Major sources of error of air displacement pipettors

by Sari Mannonen[1], Ph.D. and Tapani Tiusanen[1], Ph.D. and Osmo Suovaniemi[1], M.D., Ph.D.

Single- and multichannel air displacement pipettors with adjustable volumes were developed by the end of the 1960s and first manufactured in the beginning of the 1970s by the company Finnpipette Osmo A. Suovaniemi(Suovaniemi, U.S. patents 3,855,868; 4,058,370 and 4,215,092). Still in the 1970s the quality of both single- and multichannel pipettors and the disposable pipettor tips that they required, was quite variable. After three decades of development, mechanical pipettors exhibit excellent accuracy and precision. Yet, results with mechanical and even electronic pipettors are still user-dependent. Experience and skill are still required to reach high-quality results, and a significant number of laboratory personnel never acquire this, despite training (Suovaniemi, 1994).

Today, the leading companies, which design and manufacture pipettors and tips, test the products according to standardised quality control procedures. Also, the designs and materials used are carefully selected to guarantee stable performance for years. Before delivery the pipettors are calibrated[2] to perform as specified by the manufacturer. The calibration performed before delivery is carried out under steady and controlled atmospheric conditions using purified water as test liquid. Moreover, the pipettor is always calibrated as a system? which is composed of both the pipettor and the tip. Also, the procedure on how calibration is performed is well-defined and done by an experienced technician.

In routine laboratory work the pipettor is inevitably used under non-standard conditions. The techniques may not be adhered to, and the fluid to be pipetted is seldom purified water. Unfortunately, very often also tips, not specified by the manufacturer, are used. If other possible sources of errors, such as the angle of pipetting, pipetting rhythm or speed, pre-wetting of the tip or temperature difference between the liquid, tip or pipettor exist, a major error in the end result is inevitable. Strain, leading to problems with the hand and thumb, caused by manual pipetting, may induce additional error. Therefore, it is important to evaluate and reduce, wherever possible, both the random and systematic errors in liquid handling. In the next chapter we will focus on two major sources of systematic error taking place with air displacement pipettors; hydrostatic pressure induced errors and the effect of temperature.

Hydrostatic pressure induced errors

With an air-displacement pipettor the piston is displaced to produce a pressure drop inside the tip touching the liquid. The atmospheric pressure will do the job by filling the tip as long as the inside pressure plus the hydrostatic pressure induced by the liquid column equal to the ambient pressure. The hydrostatic pressure, which is directly proportional to the height of the liquid column, causes a difference between the displaced air volume and the liquid volume. Therefore, the air volume displaced by the piston is bigger than the volume of the liquid in the tip. This displaced air liquid difference (Fig.1) can be expressed with the approximating Formula 1 assuming that the atmospheric pressure is very high compared to the hydrostatic pressure of the liquid.

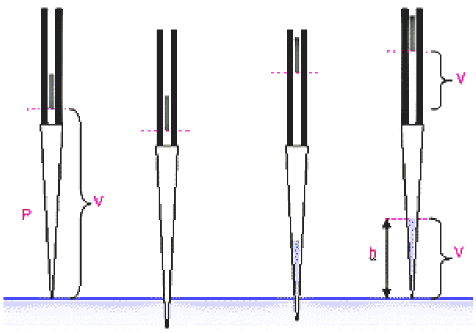

Fig. 1. The aspiration sequence

Formula 1: DV = Va – Vl» V0r g h / P0

where: DV = difference in piston displaced air volume and liquid volume in the tip
Va = piston displaced air volume
Vl = liquid volume in the tip
V0 = air volume inside the pipettor and tip (= dead volume of air)
r = density of the liquid
g = gravity constant
h = height of the liquid sample inside the tip
P0 = atmospheric pressure

The design and calibration work done by the pipettor manufacturers is based on minimising the difference DV by making the dead volume of air inside the pipettor as small as possible. The piston displacement is adjusted during the design and calibration work to provide the smallest standard deviation covering the dynamic range of the pipettor at known values of V0, r, g, h, and P0.However, if one or several of these parameters differ from the values used during design and calibration work, error on accuracy is induced. It is not that simple to detect whether the error is significant or not. However, the major sources of errors can be avoided based on analysing the formula.

Tip

For a typical conically shaped tip the liquid height h versus the liquid volume is not linear. This induces non-linear calibration, which is difficult to correct at least with a mechanical pipettor. Moreover, if a differently shaped tip is used where the height versus the volume behaves unlike in the tip used for calibration, the calibration is not correct and may cause significant errors (order of 1%) especially with small volumes. For example, the use of gel loading tips in which the long thin end of the tip differs significantly in shape from the standard tip would probably create problems in accuracy.

It is not only the shape of the tip that is important but also the nominal volume of the tip. First, if the design work and calibration is done with a tip of different nominal volume, the dead volume of air V0 will not be the same. Second, if a tip with a higher nominal volume is used, Va-Vlincreases along with V0 and less liquid is aspirated. Third, if a small tip is used to reduce the dead volume of air, too much liquid might be aspirated because the calibration has not been done using this value of V0. All this is obviously a problem with extended tips, in which the length (height) of the tip is significantly different from the standard tip with which the pipettor is usually calibrated.

In general, one should always use the smallest pipettor with proper tip available for each volume pipetted, because the percentage error after calibration is by this means minimised.

Pipetting angle

Calibration is performed by keeping the pipettor vertically oriented. In routine work it is common to tilt the pipettor during aspiration. This causes a volume greater than the volume set of liquid to enter into the tip because of the reduced effect of hydrostatic pressure. For example, a pipettor calibrated to 1000 ml at vertical position will provide 1003 ml at an angle of 45°. In practise, the angle does not stay the same during pipetting when tilted, and the difference in accuracy can be even more (Fig. 2). Nevertheless, even if the effect is usually small as such, but when combined with other systematic errors, the cumulated error may be significant.

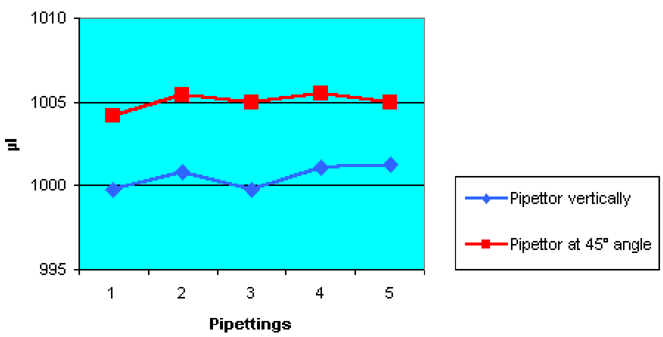

Fig. 2. Comparison of the pipetting results when the pipettor is held vertically and approximately at 45° angle. As seen, pipetting at an angle increases the volume.

Altitude

If the pipettors are calibrated at normal temperature and pressure (NTP) close to sea level, but used at areas of high altitude, less liquid is aspirated. This is because air-volume displacement error is inversely proportional to the atmospheric pressure P0, being lower at high altitudes. For example, at 5 km the atmospheric pressure is about half of the value at sea level. If the pipettor is calibrated at sea level to 1000 ml the same pipettor will provide 990 ml at 5 km. When it is not possible to do re-calibration, it is recommended to use higher settings to compensate the error caused by low atmospheric pressure at a high altitude. For example, 501 ml setting will provide 500 ml at 1 km with a pipettor calibrated at NTP.

Liquid density

If the density of the liquid is higher than that of purified water, the volume entered into the tip is smaller. The error is usually significant. For example, if 1000 µl of liquid with density of 1.2 kg/m3 is pipetted, the error is of the order of 1 µl (0.1%). The density is in fact a function of absolute temperature. However, it is evident that the change in density due to temperature does not produce a large error. For example, a 10°C increase in water temperature decreases its density so little that the error is of the order of (0.01%). Therefore, the density effect is very seldom of concern.However, if the liquid is very viscous, volatile or has a high surface tension, accuracy depends mostly on the pipetting technique used (Yläupa, 1997).

From the simple formula we can learn a lot in order to help us to avoid systematic errors. It is important to pay attention to the dead volume of air and the shape of the tip. To minimise the sources of error on accuracy caused by hydrostatic pressure, one should consider the following:

1) Use the same tips that the pipettor manufacturer uses for calibration and performance testing. If that is not possible, select a tip that is specified by the manufacturer. Most manufacturers provide a list of tips that have been tested with their products and fulfil the specifications set for the pipettors. When extended tips are being used, the pipettor should be checked for performance before beginning the pipetting.
2) Keep the pipettor at vertical position during aspiration. Tilting the pipettor at an angle produces a volume greater than the set volume of liquid to enter the tip. It is often advised not to immerse the tip more than to a depth of 2-3 mm because some liquid attached to the outer surface during immersion may come off during dispensing and cause an error.
3) Working at high altitude areas one should consider recalibration or use higher settings (0.2% per kilometre) to compensate the atmospheric pressure effect. It is not recommended to tilt the pipettor (at 45° gains 3% increase) for correcting this error.

The avoidance of the above errors does not necessarily guarantee correct volumes because the viscosity of the liquid, pipetting etc. affect the results considerably, but help to achieve better results, for sure.

Effect of temperature

Temperature affects the pipetting results considerably and, thus, it must be takien into consideration and corrected, if necessary, when using air displacement pipettors (Suovaniemi and Harjunmaa, U.S. patent 5895838). This is especially the case, if there exist a temperature difference between the pipettor, tips and the liquid to be pipetted. This is illustrated by the following Formula 2 derived for a typical two-compartment model and applying gas laws on it.

Formula 2: $DV = (1 - Tp / Tt) V$

where: DV = difference in piston displaced air volume and liquid volume in the tip

Tp = absolute temperature of the gas inside the pipettor (Kelvins)

Tt = absolute temperature of the gas inside the tip (Kelvins)

V = set value of the aspirated liquid volume

It must be noticed that the hydrostatic effect discussed in the previous chapters is omitted for clarity. Because the error is proportional to the absolute temperature, a 10°C liquid temperature difference would gain an error of 3% in volume at maximum. However, such an error does not take place immediately but needs several successive pipettings to be done. Also, the volume set, time elapsed and the design of the pipettor have an impact on the error induced.

Warm liquids are seldom of concern as are the cold ones. For example, in biotechnology work the liquids (e.g. enzyme solutions) are unstable and, therefore, must be kept in ice bath during pipetting. Also, in laboratory routines people often take liquid out from the fridge and start pipetting immediately without considering the consequences. As an example, we pipetted 1000 µl of cold water (+5°C) at room temperature. The actual volume delivered was some 940 µl (Yläupa, 1997).

The 6% error in this case must be significant for any assay and, therefore, needs to be taken into account. To work with cold samples is not that simple. The temperature of the liquid is increasing all the time towards room temperature reducing the error. At the same time some cooling may take place inside the pipettor. The process is dynamic and provides non-reproducible results in time. In order to minimise this kind of an error, it is recommended to allow the liquid to warm up to room temperature before starting the pipetting. When this is not possible the absolute error can be, in principle, reduced by setting the pipettor to a higher volume compensating this effect. However, one must be very careful because of the dynamic conditions.

Should one work with cold liquids, the following simple rules are the most recommended to achieve accurate and, above all, precise pipetting results:

1. Do not pre-rinse the tip
2. Change the tip between every pipetting

This is because pre-rinsing decreases the air temperature inside the tip (when stored at room temperature) causing the volume to drop according to the gas laws of physics. The more often the same tip is used, the colder the air gets and the smaller the aspirated volume until minimum level is achieved. As long as the tip is used only once, the air in the tip and the pipettor is warm (at room temperature), and the result is correct. In practice, even if pre-rinsing is often recommended it is seldom done because of lack of time. In fact, if samples to be dispensed are not at room temperature, one must not use pre-rinsing, because the induced temperature error is easily dominant. Also, never use tips, if stored in a fridge, before heated up to the same level of temperature as the pipettor.

Discussion

Very often people claim that their pipettors are inaccurate. As often the pipettor itself is not the problem, but the problem arises from the environment, liquid or the user. At the factory all air displacement pipettors are adjusted to give their nominal volume in a reference temperature and with distilled water as a test liquid.Competent technicians, who carry out thousands of pipettings every day, do the testing. In practical laboratory work the working process is usually different. The environment varies, the competence and skills differ from person to person, different pipetting techniques are used, and samples are different from each other. No wonder why variations in the end results occur. Of course, one should ensure that the pipettor is functioning properly and serviced regularly. As important is to check the performance and calibration regularly to ensure that the pipettor performs as specified. The importance of the correct, well-fitting tips is never emphasised too much. Still, most important of all is to realise the possibility of errors in the pipetting event and either avoid errors or, if not possible, at least reduce them wherever possible to guarantee accurate and precise results. Very often the accuracy is not as important as the precision. For example, in EIA work, more important is to pipette every sample in the same way than to get exactly the accurate volume, because standards and calibrators are included in the test and the samples are compared to them. However, one excellent option available is to use microprocessor-controlled electronic pipettors (Suovaniemi, 1994), which not only guarantee high reproducibility and accuracy, but also reduces the person-to-person variation (Fig. 3). Even an inexperienced person can get similar results with an electronic pipettor than a well-trained technician. With a mechanical pipettor this is seldom the case. Moreover, in a laboratory where thousands of pipettings must be done daily, and usually in a hurry, the electronic pipettor adds speed to the pipetting and significantly reduces the strain, even by 50-fold.

Fig 3. Electronic pipettors diminish workload, guarantee high reproducibility and accuracy, and reduce the person-to-person variation

References

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[1] Biohit Plc., Laippatie 1, 00880 Helsinki, Finland

[2] In this connection calibration refers to a procedure to test, measure and adjust to provide correct output