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# VESSEL CROSS SECTIONAL AREA EFFECT ON SMALL VOLUME MEASUREMENT USING GRAVIMETRIC METHOD

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***Abstract*** **-** A study to identify the effect of changes in vessel cross-sectional area to the mass evaporation rate of micropipette calibration without evaporation trap at nominal value of 20 µl is still being tested. Three different vessels with various diameter values of 5.9 mm, 9.8 mm and 13.7 mm were used to determine their effect to evaporation. The result of the test showed that the smaller diameter of vessel reduced the mass evaporation rate on micropipette calibration.

***Keywords*****:** evaporation, cross sectional area, vessel, small volume, micropipette

## 1. INTRODUCTION

Micropipette or pipette piston is widely used for measuring the volume in various fields such as health, chemistry, biology, pharmaceuticals and genetics. Laboratories should ensure that the measurement results using a micropipette or pipette piston is reliable. Therefore, it is necessary to calibrate the micropipette using correct methods and estimation of its uncertainty.

This study is carried out for highly sensitive measurements (with a very small amount of water) where a small mistake in pipetting may cause to large errors in the final results. One of the factors that may cause errors of determining the volume of the measuring results of micropipette is evaporation.

According to ISO 8655-6 and EURAMET cg on measurements of micropipette volume with a volume of less than 50 µL, evaporation effects need to be considered. With the evaporation feared volume measured at nominal value of less than 50 µL can be smaller than the true value. The reference documents also mention the need for evaporation trap to reduce the amount of water particles released into the atmosphere.

Evaporation trap serves to limit direct contact between the water surface and the atmosphere and in case of evaporation occurred water particle can be trapped in the equipment and the water particles still can still be known mass by weighing process [1][2][3].

The objective of this study is to reduce the effect of evaporation used smaller diameter of vessels therefore it can be seen how effective the vessel cross-sectional area can be used without using evaporation trap and calculate evaporation factor.

## 2. MECHANISM FOR REDUCING EVAPORATION

Evaporation is the process whereby liquid is converted to water vapor and removed from the evaporating surface. Evaporation is a natural physical process. Standard of ISO 8655-6 requires that the evaporation process should be taken into consideration in the calculations and decrease the possible measuring errors. For volumes below 50 µl the standard states it explicitly that a weighing vessel with a lid or other methods compensating the process should be used.

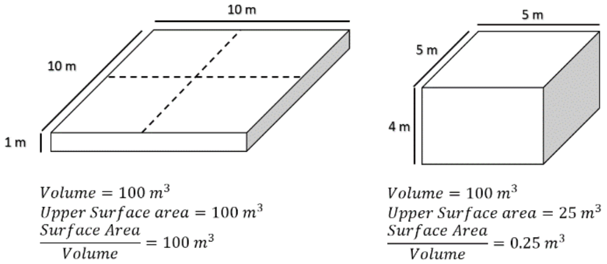


Figure 1: Surface area modification for a given volume of water and its effect of surface area to volume ratios.

The loss of water volume through the evaporation is controlled by the rate of evaporation and the surface area of the water exposed to the atmosphere. Any change to the surface area of the water transfers directly into a change in evaporation volume, i.e. 20% reduction in surface area results in a 20% reduction in evaporation volume. The volume of evaporation is so strongly controlled by surface area that there are some opportunities to exploit this factor to reduce the evaporation. For a given water storage, reducing the surface area for the available volume of water is a way to achieve this. This is illustrated in Figure 1 where the effect of changing to the configuration from the left hand design to the right hand design would be a 75% reduction in evaporation volume [6].

## 3. CALCULATION OF WATER VOLUME TEST

The gravimetric method is used to determine the volume of the test water micropipette. The method is determining the volume of water by weighing the water with calibrated balance and dividing this by the density of water, when determining the volume of water, the accuracy of measurements is affected by various factors such as ambient temperature, atmospheric pressure and relative humidity.

The equation for the volume of the test water is as follows [4]:

$$V_{20} = \frac{m}{\rho_b} \times \frac{\rho_b - \rho_a}{\rho_w - \rho_a} \times [1 - \alpha_c(t_{dw} - t_{d20})] \quad (1)$$

with

$$m = m_L - m_E \quad (2)$$

Where

$V_{20}$ volume, at temperature of 20 °C, in µl;

$m_L$ weighing result of the recipient after the pipetting, in mg;

$m_E$ weighing result of the recipient before the pipetting, in mg;

$\rho_w$ water density, at calibration temperature t, in mg/µl;

$\rho_a$ air density, in mg/µl (0.0012 mg/µl);

$\rho_b$ mass pieces density (8.0 mg/µl);

$\alpha_c$ material cubic thermal expansion coefficient of the micropipette, in 1/°C;

$t_w$ water temperature, in °C.

## 4. METHOD

A tested object was a micropipette with fixed nominal value of 20 µl. The measurement was made with empty and full three vessels with different nominal diameter of 5.9 mm (D1), 9.8 mm (D2) and 13.7 mm (D3). All the measurements and calculations were performed in accordance to ISO 8655-6.

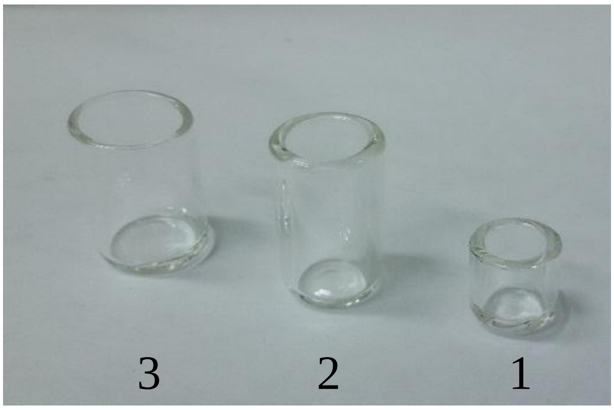


Figure 2: The various vessels with different diameter

The weighing instrument is the most important measuring instrument for the gravimetric calibration of measuring devices used to determine the volume of liquid. The Mettler Toledo electronic balance type XP 26 which has a resolution of 1 µg was used. Before taking the measurement the equipment must be conditioned in the laboratory to achieve thermal equilibrium with the environment. It is about at least three hours in conditioning.

The test was to place various vessels filled with distilled water in a weighing chamber. The mass was read out from indicator of balance in 10 seconds time intervals. The measuring time took approximately 10 minutes for each vessel.

The equation is used to calculate the mass loss as follows [7]

$$m_E = \frac{(m_{10} - m_{11})}{10} \qquad (3)$$

## 5. RESULT AND DISCUSSION

In this study determined the volume of micropipette. According to the equation (1) to determine the volume of micropipette there are parameters density of water and air.

Density of water given by Tanaka's equation, as follows

$$\rho_w = a_5 \left[ 1 - \frac{(t_w - a_1)^2 (t_w + a_2)}{a_3 (t_w + a_4)} \right] \qquad (4)$$

Where $t_w$ is temperature of water (°C)
With constants

$a_1$ = -3,983 035°C
$a_2$ = 301,797 °C
$a_3$ = 522 528,9 (°C)$^2$
$a_4$ = 69,348 81°C
$a_5$ = 999,974 950 kg/m$^3$

Density of air depend on air pressure, air temperature and relative humidity, given by Spieweck's equation, as follows

$$\rho_a = \frac{k_1 P_a + \varphi(k_2 t_a + k_3)}{t_a + t_{a0}} \qquad (5)$$

Where $t_{a0}$ = 273,15 °C, $P_a$ is air pressure (hPa), $\varphi$ is relative humidity (%), and $t_a$ is air temperature (°C).

With constants (ITS-90 scale)

$k_1$ = 0,348 44 (kg/m$^3$) °C/hPa
$k_2$ = -0,002 52 °C$^{-1}$ kg/m$^3$
$k_3$ = 0,020 582 (kg/m$^3$) °C

This equation can be used on the range air pressure 940 hPa ~ 1080 hPa, the air temperature is between 18 ° C ~ 30 ° C and a relative humidity of less than 80%. In this study, the average calculation of the density of air is 1.18 kg/m$^3$.

To determine the air density in laboratory while the measurements was conducted, environment parameter was noted. Environmental conditions during the measurement process carried out is shown in Table 1. Where the table is written in detail the condition of minimum to maximum for each parameter uncertainty in laboratory conditions along with coverage factor k = 1.

Table 1. Parameters laboratory conditions when measurements were performed

| | |
|---|---|
| Air Temp. (°C) | 19.94 ~ 20.40 ± 0.11 |
| Air Pressure (hPa) | 1001.45 ~ 1004.70 ± 0.10 |
| Rel. Humidity (%) | 49.80 ~ 57.75 ± 0.63 |
| Liq. Temp. (°C) | 19.20 ~ 20.20 ± 0.04 |

Measured volume is determined by using the equation (1). During the measurement, parameters in that equation x̶ is recorded and used to determine the volume. When determining the volume of water, the accuracy of the measurement is affected by temperature, atmospheric pressure and relative humidity. The volume measured for this study is presented in Table 2 x̶ (in µl).

Table 2. Volume measured

| No | Without Eva. Effect | | | With Eva. Effect | | |
|---|---|---|---|---|---|---|
| | D1 | D2 | D3 | D1 | D2 | D3 |
| 1 | 19.69 | 19.68 | 19.67 | 19.80 | 19.73 | 19.79 |
| 2 | 19.73 | 19.45 | 19.83 | 19.83 | 19.74 | 19.71 |
| 3 | 19.66 | 19.66 | 19.50 | 20.13 | 19.60 | 19.70 |
| 4 | 19.64 | 19.71 | 19.55 | 19.74 | 19.65 | 19.70 |
| $\overline{V_{20}}$ | 19.68 | 19.62 | 19.64 | 19.87 | 19.68 | 19.72 |

Measured volume difference between with and without evaporation effect and period of a measurement series used to determine the evaporation rate along measurement carried out. The result of mass evaporation rate is presented in Table 3.

Table 3. Mass evaporation rate for each vessel

| | | Evaporated Mass (g) | evaporation rate (µl/min) |
|---|---|---|---|
| D1 | 1 | 0.000449 | 0.09 |
| | 2 | 0.000589 | 0.12 |
| | 3 | 0.000556 | 0.11 |
| | 4 | 0.000459 | 0.10 |
| | Average | 0.000513 | 0.11 |
| D2 | 1 | 0.000893 | 0.19 |
| | 2 | 0.000779 | 0.19 |
| | 3 | 0.000925 | 0.22 |
| | 4 | 0.000907 | 0.21 |
| | Average | 0.000876 | 0.20 |
| D3 | 1 | 0.001265 | 0.32 |
| | 2 | 0.001364 | 0.34 |
| | 3 | 0.001272 | 0.31 |
| | 4 | 0.001338 | 0.34 |
| | Average | 0.001310 | 0.33 |

As result of test can be stated that smaller diameter vessel reduces the evaporation surface of water and reduces the mass evaporation rate during weighing.

Figure 3 present the influence of surface area to water evaporation with relation to diameter of weighing vessel.

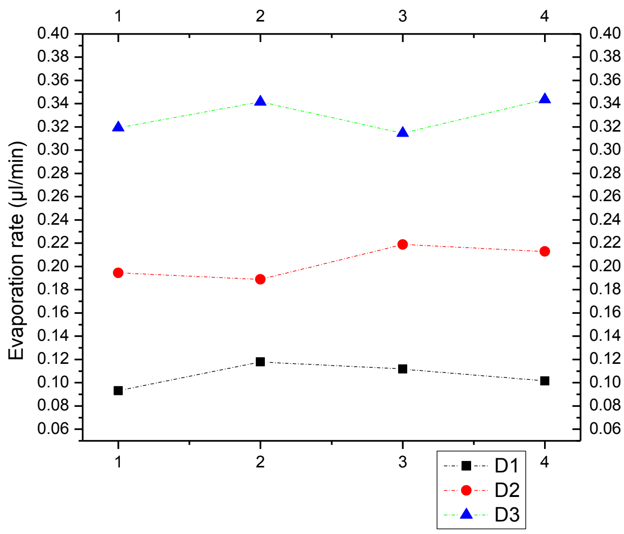


Figure 3.Relation between surface area and mass evaporation rate

Table 4 present the liquid volume evaporated during measurement carried out with relation to diameter of weighing vessel. Weighing vessel D2 and D3 has about 2 and 3 times liquid volume evaporated against D1. This indicate D1 can be reduce evaporation factor error 2 and 3 times better than D2 and D3.

Table 4. Liquid volume evaporated along measurement carried out

| Series | D1 | D2 | D3 |
|---|---|---|---|
| 1 | 0.45 | 0.89 | 1.27 |
| 2 | 0.59 | 0.78 | 1.37 |
| 3 | 0.56 | 0.93 | 1.27 |
| 4 | 0.46 | 0.91 | 1.34 |
| Average | 0.51 | 0.88 | 1.31 |

## 6. EVALUATION OF MEASUREMENT UNCERTAINTY

In this study, all measuring equipment and environmental conditions refers to the minimum requirements of ISO 8655. If all of the measuring equipment in accordance to the minimum requirements, theoretically, uncertainty component by volume determination by gravimetric methods will be smaller than the uncertainty component by volume measurement with volume meter.

Expression of the standard uncertainty associated with $V_{20}$ obtained from equation (1) using the law of propagation of uncertainty. The resulting expression as

$$V_{20}) = \left(\frac{\partial V_{20}}{\partial m}\right)^2 \times u^2(m) + \left(\frac{\partial V_{20}}{\partial \rho_w}\right)^2 \times u^2(t_w) + \left(\frac{\partial V_{20}}{\partial \rho_a}\right)^2 \times u^2(t_a) + \left(\frac{\partial V_{20}}{\partial \rho_b}\right)^2 \times u^2(\rho_b) + \left(\frac{\partial V_{20}}{\partial \alpha_C}\right)^2 \times u^2(\alpha_C) + \left(\frac{\partial V_{20}}{\partial t_d}\right)^2 \times u^2(t_d) \quad (6)$$

Equation (6) gives the expression of the uncertainty of each component of uncertainty. Uncertainty components are mutually contribute to the combined uncertainty. The uncertainty components obtained from all the measuring instruments used (scales, thermometer, barometer, etc.). These calculations require information about the uncertainty of each equipment used in the measurement and sensitivity coefficients of each component of measurement uncertainty that represent the contribution for the combined uncertainty. Information about the uncertainty of each instrument can be obtained from the instrument calibration certificate or instrument specification from the factory.

An important uncertainty contribution to the gravimetric volume determination of water stems from the evaporation of the liquid. Evaporation leads to a decrease in the water mass during weighing. In addition, the temperature of liquid and measuring instrument and the relative humidity of the air may be influenced by evaporation.

Equation 6 used to determine the measurement uncertainty, the calculations will be obtained combined uncertainty. The calculated combined uncertainty for this study is presented in Table 5 (in µl).

Table 5. Combined measurement uncertainty

| No | Without Eva. Effect | | | With Eva. Effect | | |
|---|---|---|---|---|---|---|
| | D1 | D2 | D3 | D1 | D2 | D3 |
| 1 | 0.48 | 0.51 | 0.51 | 0.47 | 0.44 | 0.50 |
| 2 | 0.51 | 0.50 | 0.34 | 0.51 | 0.48 | 0.47 |
| 3 | 0.53 | 0.47 | 0.54 | 0.45 | 0.41 | 0.57 |
| 4 | 0.54 | 0.57 | 0.47 | 0.59 | 0.43 | 0.51 |
| | 0.51 | 0.51 | 0.46 | 0.50 | 0.44 | 0.51 |

## 7. CONCLUSION

The result of this study made it possible to determine the influence of cross sectional area of vessel on reducing evaporation process. The performed calculation helped to determine how much the process was reduced. Weighing vessel D1 has smaller evaporation effect which can be reduce about 2 and 3 times better than weighing vessel D2 and D3. Weighing vessel D1 is recommended for calibration of micropipette.


## ACKNOWLEDGMENT

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