

Work with solutions

Measurement of liquid volumes

Measuring vessels and other tools in the chemical laboratory

When preparing solutions and handling liquids, we use various laboratory containers and tools. They differ in the purpose for which they are to be used and in accuracy. Maintaining the temperature is important because the density of the liquid changes with temperature.

Let's compare the density of pure water at different temperatures: At a temperature of 4 °C one liter contains almost exactly one kilogram of pure water. As the temperature increases, the density decreases, so at 20 °C it is two grams less and at 37 °C one liter of pure water weighs only about 993 grams.^[1]

Measuring vessels are usually calibrated 20 °C (in the US 25 °C). The calibration temperature is marked on each measuring container.

It should be borne in mind that the temperature of many solutions changes during mixing - for example, inorganic acids and alkalis heat up when diluted while many salts cool down when dissolved. When measuring and preparing such solutions, a significant error can occur unnoticed

Aids for measuring the volume of liquids

| Aid | Usual volume range | Accuracy |
|--------------------------|--------------------|-----------------|
| Erlenmeyer flask, beaker | 5-5000 ml | indicative only |
| Measuring flask | 5-2000 ml | high |
| Graduated cylinder | 5-2000 ml | medium |
| Burette | 1-100 ml | high |
| Pasteur pipette, dropper | 1-5 ml | small |
| Glass pipette | 1-100 ml | high |
| Automatic pipette | 5-5000 µl | high |
| Automatic dispenser | 0,1-100 ml | medium |
| Micro syringe | 0,5-1000 µl | high |
| Piston valve dispenser | 1-500 ml | medium |

Beakers

Beakers are used for indicative determination of liquid volumes. In addition to rough measuring of volumes beakers are mainly used for dissolving substances, diluting liquids, heating and other laboratory operations. Because of the low measurement accuracy, they are usually not even classified as measuring containers.



Beaker

Measuring flasks and measuring cylinders

Measuring flasks and measuring cylinders are calibrated "for filling", which is marked on them with the mark D according to the Czech "dolít" or IN according to the English "include". After filling up to the appropriate line, the liquid inside the container has the exactly indicated volume. If we pour the liquid into another container, a certain amount will remain in the form of a thin film or drops on the walls, so by pouring we transfer less than the indicated volume.

Volume is usually given in milliliters. When measuring, the container must stand on a firm, horizontal support. The correct volume is measured if the meniscus of the liquid touches the mark on the container with its lower edge. Measuring cylinders are only used for approximate measuring, measuring flasks are used to prepare solutions with a precise concentration.



Measuring cylinder and measuring flask

Burettes, pipettes, dispensers and syringes

Burettes, pipettes, dispensers and syringes measure the volume of liquid taken into another container.

Pipettes and burettes are usually **calibrated „for pouring“**, marked **V** according to Czech "vylít" or **Ex** according to the English "exclude". The liquid flowing from the respective lines has the indicated volume. We do not blow out the contents of the pipette, even if a drop remains in the tip. Its volume is taken into account during calibration.

▪ Burettes

They are used in titrations, or where the same volume of liquid is repeatedly measured. These are glass or plastic calibrated tubes closed by a tap. The burette is fixed vertically to the stand using a holder. With the tap closed, it is carefully filled with the appropriate liquid using a funnel. The funnel is removed and by slightly opening the tap, such an amount of liquid is released that its lower meniscus touches the mark. Then the burette is ready for titration. The titration agent is discharged through the tap and its volume is monitored on the scale. One of the most important actions when working with a burette is the correct reading of the volume. It is always read twice on the burette. The first time when determining the zero mark, the second time when subtracting the drained volume. Since the change in volume is subtracted, the method of subtraction does not matter much. However, it must always be subtracted in the same way.



Burettes

Automatic burettes are used in routine laboratories.

▪ Glass pipettes

They are rarely used to measure volumes in modern routine laboratories and are being replaced by semi-automatic dispensers. The volume of glass pipettes can be different, from 1 to 100 ml. They can be either undivided, designed to measure a single volume, or divided - usually in milliliters and tenths of milliliters. The scale can point from the tip towards the top edge or vice versa.

For safety reasons, we never suck the solution into the pipette by mouth - different types of attachments or pistons are used to draw it.



Glass pipettes



When suctioning, the pipette must not rest on the bottom of the container. Before measuring the sample, the pipette is first filled with the solution and the collected volume is drained into the waste container. Only then is the exact volume taken and transferred to a container for further processing. The solution must never enter the pipette tip.

▪ Automatic pipettes (pipettors, micropipettes, microdispensers)

One option for measuring small volumes is the use of automatic pipettes.



Microdispensers

▪ Microsyringes

They are used for precise dosing of small amounts (0,1–1000 μ l) of liquids. They consist of a needle attached to a graduated glass cylinder in which a piston moves. Individual types differ in needle and piston diameters.



Microsyringe

▪ Piston valve dispensers

They consist of a piston with a scale, which is mounted on storage bottle. They enable the repeated dosing of a certain volume of liquid from the storage bottle. Dosers intended for dosing of aggressive chemicals (e.g. strong acids) have glass parts made of borosilicate glass, plastic parts that are in contact with the liquid are made of PTFE, other parts are made of PE or PP. In modern types of dispensers, dosing is automated using control modules.



Piston valve dispenser

Automatic pipettes

According to the control method, we distinguish between manual and electronic **automatic pipettes**. In **manual** pipettes, the plunger is moved with the thumb using the control button. The correctness and accuracy of pipetting is significantly influenced by the experience and skill of the worker. In the case of **electronic** pipettes, the piston is moved by an electric motor. Compared to manual methods, it also offers programming of the pipetting method. Depending on the nature of the liquid, you can choose a different speed of piston movement when sucking in or expelling the liquid.

A disposable tip is attached to the body of the pipette (also called *pipettor*). The pipetted liquid comes into contact only with this tip.

According to the principle of their operation, automatic pipettes can be divided into two basic types:

▪ „Air displacement“ pipettes

This type of pipette uses the so-called air cushion principle. A certain volume of air always remains between the piston and the liquid. The volume of liquid drawn into the tip by the pipettor may differ slightly from the volume of air drawn in or pushed out by the piston, depending on the density and viscosity of the pipetted liquid, the wettability of the tip surface by the pipetted liquid, temperature and atmospheric pressure, and other influences. Therefore, the pipettor must be regularly calibrated and adjusted.

Pipettes of this type are distinguished according to their design as **single-channel** (intended for pipetting one volume of a given liquid in time) or as **multi-channel** (most often eight or twelve-channel) intended for simultaneous pipetting of the same volume of a given liquid into several wells in a microtiter plate. Each channel in multi-channel pipettes has its own piston, therefore it is not necessary to use all channels at one (less than 8 or 12 tips can be connected).

Automatic pipettes are designed either for one **fixed** volume or are **adjustable** for multiple volumes. Changing the volume setting is possible within a certain range (e.g. 10-100 µl) using the adjusting screw or knob.

▪ „Positive displacement“ pipettors

This type of pipette sucks liquid into the tip directly without creating an air cushion, i.e. the piston is in direct contact with the measured liquid. The liquid sucked into the tip (without air bubbles) is discharged all at once (syringe type) or in steps of the same volume (stepper pipette). This type of pipettor is convenient to use for highly viscous or volatile liquids, or for repetitive pipetting.

Ways to use a pipettor

Direct pipetting

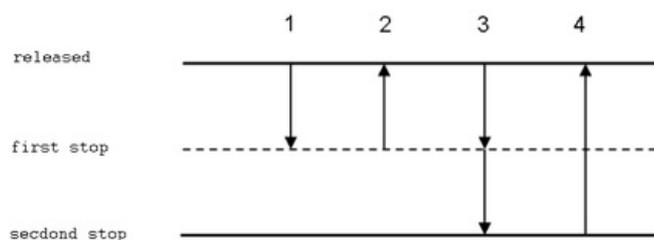
This is the most commonly used pipetting technique. During direct pipetting, a precisely set volume is sucked into the tip and in the next step it is completely pushed out of the tip into the selected container. Because a certain amount of liquid remains on the inner surface of the tip as a thin film, it is necessary to wet the tip with the measured liquid before pipetting. The direct pipetting technique is used for measuring most aqueous solutions, buffers, dilute acids and bases.



Pipetting positions

Method

1. Place the tip on the dispenser. Press the controller button to the first position (a small resistance must be overcome when pressing the button).
2. Dip the tip of the dispenser about 2-3 mm below the solution level. Slowly release the pressed button on the controller while sucking the sample into the dip.



Direct pipetting scheme

By slowly sucking the liquid into the tip, the possible formation of turbulence is limited, which can cause the formation of aerosol and gas bubbles coming out of the liquid. The optimal speed depends on the properties of the liquid (its

density, vapor tension and viscosity).



- Always check whether air bubbles have entered the tip (e.g. when the piston is opened more sharply or the tip is incorrectly fitted).



- For greater pipetting accuracy, remove your thumb completely from the controller button once it reaches the home position.
3. Slowly withdraw the tip from the liquid. Some of the contents of the tip may be lost when pulled out quickly. Wait, especially for larger 500-5000 μl pipettors, about 1-3 seconds before pulling the tip out of the liquid.
 4. When expelling the liquid, hold the tip at a slight angle against the wall of the container ($10-45^\circ$), just above the solution already in it, and smoothly press the control button with your thumb to the first position. Wait about 1 second and continue to quickly press the controller button to the second position (you will feel more resistance when pressed). Make sure that no droplets of liquid remain in the tip or splash on the wall of the container.



5. Hold down the controller button and pull the tip out along the wall of the container. Now enable the controller button.

In direct pipetting, a certain error is created by the fact that a very thin film of the transferred liquid remains on the inner surface of the tip. With the mentioned procedure, we measure a slightly smaller volume than is set on the pipette, while the error depends mainly on the properties of the pipetted liquid and the material from which the tip is made. This error can be eliminated by wetting the inner surface of the tip with the measured liquid before pipetting. In practice, this means that we first suck the solution into the tip using the procedure described above, but instead of measuring it into the target container, we return it back to the storage container. At this moment, a film of pipetted liquid is formed on the inner wall of the tip, in the case of colorless solutions it is invisible to the eye with the correct technique. This is followed by measuring the liquid exactly according to the above mentioned procedure (only we do not insert a new tip.) Since the amount of liquid that remains in the tip is practically constant, we now measure the actual set volume.

If drops remain on the outer wall of the tip, it is possible to wipe them a cotton wool with a light movement from top to bottom. Never touch the mouth of the tip with the cotton wool, otherwise you will suck out some of the liquid inside.

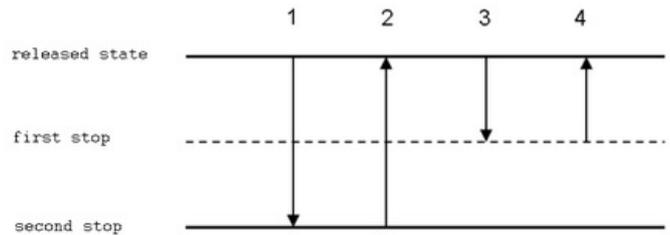


Reverse pipetting

In reverse pipetting, we draw a larger volume of liquid into the tip than we want to measure, and in the next step we push out the volume set on the pipette from the tip. This method of pipetting gives better results when working with viscous or highly volatile liquid, strongly wetting liquids and solutions that foam. It is also suitable for measuring very small volumes. After pipetting, there is always a residue of liquid in the tip, which can be squeezed back into the storage container or into the waste before removing the tip itself.

Method

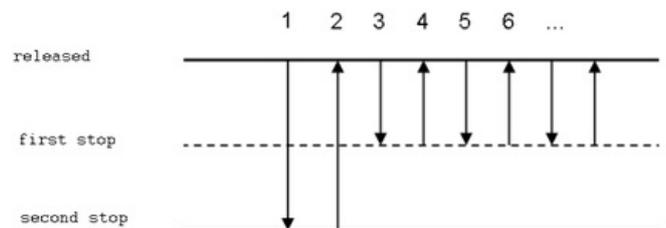
1. Press the button to the second position (you will feel first a weak and then a greater resistance of the piston when pressing the button of the controller).
2. Dip the tip of the dispenser about 2-5 mm below the surface of the solution. Slowly release the plunger while sucking the sample into the tip.
3. Slowly withdraw the tip from the liquid and remove any droplets adhering to the outer wall of the tip by touching the tip to the rim of the container.
4. When expelling a given volume of liquid, hold the tip at a slight angle against the wall of the container just above the solution, that is already in it, and slowly and smoothly press the control button to the first position with your thumb.
5. Hold the controller button down in this position and pull the tip out of the container.
6. Push the part of the liquid that remains in the tip back into the original container or into the waste by pressing the controller button to the second position.
7. Hold the controller button down and pull the tip out of the liquid, then release the controller button.



Reverse pipetting scheme

Repetitive pipetting

This method of pipetting is intended for repeated pipetting of the same volume, e.g. for adding a reagent to a series of tubes or to wells in a microtitre plate. This is actually repetitive reverse pipetting. After sucking the liquid into the tip, repeat steps 2 to 4.



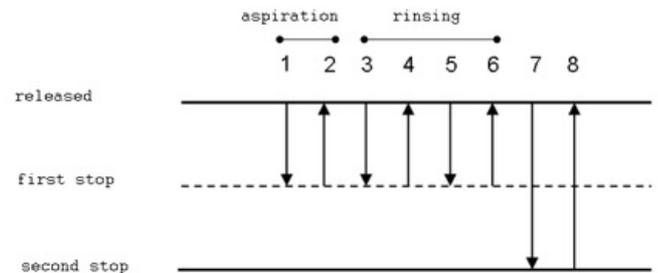
Repetitive pipetting

Pipetting of heterogeneous samples

A technique suitable for pipetting heterogeneous samples such as blood, when it is not easy to rinse the tip before pipetting. It is similar to the direct technique, but the tip is not pre-wetted with the measured liquid. Instead, after the liquid has been transferred, it is repeatedly flushed with the solution with which the measured liquid is mixed.

Method

1. Press the button to the first position and dip the tip of the dispenser about 2-5 mm below the level of the solution.
2. Slowly release the plunger while sucking the sample into the tip.
3. Slowly withdraw the tip from the liquid and remove the drops of solution adhering to the outer wall of the tip by pulling the tip along the wall of the container.
4. Dip the tip of the dispenser **into the** cílového roztoku.
5. Press the control button to the first position and then slowly release it to the original position. This will draw the solution into the tip. Do not remove the tip from the solution and repeat this step until the inside of the tip is clean.
6. Pull the tip along the wall above the solution level and empty it by pressing the controller button to the second position.
7. Hold down the controller button and pull the tip out of the container along the wall and then release the controller button.



Pipetting heterogenous samples scheme

Preparation of solutions with a given concentration

Mass concentration: $\rho_B = m_B / V$ (g/l)

Substance concentration (molarity): $c_B = n_B / V$ (mol/l)

Mass fraction: $w_B = m_B / m$

Diluting and mixing solutions: $c_1V_1 + c_2V_2 = c_3(V_1 + V_2)$

Dilution number (dilution factor): $D = (V_{\text{konečný}} / V_{\text{původní}})$

A D times diluted solution with a volume of V_{final} is prepared from 1 part of the original solution (V_{original}) and (D-1) parts of the solvent rozpouštědla (e.g. 5 times diluted solution is obtained by mixing 1 part of the original solution and 4 parts of water).

In Anglo-Saxon terminology, we also encounter the term **dilution ratio**, which expresses 1 part of the original solution to D parts of the total volume ($1/D = V_{\text{original}} / V_{\text{final}}$). According to this concept a solution diluted 1:D is the same as a solution diluted D times (e.g. a solution diluted 1:5 is a solution prepared from 1 part of the original solution and 4 parts of water, i.e. diluted 5 times).

Practical task Preparation of solutions with a given concentration (<http://portal.med.muni.cz/download.php?fid=654>)

Photometric verification of concentration

When electromagnetic radiation from the ultraviolet or visible part of the spectrum passes through the measured solution, it is absorbed. The amount of absorption depends on the wave length of the radiation, the concentration of the absorbing substance in the solution and the thickness of the measured layer. At a given wavelength of radiation, there is a direct relationship between the concentration of the absorbing substance and a quantity called absorbance (A). This dependence is expressed by the Lambert-Beer law: $A = \epsilon_{\alpha} c l$, where ϵ_{α} is the molar absorption coefficient (its value corresponds to the absorbance of a substance with a concentration of 1 mol/l and a thickness of the measured layer of 1 cm), c is the substance concentration (mol/l) and l is the thickness of the measured layer (cm). The given relationship is valid only for monochromatic radiation and for dilute homogeneous solutions. For absorbance measurement, the wavelength corresponding to the absorption maximum of the substance to be determined is usually chosen.

Practical task Photometric verification of concentration (<http://portal.med.muni.cz/download.php?fid=648>)

1. ČMELÍK, Milan – MACHONSKÝ, Lubor – ŠÍMA, Zdislav. *Fyzikální tabulky*. 2. edition. Liberec : Technická univerzita v Liberci, 2005. 60 pp. ISBN 80-7372-009-4.