Evaporation minimization at piston pipettes calibration by gravimetric method thanks to using evaporation trap.

The article presents the survey results and a solution to minimization of liquid evaporation process at piston pipettes calibration by gravimetric method according to the requirements of ISO 8655-6 norm. One of the elements interfering the process of piston pipettes calibration is evaporation of distilled water used for the process. Evaporation during weighments is usually minimized by using appropriate weighing instruments with a lid. In the process of weighing liquid ejected by the pipette during its calibration one of the most essential elements is the time of particular series of weighments. In order to minimize liquid evaporation and improve calibration process, we used a special weighing instrument called ‘evaporation trap’.

The article describes the results of the tests using the ‘evaporation trap’ carried out in RADWAG Metrology Center.

1. Introduction

For measuring the volume we use measuring unit ‘cubic meter [m$^3$]’, which is the SI derived unit of volume expressed by the product of length, width and height of the measured object. In chemical tests (e.g. volumetric analysis) it is crucial to accurately measure the liquid volume measured by means of different measuring instruments.

Development of science involves development of metrology: we constantly create more accurate weighing instruments. It also applies to the techniques of measuring liquids commonly called ‘liquid handling’. These days we can use we modern piston pipettes which are able to doze liquid in tenth parts of a microliter [µl].

At present the valid international standard for piston pipettes (and other piston-operated instruments) is a set of international norms ISO 8655:2003 Piston-operated apparatus for volume determination. The norms include the construction requirements essential for the pipettes manufacturers, MPE and gravimetric method of calibration in part 6.

Gravimetric measuring method

In order to calibrate piston pipettes by gravimetric method, it is essential to have appropriate weighing equipment whose metrological requirements are determined in norm ISO 8655-6.

Analytical balance

The basic instrument is the analytical balance. Norm ISO 8655-6 lists the requirements concerning the balances that should be used depending on the calibrated pipette volume:
<table>
<thead>
<tr>
<th>Tested volume</th>
<th>Reading unit ( d )</th>
<th>Repeatability</th>
<th>Standard uncertainty of measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V )</td>
<td>( mg )</td>
<td>( mg )</td>
<td>( mg )</td>
</tr>
<tr>
<td>1 ul ( V &lt; 10 ) ul</td>
<td>0,001</td>
<td>0,002</td>
<td>0,002</td>
</tr>
<tr>
<td>10 ul ( V &lt; 100 ) ul</td>
<td>0,01</td>
<td>0,02</td>
<td>0,02</td>
</tr>
<tr>
<td>100 ul ( V &lt; 1000 ) ul</td>
<td>0,1</td>
<td>0,2</td>
<td>0,2</td>
</tr>
<tr>
<td>1 ml ( V &lt; 10 ) ml</td>
<td>0,1</td>
<td>0,2</td>
<td>0,2</td>
</tr>
<tr>
<td>10 ml ( V &lt; 200 ) ml</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

*Table 1 Minimal requirements for balances (acc. to ISO 8655-6)*

**Ambient conditions at pipettes calibration**

According to the norm air temperature should be constant allowing a deviation of \( \pm 0,5^\circ C \) in the range between 15 °C and 30 °C; relative humidity should be approximately 50%.

The calibrated object and distilled water should be subject to temperature stabilization process in the laboratory for minimum 2 hours so that the pipette and water temperature are the same as in the laboratory.

**Evaporation**

Evaporation is a natural physical process. Norm 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 \( \mu \)l the norm states it explicitly that a weighing vessel with a lid or other methods compensating the process should be used.

2. **Using ‘evaporation trap’ in electronic balances**

The below picture depicts the construction of ‘evaporation trap’ and explains how it works

*Fig. 1. Evaporation trap construction and working principle*

Evaporation trap is placed in the weighing chamber of the microbalance or analytical balance. In the upper part there is a special vessel of ‘the evaporation trap’ covered with a lid. Evaporating water contributes to relative humidity approximately 90%. Through a hole in the vessel top you eject the measured water.
Appropriate balances for the procedure were prepared according to table 1

<table>
<thead>
<tr>
<th><strong>MYA 21.3Y.P electronic microbalance for pipettes calibration</strong></th>
<th><strong>XA 82/220.3Y electronic analytical balance with a chamber for pipettes testing</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer: RADWAG</td>
<td>Manufacturer: RADWAG</td>
</tr>
<tr>
<td>Max 21 g, $d = 0.01$ mg,</td>
<td>Max 82 g/220 g, $d = 0.01$ mg/0.1 mg,</td>
</tr>
<tr>
<td>Repeatability: 0.002 mg</td>
<td>Repeatability: 0.02 mg/0.08 mg</td>
</tr>
<tr>
<td>Stabilization time: 5 s</td>
<td>Stabilization time: 5 s</td>
</tr>
<tr>
<td>Vessel: $\phi = 14.6$ mm, $V \sim 11$ ml</td>
<td>Vessels: $\phi = 24.5$ mm, $V \sim 200$ ml</td>
</tr>
</tbody>
</table>

**Determination of humidity stabilization time in the weighing chamber**

The key element to start the measuring is stabilization of humidity in the weighing chamber with a filled ‘evaporation trap’.

The tests were carried out on the MYA 2.3Y.P analytical balance with ‘evaporation trap’, a THB probe and a stopwatch. The hygrometer probe is placed in the weighing chamber (see the picture). Humidity stabilization time was **Determined on the basis of humidity tests in the weighing chamber**.
Figure 2 depicts the graph of humidity changes in the weighing chamber in time. For the analysis the following labels were used:

- \( t_1 \) – time [min] of filling the ‘evaporation trap’
- \( t_2 \) – time [min] of humidity stabilization
- \( \Delta t_1 \) – period of humidity stabilization in the weighing chamber

\[
\Delta t_1 = t_2 - t_1 \text{ [min]}
\]  
(1)

The test allows to determine stabilization time from equation (1) which is 50 minutes.

The influence of ‘evaporation trap’ on the speed of liquid evaporation depending on evaporation surface

The tests were carried out in the time periods \( t=10 \) seconds. It implied placing particular vessels with distilled water in the weighing chamber (a small vessel \( \Phi 14,5\text{mm} \) for MYA 21.3Y.P balance and bigger vessel \( \Phi 22,5\text{mm} \) for XA 82/220.3Y balance). The water mass weighing results were read in time periods of 10 seconds. The test was carried out for approximately 600 seconds (10 minutes) for every vessel. The test results were registered in table 2 and on the graphs.
Table 2 Mass changes in time as a result of liquid evaporation using evaporation trap.

<table>
<thead>
<tr>
<th>Vessel diameter</th>
<th>Vessel diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ø 14,5mm</td>
<td>Ø 22,5mm</td>
</tr>
<tr>
<td>With evaporation trap</td>
<td>Without evaporation trap</td>
</tr>
<tr>
<td>Δm/t</td>
<td>0,003mg / 10 min.</td>
</tr>
</tbody>
</table>

Table 2 Mass changes in time as a result of liquid evaporation using evaporation trap.

Fig. 3. Influence of ‘evaporation trap’ on evaporation: a) MYA 21.3Y.P balance, b) XA82/220.3Y balance

Looking at the graphs it becomes clear that using the trap in both cases either slowed down or completely eliminated evaporation effect. Long-term tests revealed that evaporation effect takes place later, but for the procedure of pipettes calibration time periods are short.
Analyzing evaporation speed for particular balances in unit µg/mm²·s, the results are following:

<table>
<thead>
<tr>
<th>Balance</th>
<th>Evaporation speed in unit [mg/Pp 600s]</th>
<th>Evaporation speed in unit [µg/mm²·s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYA 21.3Y.P</td>
<td>0.003</td>
<td>0.00003</td>
</tr>
<tr>
<td>with evaporation trap</td>
<td></td>
<td></td>
</tr>
<tr>
<td>without evaporation trap</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XA 60/220/ P</td>
<td>0.54</td>
<td>0.00226</td>
</tr>
<tr>
<td>with evaporation trap</td>
<td></td>
<td></td>
</tr>
<tr>
<td>without evaporation trap</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.87</td>
<td>0.01204</td>
</tr>
</tbody>
</table>

*Table 3. Evaporation speed for particular balances*

The test results allowed to determine the influence of ‘the evaporation trap’ on evaporation process. The previous test showed that ‘the evaporation trap’ increases humidity in the weighing chamber. The general laws of physics say that evaporation depends among others on the humidity of the environment the process is takes place. The calculations allowed to determine to which extent the process was slowed down. The results allow to determine the optimal time of a series of measurements for a single pipette volume.

**Influence of using ‘evaporation trap’ on the measurements results at pipettes testing**

The test was carried out to determine the influence of evaporation on the piston pipette MPE determined for particular volume in the international norm ISO 8655-2. The following volume was tested: 2ul, 20ul, 100ul and 1000ul. For every volume determined 10 average volumes from a series of 10 measurements using MYA 21.3Y.P balance. Table 4 presents the tests results, MPE values according to ISO 8655-2 and MPE determined by the producer.

<table>
<thead>
<tr>
<th>No</th>
<th>Tested volume – 2ul</th>
<th>Tested volume – 20ul</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Volume</td>
<td>MPE</td>
</tr>
<tr>
<td></td>
<td>Without evaporatio n trap</td>
<td>With evaporatio n trap</td>
</tr>
<tr>
<td></td>
<td>Tested volume – 100μl</td>
<td>Tested volume – 1000μl</td>
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<tr>
<td>----</td>
<td>-----------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td></td>
<td>Volume</td>
<td>MPE</td>
</tr>
<tr>
<td></td>
<td>Without evaporatio n trap</td>
<td>With evaporatio n trap</td>
</tr>
<tr>
<td>1</td>
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</tr>
<tr>
<td>2</td>
<td>98,9</td>
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</tr>
<tr>
<td>3</td>
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<tr>
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<td>99,5</td>
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</tr>
<tr>
<td>5</td>
<td>99,3</td>
<td>98,7</td>
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<tr>
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<td>99,5</td>
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<td>99,4</td>
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<tbody>
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<td>1,96</td>
<td>19,73</td>
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<td>2</td>
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<td>1,98</td>
<td>19,76</td>
<td>19,85</td>
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<td>1,95</td>
<td>1,99</td>
<td>19,73</td>
<td>19,85</td>
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<td>19,76</td>
<td>19,86</td>
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<td>1,96</td>
<td>1,97</td>
<td>19,76</td>
<td>19,85</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1,98</td>
<td>1,99</td>
<td>19,79</td>
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<td>19,87</td>
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**Average:**

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<th>[ul]</th>
<th>[ul]</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1,98</td>
<td>19,78</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>19,85</td>
</tr>
</tbody>
</table>
Table 4. The results of volume determination with and without ‘evaporation trap’

<table>
<thead>
<tr>
<th>Volume (ul)</th>
<th>Without ‘evaporation trap’</th>
<th>With ‘evaporation trap’</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>99,5</td>
<td>99,6</td>
</tr>
<tr>
<td>20</td>
<td>99,3</td>
<td>99,2</td>
</tr>
<tr>
<td>100</td>
<td>99,3</td>
<td>99,3</td>
</tr>
<tr>
<td>1000</td>
<td>99,29</td>
<td>99,34</td>
</tr>
</tbody>
</table>

The analysis of results and MPE shows that:

- In case of volume 2ul, contribution of pipette indication error caused by distilled water evaporation during the test is 0.02ul at MPE 0.04ul, which is 50% of error according to the norm and the producer’s specification;
- In case of volume 20ul, contribution of pipette indication error caused by distilled water evaporation during the test is 0.13ul at MPE 0.01ul, which is 35% of error according to the norm and 117% according to the producer’s specification;
- In case of volume 100ul, contribution of pipette indication error caused by distilled water evaporation during the test is 0.05ul at MPE 0.03ul, which is 17% of error according to the norm and 33% according to the producer’s specification;
- In case of volume 1000ul, contribution of pipette indication error caused by distilled water evaporation during the test is 0.67ul at MPE 3.0ul, which is 22% of error according to the norm and 45% according to the producer’s specification;

A similar test was carried out using the kit for XA 82/220.3Y balance. The test implied determination of influence of additional kit for pipettes testing for RADWAG analytical balances. The kit features additional weighing chamber with ‘the evaporation trap’ which is positioned inside the draft shield (weighing chamber) of the analytical balance. It was designed in order to minimize evaporation during liquid weighing,
<table>
<thead>
<tr>
<th>Lp.</th>
<th>Tested volume – 20ul</th>
<th>Tested volume – 100ul</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Volume</td>
<td>MPE</td>
</tr>
<tr>
<td></td>
<td>Without evaporatio n trap</td>
<td>With evaporatio n trap</td>
</tr>
<tr>
<td></td>
<td>[ul]</td>
<td>[ul]</td>
</tr>
<tr>
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<td>19,83</td>
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<td>19,66</td>
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<td>3</td>
<td>19,66</td>
<td>19,82</td>
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<tr>
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<td>19,63</td>
<td>19,78</td>
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<td>19,73</td>
</tr>
<tr>
<td>10</td>
<td>19,64</td>
<td>19,79</td>
</tr>
<tr>
<td>average</td>
<td>19,67</td>
<td>19,78</td>
</tr>
</tbody>
</table>

**Table 5. The results of volume testing using the pipettes testing kit**

The analysis of results and MPE shows that:

- In case of volume 20ul, contribution of pipette indication error caused by distilled water evaporation during the test is 0,13ul at MPE 0,1ul, which is 55% of error according to the norm and 183% according to the producer’s specification;
- In case of volume 100ul, contribution of pipette indication error caused by distilled water evaporation during the test is 0,05ul at MPE 0,3ul, which is 77% of error according to the norm and 153% according to the producer’s specification;

According to norm ISO 8655, errors caused by distilled water evaporation should be taken into consideration. Therefore, for small volumes below 50ul it is recommended to use weighing chambers with a lid or additional ‘evaporation trap’. The norm requirements have been supported by the above tests. Apart from that, measuring time is also essential: the measuring cycle should be as short as possible. The full cycle of testing, collecting and ejecting the liquid should be regular.
CONCLUSIONS

Using ‘the evaporation trap’ considerably contributes to the minimization of liquid evaporation during piston pipettes calibration by gravimetric method (while speeding up the weighment process). Speeding up the whole process allows to shorten the time of carrying out the required series of 10 measurements of tested volume.

Liquid evaporation during the tests at piston pipettes calibration by gravimetric method is one of the elements negatively influencing the tests. It is also essential to remember the following elements:

- Inadequate balance used,
- Inadequate weighing vessel,
- Liquid evaporation during the test,
- Inadequate ambient conditions in the room or lack of their constant monitoring,
- Inadequate environment (influence of vibrations and drafts),
- Inadequate liquid used for calibration,
- Calculation errors,
- The wrongly selected pipettes tips (other than required by the producer),
- The abnormal physiological condition of the operator (too low or too high body temperature),
- Non-ergonomic workstation.

The application described in the article was used in RADWAG balances.

References:


