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Influence of physical parameters on the dispensed volume of air-cushion pipette

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Introduction

Physical parameters play an essential role for dispensing with air-cushion pipettes. Results of analysis are directly impacted by the density of the pipetted liquid.

Also, parameters like vapor pressure, viscosity and, in particular, temperature differences have to be regarded as well.

Materials and Methods

Density of liquid

As piston-stroke pipettes are mainly used to pipette aqueous solutions, they are adjusted using distilled water as the test medium. Expansion of the air volume via the liquid varies, depending on the density of the liquid (Tab. 1). This means that when pipetting liquids that are denser than water, the volume of liquid aspirated into the tip is typically less than the set volume (Fig. 1).

As the density and vapor pressure of the liquid can also differ with a change in liquid, the sole influence of density can only be estimated on a theoretical basis. When using a pipette with a nominal volume of e.g. 1000 µl, a deviation of +2.022 µl (+0.2 %) results for methanol (r = 0.79 mg/µl), and a deviation of –7.810 µl (–0.78 %) results for concentrated sulfuric acid (r = 1.84 mg/µl) [1]. This deviation depends on the hydrostatic pressure of the liquid (height of liquid column) and the dead volume. Due to the increase in the dead volume the relative deviation that occurs with small pipetting volumes is greater, thus with a 100 µl pipette, the relative error with a 10 µl pipetting volume is greater than it is with 100 µl volume. This influence can be disregarded in the case of aqueous solutions.

Dense liquids can be dispensed properly with adjustable volume pipettes if the error of density is compensated for e.g. by conscious correction of the digital indicator setting. This correction then only applies to this individual value and not over the entire volume range of the pipette. Modern fixed-volume pipettes can also be corrected.

In practice, the deviations described are not generally problematic as work is not often performed under such extreme conditions. Dispensing systems with positive displacement can be used instead or the error for the method or dispensing process can be tolerated.

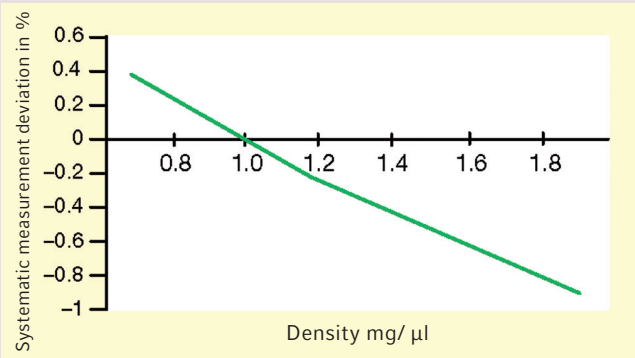

Figure 1: Systematic measurement deviation (inaccuracy) with different liquid densities, calibration with distilled water.

Table 1: Physical data of liquids (relevant examples)

Substance	Formula	Boiling point (°C)	Density mg/µl (20 °C)	Vapor pressure hPa (20 °C)	Viscosity mPas (20 °C)
2-Propanol (Isopropanol)	C_3H_7OH	82.4	0.78	42.5	2.2
Acetic acid	CH_3COOH	118	1.06	15.4	1.53 (25 °C)
Acetone	C_3H_6O	56.5	0.79	233	0.32
Ammonia 25%	NH_3	37.7	0.91	500	
Butanol	C_4H_9OH	117.2	0.81	6.7	
Chloroacetic acid	$CH_2ClCOOH$	189	1.6		
Chloroform	$CHCl_3$	61,7	1.47	213	
Ethanol	C_2H_5OH	78.5	0.79	59	1.2
Formic Acid	HCOOH	100.7	1.23	42	1.8
Glycerol	$C_3H_8O_3$	290	1.26		
Hydrochloric acid	HCl		1.15	213	2
Hydrofluoric acid	HF	112	1.13		
Methanol	CH_3OH	65	0,79	128	0.597
Nitric acid	HNO_3	121.8	1.41	9	1.49 (40 °C)
Phenol	C_6H_5OH	181.4	1.06		1.099 (100 °C)
Phosphoric acid	H_3PO_4		1.71	2	
Potassium hydroxide solution	KOH		1.29		
Sodium hydroxide solution	NaOH		1.33		19
Sulphuric acid	H_2SO_4		1.84	0.0016	26.9
Trichloroacetic acid	CCl_3COOH	196	1.62		

Viscosity

Difficult wetting and flow behavior can be seen through the formation of droplets when dispensing liquids. This may cause a considerable volume to remain in the tip.
In addition to high viscosity (e.g., glycerol), surface-active substances (e.g. surfactants, proteins) may often be responsible for such problems as well. In such cases another frequent problem is the formation of foam. The best ways to counteract this are to use a very slow aspiration and discharge speed as well as reverse pipetting technique [2].

Pre-wetting (premoistening)

Pre-wetting (filling and emptying) of the tip – when pipetting water or aqueous solutions – increases the water vapor saturation level of the air in the tip and in the interior of the pipette [1]. If a measurement series is started without previously wetting the tip, the volumes dispensed will increase in the course of the measurement series until an equilibrium value is reached. This increase is due to the rise in the relative humidity in the tip and interior of the pipette with the number of pipetting steps. To raise the humidity of the interior of the pipette the first tip should be pre-wetted several times at the start of the measurement series. When subsequently changing tips with aqueous solutions it is then sufficient to pre-wet the new tip once to minimize evaporation.

Relative humidity

Despite premoistening of the pipette tip the volume pipetted depends on the humidity of the ambient air to a marked extent [1]. If the relative humidity of the ambient air falls from 80 % to 20 %, the volume dispensed falls by

> 2.1 % to 3.5 % for 10 µl pipettes
> 0.3 % to 0.6 % for 200 µl pipettes with a yellow pipette tip (low dead volume)
> 0.9 % to 1.2 % for 200 µl pipettes with a blue pipette tip (high dead volume).

However, the relative errors specified per percent of the change in humidity should not be viewed without reservation.

Vapor pressure

When using air-cushion pipettes, liquids with a high vapor pressure, such as solvents (see Tab. 1) cannot be dispensed with the accuracy and precision specified for distilled water. When pipetting such liquids, evaporation influences make themselves particularly noticeable for the following reasons: the vapor pressure is higher than it is with water, and the air volume present in the tip is entirely unsaturated at the start [1]. To ensure the highest possible saturation level in the interior of the pipette with the vapor phase, the tip should be pre-wetted for a very long period (1 min or longer). The pipetted volume will nevertheless always be lower than the nominal volume.

System temperature, temperature differences and temperature gradients

The dispensed volume is virtually independent of the system temperature as long as there is no temperature difference within the pipette-liquid-ambient air system [1]. On the other hand, due to the high thermal coefficient of air expansion minor temperature variations of the air cushion while the pipette tip is immersed in the liquid may result in relatively large errors. Such temperature variations are caused by different temperatures of the pipette, the liquid to be pipetted and the ambient air.

If the pipette is warmer than the liquid and the ambient air, then the air taken in during aspiration of the liquid is heated through contact with the warmer pipette, causing it to expand. While the pipette is immersed in the liquid, liquid is displaced from the tip, ultimately reducing the volume aspirated and, thus, also the volume dispensed. Conversely, with an opposite temperature gradient a greater volume is observed.

As any increase in the pipette temperature might result in a drastic reduction in the volume dispensed, the transfer of heat from the user's hand to the pipette piston must be prevented by means of design measures. This problem has been more or less solved with modern pipettes by creating sufficient spatial distance between the piston and the handle as well as thermal shielding or insulation (Fig. 2).

If the room temperature increases while the temperature of the pipette and liquid remains constant, the pipetted volume will increase. The effect is, however, much less pronounced than with variation of the pipette temperature.

If the temperature of the liquid increases while the temperature of the pipette and the room temperature remain constant, the pipetted volume will increase (Fig. 3). This initially seems to entirely contradict what has just been stated.

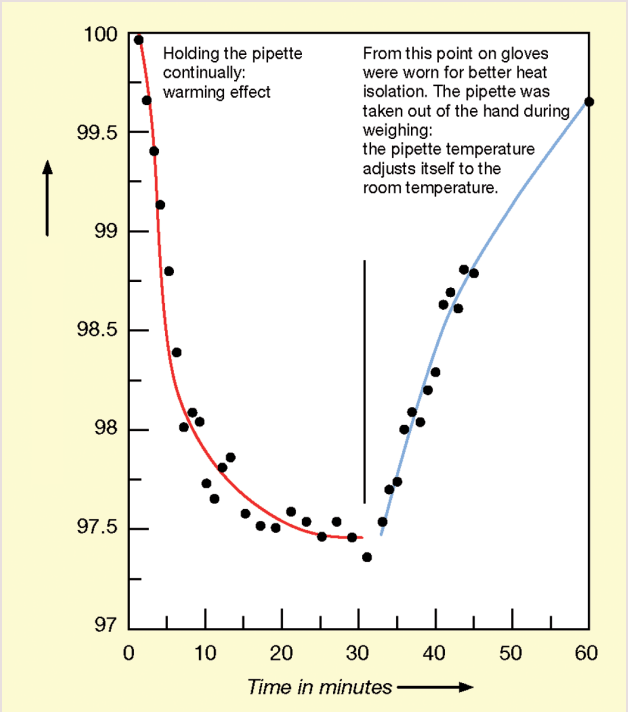

Figure 2: Influence of hand temperature on pipetted volume with a 100-µl pipette with inadequate heat insulation [1]

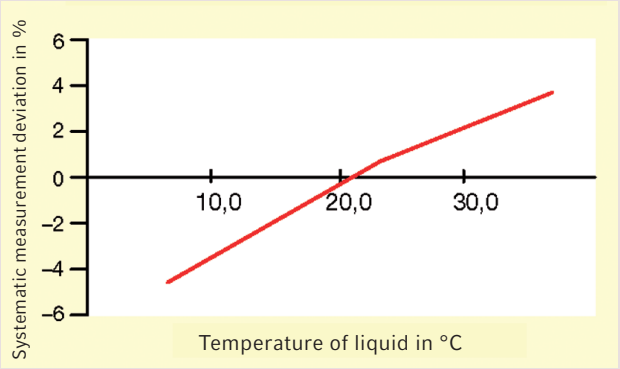

Figure 3: Systematic measurement deviation (inaccuracy) at different liquid temperature; calibration at 22 °C

One would expect the air cushion to become warmer during aspiration, resulting in its expansion and displacement of part of the liquid - and as long as pre-wetting is not carried out, this assumption is correct. While a pre-wetted tip results in an increase in the volume dispensed, the exact opposite is true for a pipette tip which is not pre-wetted.

To rule out temperature variations in the air cushion during, pipetting, the temperatures of the pipette, the liquid and the room temperature should be the same. The smaller the temperature differences, the more accurate the results will be. However, the ideal case where the temperatures of the components involved are identical tends to be the exception in laboratory practice, in particular, when liquids that are chilled or at body temperature need to be pipetted during clinical or biochemical applications. It is recommended to calculate the resulting and frequently inevitable error by means of control measurements and taking this into account during the analysis.

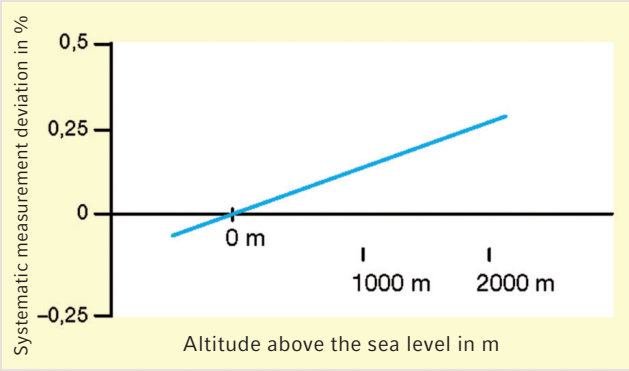

Figure 4: Systematic measurement deviation (inaccuracy) at different altitudes (air pressure). Calibration at 0 m above sea level

Air pressure and sea level

The mean air pressure of a location depends on its height above sea level. If a pipette is adjusted e.g. in Hamburg but used in Munich, the differing sea levels result in an annual mean difference in air pressure of –63 mbar [1]. For a 1000 µl pipette for example, this results in a volume reduction of 0.064 %. For a 50 µl pipette the difference is –0.14 %.
If the air pressure fluctuates by ±25 mbar due to major changes in the weather, this results in a further difference of ±0.024 % with a 1000 µl pipette and ±0.056 % with a 50 µl pipette (Fig. 4). When testing (calibrating) pipettes, it is thus important to take fluctuations in pressure into account.

Literature

[1] Lochner K H, Ballweg, T, Fahrenkrog, H-H: Untersuchungen zur Messgenauigkeit von Kolbenhubpipetten mit Luftpolster. Lab Med. 1996; 20, No. 7/8:. 430–440.
[2] Ewald, K: Liquid Handling – Laboratory Practice. Munich; Germany: Verlag Moderne Industrie; 2005.

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