BIS 2012).

Despite establishment of uncertainty or working range of ambient conditions, there is no study in the existing literature which has been done to analyze the effects of ambient conditions and water temperature on volume calibration of small laboratory glassware. The current study employed a multiple linear regression model to investigate the influence of ambient conditions and water temperature in volume calibration of small laboratory glassware. The results of this study will allow the improvement of the calibration procedures of small laboratory glassware and harmonization of the results between laboratories for better comparable results.

Material and Methods

The current study used secondary data from metrology laboratory of Tanzania Bureau of Standards (TBS) and the collection permission was assisted by a Senior Metrologist in that laboratory. Seventy (70) observations were collected from a series of repeated observations which were carried out during 1000 mL strike measure calibration. The data included calibration volume (cvol) as a response variable and four predictor variables namely ambient temperature (ambtemp), ambient pressure (ambpres), ambient relative humidity (ambrh) and water temperature (wtemp). The data were analyzed descriptively in terms of measures of central tendency and measures of variability. The measures of central tendency include the mean, median and mode. The measures of variability include standard deviation, skewness and kurtosis. This analysis of data is necessary as it helps to determine the normality of the distribution.

The response variable assumed to be directly related to a linear combination of explanatory variables. Multiple Linear Regression (MLR) model was used to fit the data. The model aimed at establishing the relationship between calibration volume of small laboratory glassware as response variable denoted by Y and the explanatory variables which are ambient temperature X_1 ambient pressure X_2, ambient relative air humidity X_3 and water temperature X_4 together with their interaction terms $(X_{15} + \cdots + X_{ij})$. The interaction terms represent the dependence contribution of one explanatory variable on a certain level or value of one or more explanatory variables (Fitzmaurice 2000). The relationship between the response variable and the explanatory variables is represented by the following equation:

$$Y_i = \beta_0 + \beta_1 X_{i\,1} + \beta_2 X_{i\,2} + \beta_3 X_{i\,3} + \beta_4 X_{i\,4} + \beta_5 X_{i\,5} + \ldots + \beta_j X_{i\,j} + \varepsilon_i \,, \qquad (1)$$

where $\beta_j; j = 0, 1, 2, 3, \ldots, k$ are regression coefficients for k explanatory variables, the subscript i denote the number of observations, ε is an error term assumed to be normally distributed with the properties that $E(\varepsilon_i) = 0$, the errors have constant variance (i.e., $Var(\varepsilon_i) = \sigma^2$) and $Cov(\varepsilon_i, \varepsilon_j) = 0$ for $i \neq j$. From this we obtain

$$\hat{Y}_i = E(Y|X = x_i) = \beta_0 + \beta_1 x_{i\,1} + \ \ldots \ + \beta_j x_{i\,j} \,.$$

The MLR model was developed starting with the linear combination of the response variable with the explanatory variables without interaction terms (model equation (2)),

$$Y_i = \beta_0 + \beta_1 X_{i\,1} + \beta_2 X_{i\,2} + \beta_3 X_{i\,3} + \beta_4 X_{i\,4} + \varepsilon_i, \qquad (2)$$

followed by a model with addition of interaction terms (model equation (1)). Addition of interaction terms followed hierarchical approach, which enters variables in a series of blocks of variables to examine whether each new block adds anything to the prediction produced by the previous block.

The independent effects of the explanatory variable on the response variable were not considered since the calibration practice takes place in the environment where all these explanatory variables coexist. The process of developing and analyzing the model were performed using Stata.

The correct use of the MLR model requires that several critical assumptions be satisfied in order to apply the model and establish validity. The assumptions include linearity, independence of errors, homoscedasticity, normality, and collinearity (Garson 2012). The test for these assumptions was performed using Statistical Package for the Social Sciences (SPSS) and Stata software package.

The linearity relationship between each explanatory variable and the response variable were determined by constructing a scatter plot for the explanatory variables against the response variable Y. Multicollinearity among a set of explanatory variables were examined by variance inflation factor (VIF). The VIFs above 10 are seen as a cause of multicollinearity among explanatory variables. The explanatory variables with high VIF (VIF above 10) imply that their effects in the model can be explained by another explanatory variable within the model and they are excluded (Landau and Everitt 2004).

The Durbin-Watson statistic was used to determine the independence of error. The Durbin-Watson statistic is generally ranging from 0 to 4. The values between 1.5 and 2.5 mean that the errors are independent of one another (uncorrelated), and if the value approaches 0, it indicates increasingly stronger positive correlation and values towards 4 indicate increasingly stronger negative correlations (Garson 2012, Stirba 2016). Furthermore, the plot of the standardized residuals (the errors) against the standardized predicted values was used to test variance of error term (homoscedasticity). When this assumption is satisfied, residuals normally form a non-pattern cloud of dots around the regression line (Keith 2014).

When the assumptions of the MLR model (1) were satisfied, the method of least squares was used to find the optimal estimator of the unknown regression coefficients β_j's of the model. The estimates $\hat{\beta}_j$'s of the model parameters were estimated using sample data in Stata software to give the best fit of the observations (Montgomery and Runger 2014).

The coefficient of determination R^2 and adjusted coefficient of determination $\bar{R}^2$ were computed. The R^2 was used to measure usefulness of the model for predicting calibration volume as a response variable. The R^2 depicts how well the response variable can be explained by explanatory variables. $\bar{R}^2$ has similar interpretation as R^2, however it attempts to improve estimation of R^2. R^2 takes on values between 0 and 1, and $\bar{R}^2$ is always smaller than R^2. The predictive power of explanatory variables increases as the values of R^2 move from 0 to 1. If the extreme value of the coefficient of determination is zero, it implies that the model explains none of the variability of the response data around its mean, and if it is one, it implies that all variations in the suggested model are explained by the predictor variables and that the fit is perfect.

Furthermore, the Analysis of Variance (ANOVA) was performed to examine the significance of the model. ANOVA is a statistical test that allows consideration of parameters of several populations at once, by testing hypothesis on two or more parameters at a time. It tests the null hypothesis that H_0: $\beta_1 = \beta_2 = \beta_3 = \cdots = \beta_j = 0$ (intercept only model) against the alternative hypothesis H_1: At least one of the β parameters listed in H_0 differs from 0 (predictor dependence model) (Graybill and Iyer 1994, Mendenhall and Sincich 2012). The *p*–value for *F* statistic was used to test the significance of the model at the level of significance of α = 0.05. The model with predictors is considered to be significant if the *F*–value is greater than the level of significance (i.e., there exists relationships between response variable and explanatory variables). If the *F*–value is less than the level of significance, it implies that the model with no predictor is significant (no relationship between response variable and explanatory variables) (Graybill and Iyer 1994, Mendenhall and Sincich 2012).

Results

As shown in Table 1, the average values of the ambient temperature, pressure, relative humidity and water temperature from the sample data are 22.48 °C, 961.96 *hPa*, 59.8% and 22.59 °C, respectively. Also, the middle values (p50) of the ambient temperature, pressure, relative humidity and water temperature are 21.96 °C, 1000.35 *hPa*, 57.6% and 22.20 °C, respectively.

The standard deviation (sd) for the ambient temperature, pressure, relative humidity and water temperature are 4.73, 79.34, 12.85 and 4.23, respectively. Because standard deviation is a measure of the variability about the mean, this is shown as the mean plus or minus one or two standard deviations($i.e., mean \pm sd$ or $mean \pm 2sd$). As shown in Figure 1 and Figure 2, majority of the observations are within one standard deviation of the mean, and nearly all within two standard deviations of the mean.

Table 1: Descriptive statistics of ambient conditions and water temperature

Statistics	ambtemp	ambpres	ambrh	wtemp
N	70	70	70	70
Mean	22.48071	961.9556	59.80629	22.59414
p50	21.955	1000.35	57.63	22.195
Sd	4.730788	79.3436	12.84925	4.227372
Min	14.81	789.28	31.6	15.39
Max	31.1	1056.8	86.3	29.89
Range	16.29	267.52	54.7	14.5
Skewness	0.2062174	-0.5584883	0.0189588	0.0726298
Kurtosis	1.856683	1.73412	2.549749	1.888542

The coefficient of skewness is and indicator for symmetrical or asymmetrical distributions. The coefficient of skewness for the ambient relative humidity and water temperature are very small (relatively close to zero) such that their distributions look fairly normal as shown in Figure 1.

The distribution of the ambient temperature is slightly skewed to the right (slightly positively skewed) with coefficient of skewness of 0.21 and that of the ambient pressure is slightly skewed to the left (slightly negatively skewed) with the coefficient of skewness –0.56. Figure 2 shows the distributions of ambient temperature and pressure.

Lack for normality of ambient temperature and pressure were corrected by transformation of these data. Square root transformation was done for ambient temperature values. For ambient pressure, its values were first reflected and then transformed by taking square root of the reflected values (Howell 2010, Tabachnick et al. 2019). The resulted distributions for both transformed ambient temperature (tr.amtemp), X_1^* data values and transformed ambient pressure (tr.ambpres), X_2^* data values are shown in Figure 3.

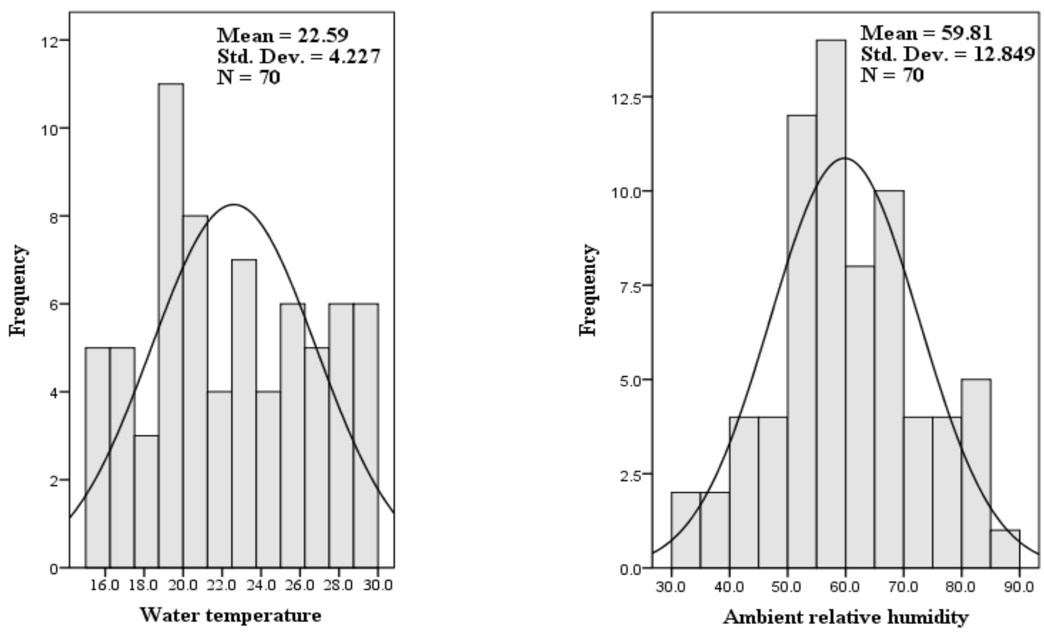


Figure 1: Histograms for the distributions of water temperature and ambient relative humidity.

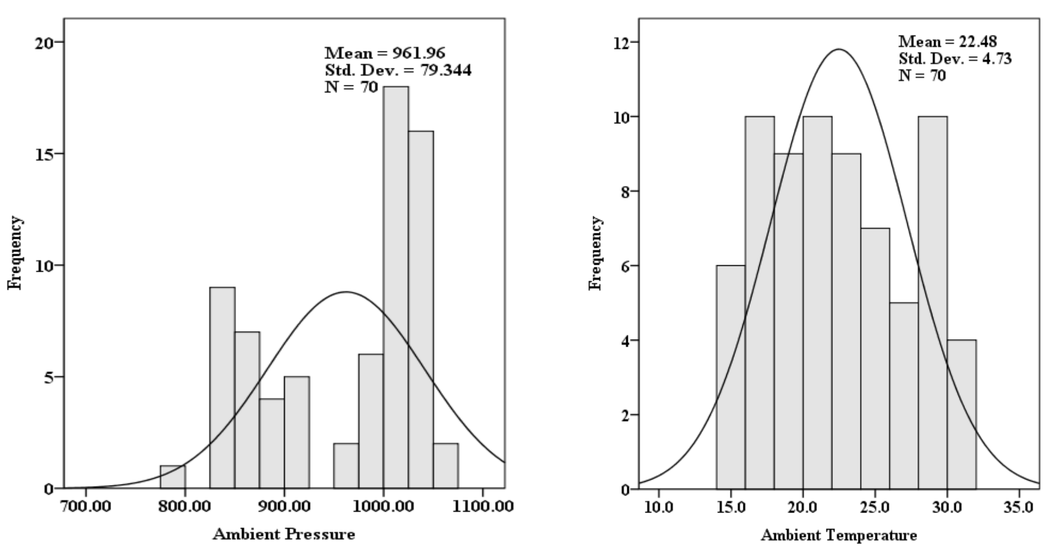


Figure 2: Histogram for the distributions of ambient temperature and ambient pressure.

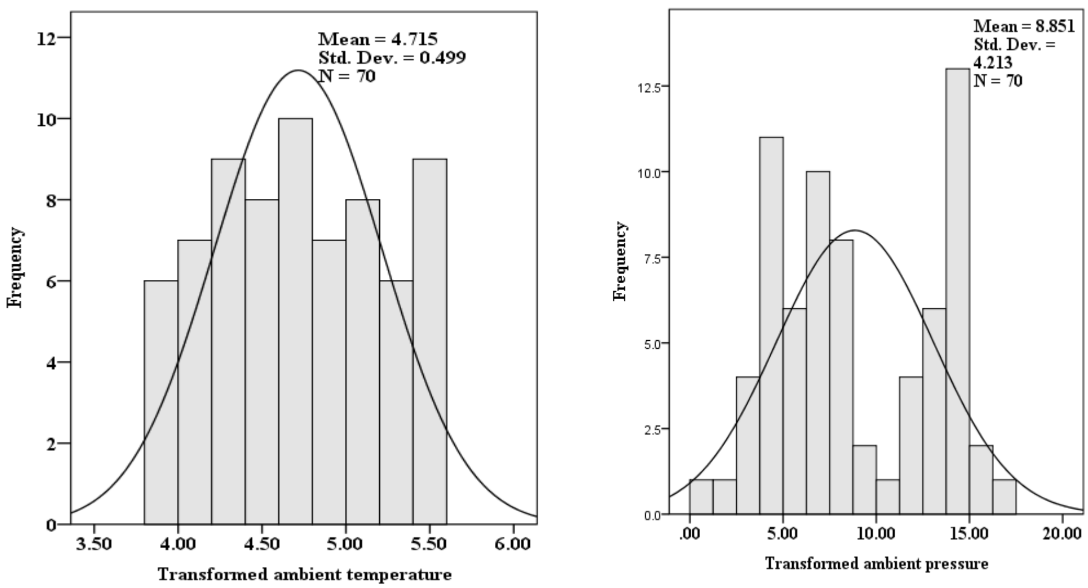


Figure 3: Histogram for the transformed distributions of ambient temperature and pressure.

Model assumptions

Multicollinearity

Test for multicollinearity among explanatory variables is very important. Table 2 shows variance inflation factors for the four explanatory variables, transformed ambient temperature, transformed ambient pressure, relative humidity and water temperature.

The VIFs for the ambient temperature and water temperature are very high; this indicates the presence of multicollinearity between them. Therefore, in this study water temperature was excluded in the model as its effects can be explained by ambient temperature.

Table 2: Variance inflation factors for the explanatory variables

Variables	Multicollinearity status	
	Tolerance	VIF
tr.ambtemp	0.059	17.082
tr.ambpres	0.545	1.836
ambrh	0.748	1.336
wtemp	0.071	14.152

Linearity

Figure 4 shows that all the explanatory variables have linear relationships with the response variable, hence the assumption is confirmed.

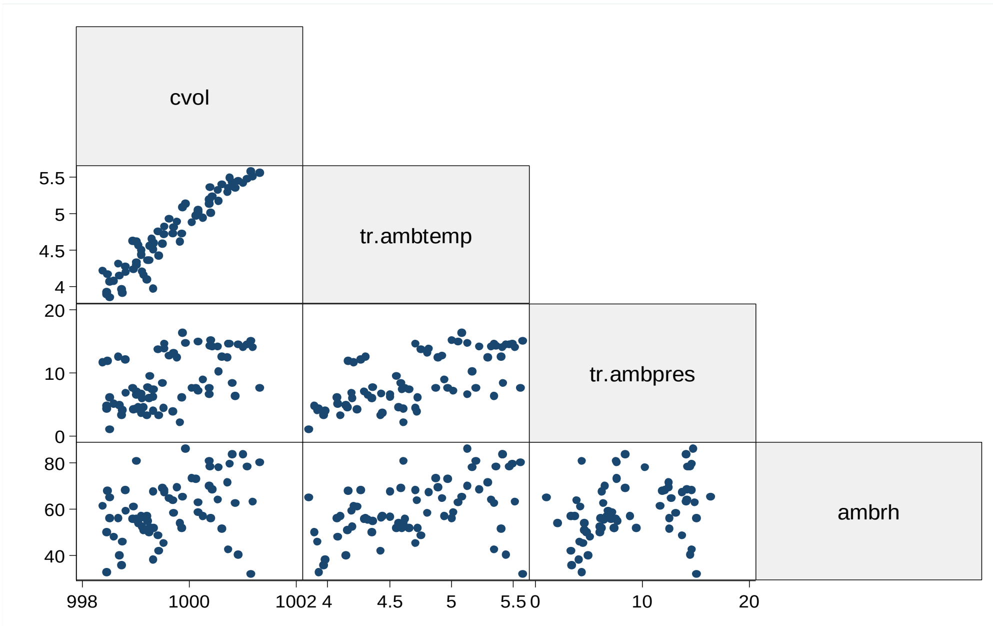


Figure 4: Scatter plots of every predictor variable against response variable.

The matrix scatter plot shows a stronger linear relationship between calibration volume and ambient temperature, moderate linear relationship between calibration volume and ambient pressure, and slightly linear relationship between calibration volume and ambient relative humidity. Though ambient temperature shows a linear relationship with ambient pressure and relative humidity, its correlation is less than 80% which cannot affect much the regression.

Independent errors

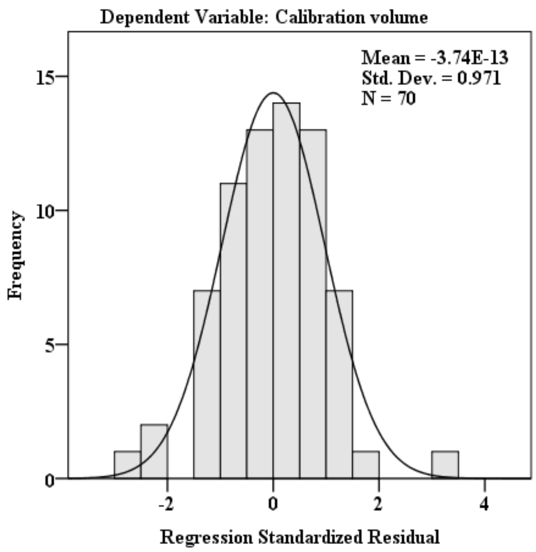
In this study the Durbin-Watson statistic is 2.177 which is between 1.5 and 2.5 and very close to 2 as shown in the last right column of Table 3; therefore the errors are independent of one another.

Table 3: Autocorrelation analysis of errors by Durbin-Watson

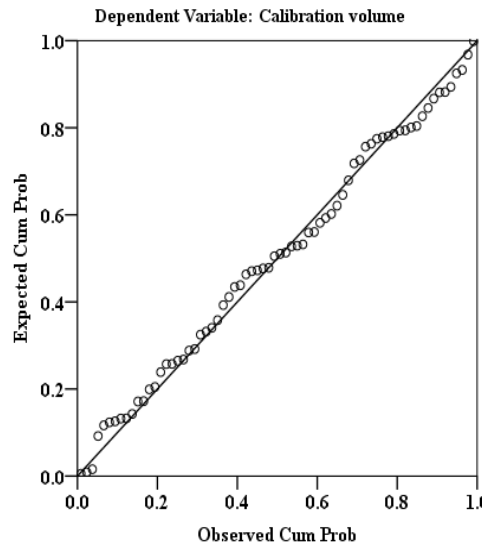
Model	R	R square	Adjusted R square	Std. error of the estimate	Durbin-Watson
1	0.966	0.933	0.929	0.217500612019461	2.177

Normality

In Figure 5, the residual plots in the histogram show that the distribution of errors follows a normal distribution (i.e., the residuals are normally distributed around zero). The expected and observed cumulative probabilities in normal probability plot are fairly normal as most of the points cluster around the straight line. This analysis shows small deviations of observed calibration volume from predicted calibration volume. In this case, the assumption is not violated.



(a) Histogram of the standardized residual.



(b) Normal probability plot of the residue.

Figure 5: Residue analysis.

Homoscedasticity

The plot in Figure 6 shows no pattern, and thus the data points seem fairly randomly distributed with a fairly even spread of residuals at all predicted values. Therefore, the error variation of the sample data under investigation is the same for the entire range of response variable (homoscedasticity).

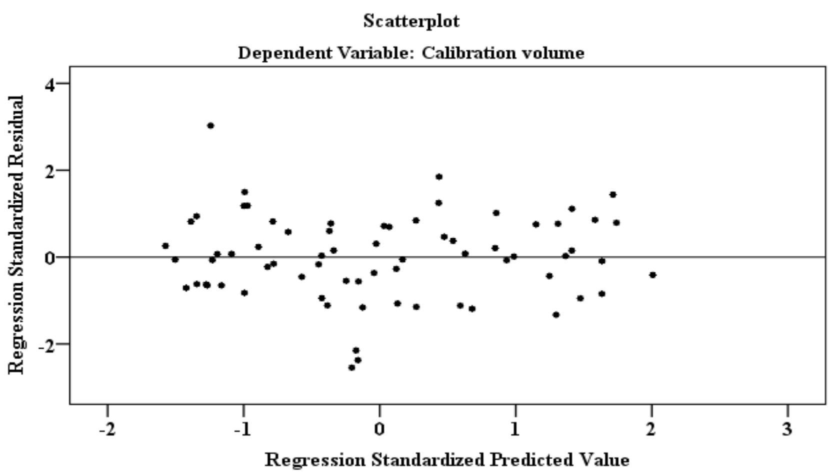


Figure 6: Residual plot.

Regression model

The Stata output for fitting the multiple regression model of the response variable Y with three explanatory variables (without interaction) X_1^*, X_2^* and X_3 are presented in Table 4. The results in Table 4 show that the combinations of explanatory variables significantly contribute in volume calibration. The R^2 using all explanatory variables (X_1^*, X_2^* and X_3) simultaneously is 0.92. This indicates that 92% of variation in volume calibration of small laboratory glassware was explained by the model. The *F–statistic* (F (3, 66) = 255.41) with *p–value* < 0.00001 at the level of significance α = 0.05, suggest that the combination of these explanatory variables contribute significantly to the calibration volume of the small laboratory glassware. Only ambient temperature and pressure are significantly contributing to the volume calibration at the significance level of $p < 0.05$. That is, ambient temperature and pressure together explain 92% of the variations in volume calibration of small laboratory glassware. But this does not imply that ambient relative humidity is not important factor in calibration. It has contribution, though it is little compared to the other two explanatory variables. The resulted model after substituting parameter coefficient in model (2) as estimated by least square method is

$$\hat{Y} = 991.84 + 1.77X_1^* - 0.03X_2^* - 0.005X_3. \quad (3)$$

The parameter coefficients in model (3) suggest that ambient temperature contribute more in volume calibration of small laboratory glassware.

Table 4: Stata output fitting calibration volume to ambient temperature, pressure and relative humidity

Source	SS	df	MS
Model	42.2079686	3	14.0693229
Residual	3.63567494	66	0.055085984
Total	45.8436436	69	0.664400632

Number of obs	= 70
F (3, 66)	= 255.41
Prob > F	= 0.0000
R-squared	= 0.9207
Adj R-squared	= 0.9171
Root MSE	= 0.2347

Y	$\hat{\beta}_j$ Coef.	Std. Err.	t	P>$\|t\|$	[95% Conf.	Interval]
X_1^*	1.774911	0.0810247	21.91	0.000	1.61314	1.936682
X_2^*	-0.0294398	0.0089588	-3.29	0.002	-0.0473267	-0.01155
X_3	-0.0048241	0.0024892	-1.94	0.057	-0.009794	0.000146
$Constant$	991.835	0.3112123	3187.01	0.000	991.2137	992.4564

When interaction terms were added to model (3) only one block variables were significantly adding value to the prediction produced by previous explanatory variables. Table 5 shows the model results of the MLR model (3) when the interaction terms were added hierarchically. The addition of interaction term between ambient temperature and pressure to model (3), has shown a significant improvement on the calibration of small laboratory glassware, (F (4, 65) = 226.15, $p < 0.00001$. The R^2 has increased from

92% to 93% and the residual has decreased by 0.57. That is, addition of the interaction term between ambient temperature and pressure explains 1% more of the variation in volume calibration than explained by model (3). Only 7% of variation in volume calibration can be explained by other factors not included in the model. The parameter coefficient for the ambient temperature, ambient pressure and that of the interaction between ambient temperature and pressure were tested significantly at the level of significance α = 0.05. The resulted model is

$$\hat{Y} = 993.88 - 0.27X_1^* + 1.30X_2^* - 0.002X_3 + 0.05X_1^*X_2^*. \quad (4)$$

Table 5: Stata output fitting calibration volume to ambient temperature, pressure, relative humidity and interaction terms

Source	SS	df	MS
Model	42.7703614	4	10.6925903
Residual	3.07328219	65	0.047281264
Total	45.8436436	69	0.664400632

Number of obs	= 70
F (4, 65)	= 226.15
Prob > F	= 0.0000
R-squared	= 0.933
Adj R-squared	= 0.9288
Root MSE	= 0.21744

Y	$\hat{\beta}_j$ Coef.	Std. Err.	t	P>$\|t\|$	[95% Conf. Interval]	
X_1^*	-0.2748404	0.0716366	-3.84	0.000	-0.4179086	-0.1317722
X_2^*	1.300565	0.1566886	8.30	0.000	0.9876357	1.613493
X_3	-0.0023535	0.0024149	-0.97	0.333	-0.0071763	0.0024694
X_5	0.0513645	0.0148932	3.45	0.001	0.0216207	0.0811083
$Constant$	993.8817	.6597723	1506.40	0.000	992.5641	995.1994

X_5 is the interaction between ambient temperature and pressure $X_1^*X_2^*$

The β coefficients in the MLR model (4) suggest that the ambient pressure has more influence in volume calibration of small laboratory glassware. This is different compared to model (3) where ambient temperature was the leading cause of variations when the explanatory variables were tested without interaction effects. The ambient temperature is negatively related to volume calibration while ambient pressure and the interaction of ambient temperature and pressure are positively related to volume calibration. The interaction term suggests that for every unit increase in ambient pressure, the constant term (993.88) will increase by 1.3 and the slope of ambient temperature will increase by 0.05 as shown in model (5).

$$\hat{Y} = (993.88 + 1.30) + (-0.27 + 0.05)X_1^* - 0.002X_3$$
$$\hat{Y} = 995.18 - 0.22X_1^* - 0.002X_3 \quad (5)$$

Likewise, for every unit increase in ambient temperature, the constant term will decrease by 0.27 and the slope of the ambient pressure increases by 0.05 as shown in model (6).

$$\hat{Y} = (993.88 - 0.27) + (1.30 + 0.05)X_2^* - 0.002X_3$$
$$\hat{Y} = 993.61 + 1.35X_2^* - 0.002X_3 \quad (6)$$

In model (5), the unit change in ambient pressure has shown an absolute large contribution to the constant term compared to the unit change in ambient temperature in model (6).

Discussion

The observed variations in volume calibration of small laboratory glassware can be explained in terms of ambient temperature, pressure, relative humidity and the interactions between ambient temperature and pressure. The analyses of the models (3) and (4) have shown a highly significant contribution of ambient temperature and pressure and at a small extent with relative humidity in model (3). Literature reveals that during determination of the volume of water, the accuracy of measurements is affected by ambient temperature, pressure and relative humidity (Mangukiya and Panchal 2016). These factors are usually combined to give the Z-factor used in calculation of volume of water. The Z-factor or correction factor equation can be found in de Groot (2018), though the dependence of this Z-factor from humidity is insignificant in comparison with the other parameter dependencies (de Groot 2018). The ambient relative humidity has a significant contribution in volume calibration of small laboratory glassware during mass weighing of the water delivered or contained by a vessel if evaporation is of concern (Liang et al. 2012).

In addition to that, the study by Lorefice (2009) has also shown the contribution of ambient temperature, pressure and relative humidity in volume calibration of volumetric glassware. Figure 7 summarized the interactive contribution of ambient temperature, pressure, relative humidity and water temperature.

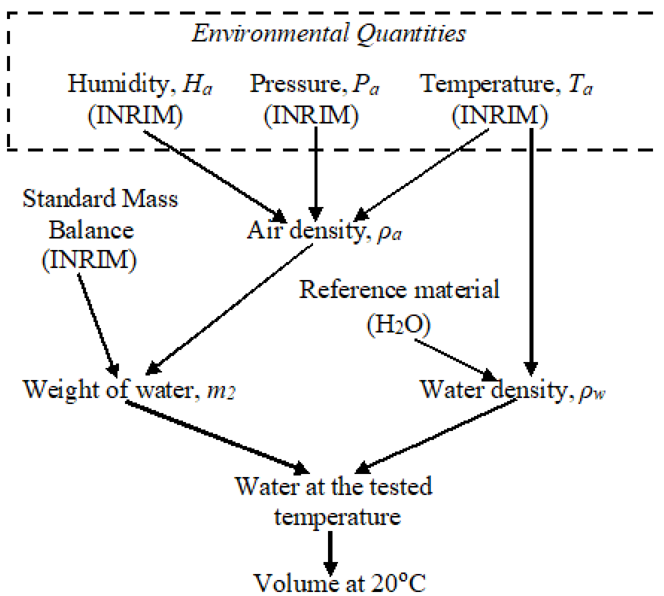


Figure 7: Traceability of uncertainty chain for volume measurements at INRIM (Lorefice 2009).

Interactions between ambient temperature and pressure have shown significance contributions in volume calibration of small laboratory glassware specifically during ambient air density measurements (Lorefice 2009, de Groot 2018). Air density is important especially when weighing the liquid mass to the highest accuracy (Lorefice 2009).

Furthermore, studies indicate that volumetric glassware calibration is affected by its make material. Volumetric glass material expands or shrinks against small change in temperature as different types of glass materials have different expansion coefficients hence affecting volumetric glassware volume calibration (Rahman et al. 2015). Also, water density is affected by ambient temperature as the water density depends on water temperature in equilibrium to ambient temperature (Rahman et al. 2015, Sutton and Reid 2018, de Groot 2018).

Conclusion

In this study, analysis of variance (ANOVA), and regression techniques were used to determine the contribution of ambient conditions and water temperature in volume calibration of small laboratory glassware. F statistics and *p*–values were used to test hypotheses about relationships between explanatory and response variables. Ambient temperature, pressure and relative humidity were considered. Both the ANOVA and regression techniques produced significance tests for ambient temperature and pressure together with their interaction term. The results have shown a potential contribution of ambient temperature, pressure and the interaction between ambient temperature and pressure in volume calibration of the small laboratory glassware. Ambient relative humidity has shown a week contribution in volume calibration when the interaction effects were not considered for a *p*–value less than the 0.05 significance level. Therefore, it is recommended to consider appropriate settings of these ambient conditions in volume calibration of small laboratory glassware to ensure that the glassware used for analysis and other practices is accurate and is within the tolerance limits of the nominal value.

Furthermore, the amount of variance explained could be maximized by including other factors such as factors originating from the balance (e.g., readability, repeatability, and departure from nominal value), influences from physical conditions of the weighing object and the use of nonlinear methods and models.

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