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5. Instructions for tasks

5.1 Task 1 – Crystallization

Tasks
1. Recrystallize a sample of crude acetanilide from hot water.
2. Recrystallize a sample of crude acetanilide from hot toluene.
3. Recrystallize a sample of crude copper(II) sulphate pentahydrate (blue vitriol) by precipitation of aqueous solution with ethanol.

Introduction
Heating
Description of different heating techniques is described in Chapter 4.7.

Crystallization
Description of different crystallization techniques is described in Chapter 4.10.

Filtration and vacuum-filtration
Description of different filtration techniques is described in Chapter 4.12.

Safety note
Used organic solvents (ethanol and toluene) are very flammable, work with them in safe distance from direct flame.

Procedure
Recrystallization of acetanilide from hot water
Weight 3.00 g of crude acetanilide and mix it in 250ml Erlenmeyer’s flask in 80 ml of distilled water. Add magnetic stirring bar. Put the flask on the heating plate of magnetic stirrer and place a small glass funnel into the flask’s neck. The funnel will work as an air-cooler and reduce evaporated amount of water from the mixture. Simultaneously, the funnel will be pre-heated which avoid crystallization of the product during filtration (if funnel would be cold, the filtered solution will be cooled and dissolved material will crystallize and clog funnel’s tube). Heat the mixture to boiling point with stirring and boil it for a short while (ca. 2 min). Filter the hot mixture through folded paper filter placed into the pre-heated funnel into a clean and dry 100ml Erlenmeyer’s flask. Hold the flask with hot solution using a piece of fabric or with a clamp. If acetanilide crystallizes already on the filter paper, pour the suspension back to 250ml Erlenmeyer’s flask, add small amount of water and heat to boiling point again. After all material dissolves complete the filtration. Close the flask with the filtrate with a plastic stopper and cool it in a sink under stream of cold water. Allow the mixture to stand for 20 min in a fridge to complete crystallization process. Isolate the crystals by vacuum-filtration.

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* Pressure is decreased in closed apparatus/flask during cooling. It can lead (in extreme cases) to implosion (when using a big Erlenmeyer’s flask with plane bottom). In addition, when lower pressure is inside, the apparatus is only hardly opened. Therefore, it is recommended to open several times the flask during cooling for pressure equilibration, or to use not-well-fitting stopper.
on a frit. If some solid material remains in the flask, wash it with a portion of the mother liquor from a filtering (Büchner) flask. Wash the product with a small amount of cold water (ca. 10 ml, cool the plastic washing bottle with water in a fridge) and pre-dry it by sucking an air through the material for 5 min. Replace the product from frit to a 100ml beaker (weighted in advance) using the spatula and place the beaker into a vacuum desiccator. Dry the product in vacuum for ca. 15 min. Weight the pure acetanilide and calculate a yield of recrystallization.

**Recrystallization of acetanilide from hot toluene**

Weight 1.00 g of crude acetanilide into a 25ml tear-shape flask. Place a magnetic stirring bar into the flask and attach the flask to a stand via a clamp. Assemble apparatus for heating under a condenser (see Fig. 27). Add 10 ml of toluene into the flask through the cooler. Use a syringe with a needle for measuring of the volume of toluene. Heat the mixture in an oil bath to boiling point. Move the apparatus from the oil bath, take off the cooler, unfasten the clamp with the flask from the stand (the clamp will serve as a holder) and filter the hot mixture through a small cotton cocoon placed into a small plastic funnel into a clean and dry 25ml Erlenmeyer’s flask. Close the flask with a stopper (see a note 4 on the previous page) and place it into a refrigerator for 15 min to finish the crystallization process. Isolate the crystals by vacuum-filtration on a frit, wash the product by 2 ml of toluene (measure volume of toluene using a syringe with a needle) and pre-dry it by sucking an air through the material for 5 min. Replace the product from frit to a 100ml beaker (weighted in advance) using the spatula and place the beaker into a vacuum desiccator. Dry the product in vacuum for ca. 15 min. Weight the pure acetanilide and calculate a yield of recrystallization.

**Recrystallization of copper(II) sulphate pentahydrate by precipitation of aqueous solution with ethanol**

Crush a sample of crude copper(II) sulphate pentahydrate (3.00 g) in a mortar. A crushing of the sample into a fine powder will speed-up its dissolution. In addition, one usually adds more solvent than necessary when dissolving big crystals, and it results in obtaining of too diluted solution. Place crushed blue vitriol into a 100ml beaker and dissolve it (with careful stirring by hand shaking) to saturated solution (add gradually water in small portions from a plastic washing bottle). As one drop of 10% aq. sulphuric acid, one small spoon of active charcoal and stir the mixture. Filter the solution through “classically” wrapped filter paper into a clean and dry 400ml beaker. Precipitate the product from the filtrate by a slow addition of ethanol from a plastic washing bottle with stirring. Add ethanol until mother liquor will be (after sedimentation of crystals) completely colourless. Isolate the crystals by vacuum-filtration on a frit, wash the product by ethanol and dry it by sucking an air through the material for 5 min. Weight the pure product and calculate a yield of recrystallization.

**Requirements for admitting of the task**

Purified materials with calculated yields of recrystallization.

Task protocol.
5.2 Task 2 – Isolation of glycine and identification of unknown amino acid

Tasks
1. Isolate free glycine in form of zwitterion from its hydrochloride using ion-exchange chromatography.
2. Identify unknown amino acid by comparative thin-layer chromatography (TLC).

Introduction
Work with rotary evaporator
For description of rotary evaporator see Chapter 3.5.6, description of work with see Chapter 4.9.

Precipitation from solution by addition of a bad solvent
Description of this technique is described in Chapter 4.10.1.

Vacuum-filtration
Description of this technique is described in Chapter 4.12.2.

Ion-exchange chromatography
In this task, you will isolate amino acid (glycine) in the free form, i.e. in form of zwitterion (betaine), \( ^+ \text{NH}_3\text{CH}_2\text{CO}_2\text{H} \). Glycine hydrochloride \([\text{NH}_3\text{CH}_2\text{CO}_2\text{H}]\text{Cl}\) will be used as a starting material. You will use strong cation exchange resin in the H\(^+\)-form (see Chapter 4.16.3). It is polymeric material with strongly acidic sulfonic acid groups (\(\text{–SO}_3\text{H}\)).

![Diagram of ion-exchange chromatography](image)

Fig. 48. Schematic representation of separation of glycine from its hydrochloride.

In glycine hydrochloride, both carboxylic and amino functions are protonated, and amino acid is in the cationic form \([\text{NH}_3\text{CH}(\text{R})\text{CO}_2\text{H}]^+\). The chloride ion Cl\(^-\) serves as a counter-ion. After pouring of solution of glycine hydrochloride onto top of a column of the ion-exchange resin, the protons of sulfonic moieties will be replaced by cations of amino acid, and released HCl will be eluted off the column with water. Under water elution, glycine will stay bound to the resin (Fig. 48). For elution of glycine from the resin, diluted aq. ammonia will be used. It will neutralize a rest of the sulfonic acid groups, and displaces bound glycine due to ammonia excess. The amino acid will be eluted in form of its ammonium salt \((\text{NH}_4)^+\text{[NH}_3\text{CH}_2\text{CO}_2^-\)). By repeated evaporation of eluate you will remove volatile ammonia from isolated amino acid and the product will be precipitated from aq. solution on addition of acetone. Precipitated
product will be isolated by vacuum-filtration on a frit. Ammonium(I) cations bound to the resin will be removed by excess of hydrochloric acid (regeneration of ion-exchange resin) and the excess of acid will be removed by washing the column with water till neutral reaction of an eluate.

Forms, in which glycine is present during whole procedure, are shown in following scheme:

**Thin layer chromatography**

For description of the technique see **Chapter 4.16.2**. You will use TLC plates with silica (dried polymeric amorphous and porous gel of silicic acid, SiO₂, containing a large number of surface –OH groups). Surface of silica particles is therefore polar, very hydrophilic, and has an acid nature. From these reasons, the surface has a high affinity to polar compounds, especially towards amines (and in general, towards cationic compounds). Amino acids are therefore bound to a silica surface very strongly, and very polar mobile phase has to be used to move spots of amino acids on the plate. Therefore, the mobile phase should be both acidic (to compete with silica surface –OH groups) or basic (when base present in the mobile phase removes adsorbed compound from the silica surface). In this task you will use the mobile phase containing ammonia. In general, the higher polarity of separated compound, the lower speed of its move. And contrary, presence of hydrophobic moiety in the molecule of separated compound lower overall polarity (and makes more difficult interaction of separated compound with silica surface) and such compounds are moving fast during chromatography.

You will use spraying of the TLC plate with ninhydrine solution for visualisation of the amino acids. Ninhydrine is an agent reacting selectively with amino groups, producing intensive purple colour according to a general scheme:

Application of detection agent solution is readily performed by spraying using a sprayer. The spots will be detected upon a gentle heating of the TLC plate in a stream of hot air from a heating gun. Before detection, you have to remove all ammonia from the mobile phase, because ammonia also reacts with ninhydrine and such reaction will disturb detection of the spots. For this reason, you have to evaporate carefully the mobile phase using the heating gun before application of ninhydrine spray. Ninhydrine causes slight irritation (and is slightly toxic) and, therefore, use gloves during the work and hold the TLC plate using a pincer or tongs. Perform a spraying with the detection solution in a sink and wash ninhydrine remains by water into sink-hole.

**Procedure**

**Isolation of glycine**

Prepare column of strong cation exchange resin into H⁺-form: wash the column with 25 ml of the exchanger by 25 ml of 10% aq. HCl. After that wash the column with water until neutral
reaction of the eluate (from time to time check pH of the eluate by a strip of pH-paper – small piece of the pH-paper dip in the eluate using a pincer, and compare the colour with a scale on a box with pH-papers). Keep relatively slow rate of elution during washing of the column with HCl (ca. 1 drop per 1 s) to allow a reaching of surface equilibrium between the resin and the eluent. A washing with water (removing HCl excess) can be done much faster. Pour a new portion of water onto a top of the column only after the previous portion is completely soaked. Only in such way you will not dilute remaining acid from above the column – if you would not wait until full adsorption, you will have to use much bigger volume of water and whole process will be much longer than needed.

Dissolve 1.00 g of glycine hydrochloride in 10 ml of water. Pour the solution (using a Pasteur pipette or plastic dropper) on top of the ion exchanger. By careful opening of the column’s valve set elution speed to ca. 1 drop per 2 s and after soaking of the sample solution wash the column with water till neutrality of the eluate (it will remove HCl). Collect an eluate into Erlenmeyer’s flask, and check pH of elute time to time with small pieces of pH-paper. In the beginning add water eluent in small portions using washing bottle in such way, that you will wash inside walls of the column. Pour a new portion of water only after a previous portion is fully soaked. Keep a flow rate small in the beginning (1 drop per 2 s), and after addition of several portions of water increase a flow by full opening of the valve to maximum. Elute glycine with 100 ml of 5% aq. NH₄OH into 250ml round-bottom flask. Also in this case add ammonia in the beginning portion-wise and add a new portion only after the previous is fully adsorbed to avoid diffusion of released amino acid to column of the eluent above the column of the resin. Evaporate the eluate on rotary vacuum evaporator till dryness, dissolve a remaining material in water, and evaporate volatiles again. This procedure removes ammonia. Wash the column with water (it will elute remaining ammonia solution which is kept between grains of the resin) and regenerate the resin to H⁺-cycle by washing with 25 ml of 10% HCl and subsequently by water till neutrality. Use time needed to wash the column and elute the product for carrying out a TLC analysis of the unknown sample (sample preparation, dropping of spots, drawing up the TLC plate, detection, see Chapter 4.16.2).

Dissolve solid remaining in the flask in minimal amount of water. Pour 80 ml of acetone into a beaker and transfer a solution of glycine into acetone using a plastic dropper. Isolate the glycine by vacuum-filtration on a frit, wash the product by acetone and dry it by sucking an air through the material for 5 min. Weight the pure product and calculate a yield of isolation.

**Identification of unknown amino acid**

Using TLC on silica plate identify unknown sample of amino acid. As the samples drop on the TLC plate the unknown sample and standards of individual amino acids – glycine, valine, phenylalanine and lysine. For application of the samples on the plate use only dedicated pipetting tips. Do not mix them to avoid mutual contamination of the samples. The spots of the samples should be ca. 2–3 mm in diameter. Use mixture of EtOH:conc. NH₄OH:H₂O = 10:1:2 as a mobile phase, and detect the spots by spraying with solution of ninhydrine. Notice not only positions of the spots, but also colours and colour change during heating of the plate.
 Requirements for admitting of the task
Weighted pure and dry glycine.
Chromatographic plate with spots of individual amino acid samples and calculated values of retention factor of individual amino acids.
Task protocol.
5.3 Task 3 – Identification and concentration determination of unknown organic acid

**Tasks**
1. Prepare volumetric stock solution of sodium hydroxide and factorize it (determine its exact concentration) using titration of primary standard, oxalic acid dihydrate.
2. Determine total concentration of protons in solution of unknown organic acid using acido-basic titration with visual detection of equivalence.
3. Determine dissociation constant $pK_{A1}$ or $pK_{A2}$ of unknown organic acid. Identify the unknown acid using determined value of $pK_A$ and calculate its concentration.

**Introduction**

*Work with pH-meter*

For description of pH-meter see Chapter 3.5.5, principle of measurement and how to work with is described in Chapter 4.6. Detailed instructions how to measure pH are nearside to pH-meter.

*Acido-basic titration*

During acido-basic titration, amount of an acid present in the sample is determined using titration with stock solution of a base (alkalimetry), or contrary, an amount of a base is determined using titration with stock solution of an acid (acidimetry). During this task you will perform alkalimetric titration using phenolphthalein as an indicator.

In general, acido-basic indicator is a compound, which behaves as acid or base (*i.e.* it is protonated/deprotonated during titration), and differently protonated forms thereof are differently coloured. In the case of titration of (generally weak) organic acid by strong alkali hydroxide, the equivalence point$^5$ lays at weakly basic region. Therefore, phenolphthalein will be used as an indicator, as it shows a colour change at $pH \approx 8.5–10$. In the case of phenolphthalein, the forms present in acidic and basic solutions have structures shown on following scheme:

![Colour change scheme](image)

For other details about titrations see Chapter 4.23.

*Determination of exact concentration of stock solution*

Reaction taking place during determination of concentration (see Chapter 4.23.2) of NaOH solution using oxalic acid is:

\[
(CO_2H)_2 + 2 \text{NaOH} \rightarrow (CO_2\text{Na})_2 + 2 \text{H}_2\text{O}.
\]

After neutralization of all oxalic acid is pH steeply increased with the first drop of excess of titration agent (NaOH) and the mixture changes its colour from colourless to vivid purplish red (pink when very diluted) due to acido-basic indicator added (phenolphthalein). As evident

$^5$ In the equivalence point, total molar amount of an acid is equal to a total molar amount of a base, *i.e.* formally, pure salt is present in the solution. However, the solution in equivalence point is not generally neutral ($pH$ need not to be 7), as due to hydrolysis of cation or anion the concentrations of $H_3O^+$ and $OH^-$ ions need not to be equal.
from balanced chemical equation, the molar amount of consumed hydroxide is in the
equivalence point doubled with respect to molar amount of oxalic acid:
\[ n(\text{NaOH}) = 2n(\text{H}_2\text{CO}_4) \].

As the mass of oxalic acid will be known and used volume for neutralization [titration agent
consumption, \( V(\text{NaOH}) \)] will be read from burette, you can easily calculate the molar
concentration of hydroxide using relationship:
\[ c(\text{NaOH}) = \frac{2n(\text{H}_2\text{CO}_4)}{V(\text{NaOH})} = \frac{2m(\text{H}_2\text{CO}_4)}{M(\text{H}_2\text{CO}_4) \cdot V(\text{NaOH})} = \frac{2m(\text{H}_2\text{CO}_4)}{126.07 \text{ g} \cdot \text{mol}^{-1} \cdot V(\text{NaOH})}. \]
The used form of oxalic acid is its dihydrate, therefore, corresponding molar mass is already
considered in the equation above. Due to a fact, that molar concentration is usually expressed
in units of mol\(\text{dm}^{-3}\), it is necessary to convert volume to appropriate unit (dm\(^3\)).

**Acido-basic equilibrium**

Arrhenius (and Brønsted) acids in aqueous solution undergo a dissociation process according
to equation:
\[ \text{HA} + \text{H}_2\text{O} \rightleftharpoons \text{H}_3\text{O}^+ + \text{A}^- \]

(shortly \( \text{HA} \rightleftharpoons \text{H}^+ + \text{A}^- \)).

We can define corresponding **dissociation constant** \( K_A \) for this equilibrium by equation:
\[ K_A(\text{HA}) = \frac{[\text{H}^+] \cdot [\text{A}^-]}{[\text{HA}]} . \]

In general, we can define dissociation constants for any chosen \( n \)-degree of \( m \)-protic acid \( \text{H}_n\text{A} \) using relationships:
\[ K_A(\text{H}_n\text{A}^{(m-n)-}) = \frac{[\text{H}^+] \cdot [\text{H}_{n-1}\text{A}^{(m-n+1)-}]}{[\text{H}_n\text{A}^{(m-n)-}]} . \]

Values of dissociation constants are of different orders, and therefore, for practical reasons,
they are tabulated in form of their negative logarithms as \( -\log K_A = pK_A \). If the acid is (nearly)
fully dissociated in its aqueous solution (\( i.e. \) equilibrium in the above-mentioned equation is
significantly shifted towards products \( \text{H}^+ \) and \( \text{A}^- \)), value of its \( K_A \) is \( \gg 1 \), as can be easily
seen from definition of the dissociation constant, and value of \( pK_A \) is negative. For example,
\( \text{HClO}_4 \) and \( \text{HI} \) have \( pK_A \approx -10 \). Contrary, acids, whose small fraction is dissociated only (\( i.e. \)
above-mentioned equilibrium is shifted towards non-dissociated form \( \text{HA} \)), have \( K_A \ll 1 \) and
values of \( pK_A \) are positive. Since a number of different acids is very high, a scale of \( pK_A \) is
continuous. Acids with \( pK_A < 2 \) are called strong, acids with \( pK_A \) in range 2–4 are called
medium-strong, in range \( pK_A = 4–9 \) are weak acids, and acids with \( pK_A > 9 \) are very weak.
The lower value of \( pK_A \), the stronger acid is, and better dissociation in aqueous solution
occurs.

By logarithm of equation defining dissociation constant we obtain:
\[ \log K_A(\text{HA}) = \log[\text{H}^+] + \log[\text{A}^-] - \log[\text{HA}] , \]
\[ \text{pH} = pK_A + \log\frac{[\text{A}^-]}{[\text{HA}]} . \]

This equation is called **Henderson-Hasselbalch equation** and finds a wide use in analytical
chemistry. It is obvious that if concentrations of dissociated and protonated forms of acid are
equal, their ratio is one, and the logarithmic addend in the equation above is equal to zero.
Then \( \text{pH} = pK_A \).

Organic acids are typically weak acids. Therefore, concentration of dissociated form \( \text{A}^- \)
which originates from direct dissociation of \( \text{HA} \) can be neglected. When some base is
gradually added to solution of \( \text{HA} \), the species \( \text{A}^- \) is generated only in such amount which
corresponds to amount of added base according to equation:
\[ \text{HA} + \text{OH}^- \rightleftharpoons \text{H}_2\text{O} + \text{A}^- , \]
i.e. \([A^-] = [OH^-]\). Condition \([HA] = [A^-]\) is therefore fulfilled in the case, when exactly one half of solution of base needed for full neutralization is added to the solution of acid (one half of acid is neutralized to salt/anion \(A^-\) and second half stays in the non-dissociated form HA). Therefore, for weak acids (**attention, only! for weak acids**) one can deduce:

\[
pK_A = \text{pH at point where consumption/volume of the base is } \frac{1}{2}V(\text{ekv}).
\]

Typical course of acido-basic titration of monoprotic acid is shown on **Fig. 49**. If the base is gradually added to weak acid solution, neutralization occurs and pH of the mixture is slowly increasing. Concentration of \(A^-\) is increasing correspondingly. Such a part of titration curve is called **buffering region** – dissolved compound behaves as the buffer – i.e. pH is changing only slowly on addition of titration agent (in general acid or base). Near to an **equivalence point** – i.e. point, in which added molar amount of the base is exactly the same as original molar amount of the acid – is derivation of the titration curve steeply increased (high pH change), and in the region with a large excess of the base is pH changed again only slowly. From the above-derived equation, pH value at half volume needed for total neutralization correspond to \(pK_A\). Therefore, for monoprotic weak acid, the value of \(pK_A\) can be directly read as the pH value corresponding to the half of the hydroxide volume needed for exact neutralization (i.e. with respect to equivalence point). From **Fig. 49** it is also obvious, that the smallest slope of titration curve (i.e. the highest buffering capacity) is in the solution with pH close to value of \(pK_A\). In calculated distribution diagram, one can see that abundances/concentrations of HA and \(A^-\) are equal at \(pH = pK_A\).

**Fig. 49.** Titration curve of titration of a weak monoprotic acid HA with a strong base (top), and distribution of HA and \(A^-\) species in dependence on pH (bottom). Figures in left column were simulated for \(pK_A = 3.3\), figures in right column were simulated for \(pK_A = 5.7\). For simulation of titration curves, concentration \(c(\text{HA}) = 0.1 \text{ M}\) and volume \(V(\text{HA}) = 10 \text{ ml}\) were used. As a titration agent, solution of NaOH with concentration 0.1 M was defined. Equivalence point therefore occurs after addition of 10 ml of titrating agent.

A situation somewhat complicates when working with polyprotic acid. A neutralization of the first proton from electroneutral acid molecule occurs according to equilibrium constant \(pK_{A_1}\). After that, the second proton is dissociated/neutralized with \(pK_{A_2}\), the third one is dissociated
with \( K_{A3} \), \( K_{A2} \), etc. In general, \( pK_{A1} < pK_{A2} < pK_{A3} \), ..., as the second proton is bound in monovalent anion, and is bound in the acid molecule more tightly due to stronger electrostatic (Coulombic) interaction than the first one, which was bound in the electroneutral molecule. Similarly, the third proton is bound to the acid divalent anion stronger than the second one due to stronger Coulombic interaction, etc. As the value of \( pK_A \) correspond to pH, at which exactly one half of given protonation state is dissociated/neutralized, it is obvious, that dissociation of the second proton occurs at higher pH than that of the first one, etc. If difference between \( pK_A \) of gradual deprotonations is high, we can see both equivalence steps as consequent waves, as shown in Fig. 50. Sequential deprotonation of the diprotic acid molecule can be seen also from changes of abundance of individual species with increasing pH, as shown in distribution diagram shown in Fig. 50.

![Fig. 50](image)

**Fig. 50.** Titration curve of weak diprotic acid \( H_2A \) by strong base (left), and distribution diagram of species \( H_2A, HA^- \) and \( A^{2-} \) in dependence on pH (right). Figures were simulated for \( pK_{A1} = 3.3 \) and \( pK_{A2} = 5.7 \). For simulation of titration curve, concentration \( c(H_2A) = 0.05 \text{ M} \) and volume \( V(H_2A) = 10 \text{ ml} \) were used. As a titration agent, solution of NaOH with concentration \( 0.1 \text{ M} \) was defined. At \( V(\text{NaOH}) = 5 \text{ ml} \), \( H_2A \) is neutralized to \( HA^- \), and at \( V(\text{NaOH}) = 10 \text{ ml} \), full neutralization to \( A^{2-} \) occurs.

From point of view of Brønsted acid/base theory, it is possible to consider protonated base \( HB^+ \) as “acids“. Therefore, it is possible to define protonation/dissociation constants even for bases. Corresponding equilibrium reaction is then:

\[
HB^+ \rightleftharpoons H^+ + B,
\]

and dissociation constant is defined by relationship:

\[
K_A(HB^+) = \frac{[H^+] \cdot [B]}{[HB^+]}.
\]

**Procedure**

**Preparation of the stock solution of sodium hydroxide**
Prepare 250 ml of sodium hydroxide solution with concentration ca. 0.1 mol·l⁻¹ (see Chapter 4.23.1): calculate corresponding amount of NaOH and weigh it into a beaker. Dissolve the weighted amount in ~50 ml of water and pour the solution into a 250ml volumetric flask. Fill the flask until a mark at flask’s neck. Close the flask and mix well its content by up-down shaking.

**Determination of concentration of the sodium hydroxide stock solution**
Weigh ca. 0.1 g of oxalic acid dihydrate (\( M = 126.07 \text{ g\cdotmol}^{-1} \)) as accurately as possible. The phrase “ca. ... as accurately as possible“ means about this value, but with maximal accuracy – in this case it means to weigh the standard on analytical balances with accuracy on four decimal places. Knock down weighted acid into a titration flask and its remains carefully wash by stream of water from washing bottle into the same titration flask – by such procedure
you transfer your sample quantitatively into the titration flask. Dissolve the oxalic acid in ca. 50 ml of distilled water and add few drops of phenolphthalein solution.

Fill the burette with prepared solution of NaOH (pour the solution carefully and slowly using conic funnel in order to avoid formation of bubbles on the walls inside the burette; place a beaker below the burette). Remove the funnel from top of the burette (some potential drops from the funnel’s stem would make an error in the volume read). Carefully drop down the level of titration agent in the burette to zero (to allow bottom of meniscus reach the mark of zero, see Chapter 4.3). Titrate the solution of oxalic acid with NaOH solution from burette until the mixture will change its colour to purple. In the beginning, the mixture will be purple-coloured in the place of drop’s impact, but it will decolorize fast, and during titration a decolourization process will slow down. Therefore, close to end of the titration add solution from the burette in drop-wise fashion and shake intensively. Before end of the titration wash walls of titration flask by distilled water from washing bottle. In an ideal case a colour of titrated mixture will be changed by one drop of the titrating agent. Read volume of the consumed hydroxide and calculate the concentration of NaOH. Perform the titration two more times and calculate averaged value. For further calculations consider the average as the exact concentration. If some value differs from others by more than ±1 %, repeat the titration once again and do not use the outlying value for averaging. Do not average weights of the standard and volumes of the titration agent – in such way you can include also outlying result of titration and the averaged value will not be correct.

**Determination of total acidity in the solution of unknown organic acid**

Pipette exactly 20.0 ml of unknown acid solution (sample) into a titration flask, and dilute it by further addition of distilled water (≈30 ml). Add few drops of phenolphthalein solution. Titrate the sample with factorized solution of NaOH till purple colour similarly as described above. Perform the titration two more times and for each titration calculate concentration of the acid. If some value differs from others by more than ±1 %, repeat the titration once again and do not use the outlying value for averaging. The average consider as the exact concentration of acid protons in the sample, $V_{eq}$.

**Calibration of pH-meter**

Electrode – rinsed by distilled water and carefully dried by wood pulp – immerse into neutral buffer with known value of pH, and set intercept of linear function pH on potential (button “Offset“) in such way that given pH value will be displayed on the instrument’s display. Rinse the electrode by distilled water, dry it using wood pulp and immerse it into acidic buffer. For displaying of requested pH (pH of the buffer) adjust in this time the slope (button “Slope”). Check if the calibration is appropriate by re-measuring of pH of the first (neutral) buffer – the difference from set value should not exceed 0.02. Always keep the electrode in vertical position and do not leave it in air for unnecessary time – after measurements immerse immediately the electrode to test tube with water.

**Determination of dissociation constant and identification of unknown acid**

Pipette exactly 20.0 ml of acetic acid solution (sample) into a small beaker, and dilute it by further addition of distilled water (≈10 ml). Put small magnetic stirring bar into the beaker and place beaker onto magnetic stirrer. Rinse freshly calibrated electrode by water, dry it by wood pulp and immerse it into solution of the sample. If the layer of the solution will not reach electrode frit (bridge to reference electrode), add more distilled water to assure that frit will be under layer of the sample solution (see Chapter 4.6). Be careful when immersing electrode into solution to prevent contact of stirring bar with glass membrane of the electrode. It is fragile and can be damaged easily by a stirring bar. Read pH value. Add 1.00 ml of NaOH
stock solution from the burette into titrated sample. After stabilization of an electrode read the value. In such a way read pH values after gradual addition of 1.00 ml portions of NaOH solution until volume lower by 5 ml than was volume needed for neutralization determined in titration with visual detection using phenolphthalein (see above). In region \( \pm 5 \) ml around the equivalence point determined in the previous titrations (i.e. from \( V_{eq} - 5 \) ml to \( V_{eq} + 5 \) ml) use smaller step of added NaOH of 0.50 ml. After that use step of 1.00 ml and add further portions of NaOH until total volume of titration agent \( V_{eq} + 15 \) ml will be reached. Draw titration curve (i.e. dependence of pH on volume of titration agent). Decide from a shape of the titration curve, whether unknown acid is monoprotic or diprotic. If the sample is monoprotic acid, determine its \( pK_A \) as the pH value at volume \( V_{eq}/2 \) (see Fig. 49). In the case, that unknown acid is diprotic, determine a value of \( pK_{A2} \), see Fig. 50 (value of \( pK_{A1} \) cannot be determined from direct reading from titration curve, as possible organic acids are not weak in their first protonation state, and condition \([HA^-] = [OH^-]\) mentioned in Introduction is not fulfilled). Identify by comparison with the values shown in Tab. 2, which organic acid was present in your sample. Calculate the concentration of the acid from total proton concentration determined by titrations with visual detection of the equivalence point (i.e. take into account relation between proton concentration and acid concentration when acid is monoprotic or diprotic).

**Tab. 2.** Values of \( pK_A \) of selected organic acids.

<table>
<thead>
<tr>
<th>acid</th>
<th>( pK_{A1} )</th>
<th>( pK_{A2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>formic</td>
<td>3.75</td>
<td>–</td>
</tr>
<tr>
<td>benzoic</td>
<td>4.21</td>
<td>–</td>
</tr>
<tr>
<td>acetic</td>
<td>4.76</td>
<td>–</td>
</tr>
<tr>
<td>oxalic</td>
<td>1.25</td>
<td>3.92</td>
</tr>
<tr>
<td>( L-(-))tartaric ((2R,3R))</td>
<td>2.89</td>
<td>4.40</td>
</tr>
<tr>
<td>( meso)tartaric ((2R,3S))</td>
<td>3.22</td>
<td>4.85</td>
</tr>
<tr>
<td>malic</td>
<td>3.26</td>
<td>5.21</td>
</tr>
<tr>
<td>maleic</td>
<td>1.90</td>
<td>6.07</td>
</tr>
</tbody>
</table>

**Requirements for admitting of the task**

- Titration curve (dependence of pH on added volume of titration solution of hydroxide).
- Value of dissociation constant (\( pK_A \)) of unknown acid read from the titration curve.
- Identification of unknown organic acid.
- Calculated concentration of organic acid.
- Task protocol.
5.4 Task 4 – Determination of melting point

**Tasks**
1. Determine melting points of given samples A and B. Identify these compounds.
2. Determine melting temperatures of mixtures of A and B with content of compound A (by weight) 10, 25, 50, 75 and 90% (content of compound B is complemental, *i.e.* 90, 75, 50, 25 and 10%).

**Introduction**

*Work with melting point apparatus*

For description of melting point apparatus and how to work with see Chapter 3.5.7, principle of given measurement is given in Chapter 4.18. Detailed instructions how to measure melting points are nearside to melting point apparatus.

**Procedure**

*Determination of melting points of unknown samples and their mixtures*

For measurement of melting points, moisture is undesirable contaminant. Therefore, keep the grinded samples in desiccator when you are not working with them.

Add about 120 mg of sample A into a clean and dry mortar. Grind up it carefully with a pestle and transfer the powdered material onto a small Petri dish or clock glass and put it into a desiccator. Carefully wash the mortar and the pestle by warm water and dry it. Grind about 120 mg of sample B. Pay attention in order to avoid mutual contamination of the samples. Do not forget label Petri dishes/clock glasses to avoid confusion of the samples. Keep the samples in the desiccator until you will start to prepare their mixtures or until filling capillaries with the samples. Weight appropriate amounts of grinded samples A and B into small vials and prepare their mixtures according to Tab. 3. Weight with accuracy of tenth of milligram. Use real weights for calculation of exact composition of the mixed samples [*e.g.* if you will weight 4.7 mg of compound A and 46.2 mg of compound B, you will use real weight fraction \( w(A) = \frac{100\% \cdot 4.7}{4.7 + 46.2} = 9.2\% \) instead of 10%; real composition of the samples should not differ from prescribed values given in Tab. 3 by more than 5%].

<table>
<thead>
<tr>
<th>sample</th>
<th>weight of A</th>
<th>weight of B</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 % A</td>
<td>5 mg</td>
<td>45 mg</td>
</tr>
<tr>
<td>25 % A</td>
<td>10 mg</td>
<td>30 mg</td>
</tr>
<tr>
<td>50 % A</td>
<td>10 mg</td>
<td>10 mg</td>
</tr>
<tr>
<td>75 % A</td>
<td>30 mg</td>
<td>10 mg</td>
</tr>
<tr>
<td>90 % A</td>
<td>45 mg</td>
<td>5 mg</td>
</tr>
</tbody>
</table>

Grind up mixtures item-by-item carefully in a clean and dry mortar – it is necessary to homogenize the mixtures before further measurement. Give the mixtures back into corresponding vials. Clean and dry the mortar and the pestle carefully between grinding of different samples.

Fill capillaries by samples (pure samples A and B and their mixtures containing about 10, 25, 50, 75 and 90% of compound A). Use two capillaries for each sample. Gather up small amount of the powdered samples by open end of the capillary. After that, let the capillary fall down through a long glass tube (about 1 m) positioned vertically onto the floor for several times (about 5×). The sample will be stuffed into a small volume on a bottom of the capillary by moment of inertia and will be heated uniformly. The sample should form about 2 mm high column.
Determine melting point of the prepared samples using melting point apparatus. At first, determine melting point of pure samples A and B. After that, determine melting points of their mixtures. Use average of values obtained for both capillaries with the same sample. Perform individual measurements only after sufficient cooling of the melting point apparatus. Construct a chart of dependence of melting temperature on composition of the samples (i.e. use weight fraction of compound A for x axis in scale 0–100%, and depict measured melting points on y axis; similar type of graph is shown on Fig. 33 in Chapter 4.14.2). Do not forget include also melting points of pure compounds A and B into the chart. Remaining samples discard into appropriate container. **Do not give them back into store flasks with pure samples.**

Identify samples A and B by comparison with values outlined in **Tab. 4**.

**Tab. 4.** Melting points of selected compounds – possible samples A and B.

<table>
<thead>
<tr>
<th>compound</th>
<th>$t_i$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>benzoquinone</td>
<td>115</td>
</tr>
<tr>
<td>benzoic acid</td>
<td>122</td>
</tr>
<tr>
<td>benzamide</td>
<td>128</td>
</tr>
<tr>
<td>nicotinamide</td>
<td>130</td>
</tr>
<tr>
<td>salicylic acid</td>
<td>158</td>
</tr>
<tr>
<td>hydroquinone</td>
<td>172</td>
</tr>
<tr>
<td>phthalic acid</td>
<td>207</td>
</tr>
<tr>
<td>phthalimide</td>
<td>238</td>
</tr>
</tbody>
</table>

**Requirements for admitting of the task**

Identification of compounds A and B.

Graph showing dependence of melting point on composition of the A–B mixture.

Task protocol.
5.5 Task 5 – Spectrophotometric quantitative analysis

Tasks
1. Measure calibration curve for determination of concentration of copper(II) ions.
2. Identify the unknown solid sample by determination of copper(II) content.

Introduction
Absorbance measurement
Principle of spectrophotometric measurement and theory is described in Chapters 3.5.9 and 4.25. Detailed instructions how to use spectrophotometer are nearside to the device.

Spectrophotometric determination of concentration
Concentrations of copper(II) ions in solution of unknown compound will be determined using Lambert-Beer’s law (see Chapter 4.25). Concentrations of the samples will be determined by comparison of absorbance of unknown samples with calibration curves (calibration curve is a dependence of absorbance on concentration, i.e. line defined by Lambert-Beer’s law) constructed using series of stock solutions with known concentration of blue vitriol. For this purpose, it is necessary to convert free copper(II) aqua ion, which has only a weak absorption, into strongly absorbing complex tetraammincopper(II) cation with a high extinction coefficient. Therefore, you will add excess of ammonia into solutions of copper(II) salt.

Procedure
Preparation of standard solutions of blue vitriol
Prepare 5 standard solutions of blue vitriol with concentrations 0.005, 0.010, 0.015, 0.020 and 0.025 M and volume of 10 ml by defined dilution of the stock solution of CuSO₄ (c = 0.150 M): pour the stock solution of the blue vitriol into a small clean beaker and measure calculated volumes of the stock solutions using an adjustable automatic pipette and add the measured amounts into 10 ml volumetric flasks. Mark flasks with individual standard solutions using a marker-pen, e.g. by labels CuA–CuE. Fill the flasks to mark of 10 ml by distilled water. Mix the standards thoroughly by bottom-up shaking. If it is not possible to measure calculated volume of the stock solution by given automatic pipette, set the closest value and re-calculate concentration of the standard properly. For example:
For preparation of 10.0 ml of 0.0100 M standard solution, one needs to pipette such volume of the stock solution of concentration 0.150 M to reach equality of the molar amounts of CuSO₄ in both solutions, i.e.:

\[ n(\text{CuSO}_4) = c_1 \cdot V_1 = c_2 \cdot V_2 \]

\[ (0.010 \text{ M}) \cdot (10.0 \text{ ml}) = (0.150 \text{ M}) \cdot V_2 \]

\[ V_2 = 0.667 \text{ ml} \]

The closest value adjustable on the automatic pipette is 0.650 ml. Therefore, such amount (0.650 ml) is pipetted into 10 ml volumetric flask, giving exact concentration of the final standard solution:

\[ c_1 = \frac{c_2 \cdot V_2}{V_1} = \frac{0.150 \text{ M} \cdot 0.650 \text{ ml}}{10.0 \text{ ml}} = 0.00975 \text{ M} \]

Calculate other required amounts of the stock solution needed for preparation of remaining standard solutions in a similar way.

Pour prepared solutions of standard concentrations into dry and clean vials labelled with marker as CuA–CuE. Using the automatic pipette, measure 5.00 ml of each standard into a set of new clean vials labelled CuST1–CuST5. To these solutions, add 1.00 ml of 5% aq. ammonia (measure this volume by 1 ml automatic pipette). Close the vials and mix the content thoroughly. Prepare the reference – measure 5.00 ml of distilled water and 1.00 ml of 5% aq. ammonia.
ammonia into a vial labelled REF, close the vial and mix its content.

**Preparation of solution of the unknown sample**

Weight (on the weighting boat) ca. 75 mg of the unknown sample as accurately as possible. The phrase “ca. ... as accurately as possible” means about this value, but with maximal accuracy – in this case it means to **weigh the standard on analytical balances with accuracy on four decimal places**. Wash the solid sample by small amount of distilled water from washing bottle into clean 25ml volumetric flask (use a glass funnel). Fill the volumetric flask till the mark, close it and **carefully mix the content** by bottom-up shaking for several times. Pour part of the solution into a clean and dry vial. Measure 5.00 ml of 5% aq. ammonia (using 1ml automatic pipette), close the vial and shake the content.

**Selection of optimal wavelength for spectrophotometric measurement**

Wash two cuvettes. Pour distilled water into the cuvettes – fill the cuvettes using a plastic pipette to reach level about ≈6 mm below an upper rim of the cuvette. Insert the cuvettes in the sample holders – one into the holder labelled “M” (“measure”), the second into the holder labelled “R” (“reference”). Take care about orientation of the cuvettes – they must be oriented in the beam by their shiny side. Measure baseline (“blank”) – i.e. device will compare absorbance of both cuvettes with the same solvent and will set zero absorbance. Empty the cuvette in “R”-holder and fill it by reference solution (REF). Empty the cuvette from the “M”-holder and wash it 2× with the first measured sample – it will be the Cu(II) standard with the highest concentration. Fill the cuvette with the standard and place it back in the holder. Measure a spectrum of Cu(II)–ammonia complex in the range 450–750 nm using a step of 1 nm. Observed absorbance is depicted automatically by the device on its display as a function of the wavelength (i.e. as absorption spectrum). Export data to ASCII/CSV format and treat the exported file using suitable table editor (Excel, Origin, etc.) to create a spectrum. **Spectrum is mandatory part of the protocol.** Optimal wavelength for further measurements is a wavelength of the absorption maximum – at this wavelength is the maximal response of the spectrophotometer to change in concentration.

**Construction of calibration curve**

Measure absorbance of all standards at the wavelength corresponding to the absorption maximum. Measure at first absorbance of the most concentrated standard solution. Empty the cuvette, wash it 2× with a new solution of the closest lower concentration, fill the cuvette and measure absorbance again. Proceed this way for all other remaining standard solutions. This method will reduce possible errors originating in non-perfect replacement of the old standard by the new one.

Construct calibration curve (line) using suitable table editor. Use the editor for calculation of linear regression of the data points (e.g. in Excel, mark the data points series, and choose “Chart” → “Add trend-line” – it will calculate the regression line; the parameters of the line can be displayed by editing of dialog window and cross-check “Display equation”). You can calculate concentrations of the unknown samples from the equation of regression. **Calibration curves must be shown in the protocol.** The lines should be going through the origin [0,0], as absorbance of solution with zero concentration of the determined compound should be zero. Therefore, the point [0,0] should be included in the regression.

**Determination of copper(II) concentration in the solution of the unknown sample and its identification**

Measure absorbance of solution of the unknown sample at wavelength chosen for
construction of the calibration curve. Of course, modify the sample of blue vitriol in the same way as was used for standard solutions (defined addition of ammonia solution). Calculate concentrations of the unknown samples using the calibration curves. From the concentration and known weight of the sample calculate weight fraction of the copper in the sample and molar mass of the sample. Using data from **Tab. 5** identify the unknown sample.

**Tab. 5.** Copper(II) salts – possible unknown samples.

<table>
<thead>
<tr>
<th>compound</th>
<th>$w_{Cu} (%)$</th>
<th>$M_r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CuCl$_2$·2H$_2$O</td>
<td>37,28</td>
<td>170,48</td>
</tr>
<tr>
<td>Cu(CH$_3$CO$_2$)$_2$·H$_2$O</td>
<td>31,83</td>
<td>199,65</td>
</tr>
<tr>
<td>CuBr$_2$</td>
<td>28,45</td>
<td>223,35</td>
</tr>
<tr>
<td>CuSO$_4$·5H$_2$O</td>
<td>25,45</td>
<td>249,68</td>
</tr>
</tbody>
</table>

**Requirements for admitting of the task**
Absorption spectrum of blue vitriol dissolved in ammonia solution.  
Calibration curve – dependences of absorbance of standards of blue vitriol on concentration.  
Calculated value of extinction coefficient in absorption maxima of sample prepared from blue vitriol.  
Calculation of molar concentration of solution of the unknown sample.  
Identification of the unknown sample.  
Task protocol.
5.6 Task 6 – Electrolytic preparation and electrogravimetry

**Tasks**
1. Prepare potassium peroxodisulphate by anodic oxidation of solution of potassium sulphate in sulphuric acid and test its reactivity according to instructions.
2. Coat metal sheet by electroplating with copper; verify validity of Faraday’s law by determination of relative atomic mass of copper.
3. Use copper-coated sheet as cathode for electrogravimetric determination of molar concentration of nickel in sample solution.

**Introduction**
Theoretical introduction to electrochemical reaction see Chapter 4.24.

**Vacuum-filtration**
For description of this technique see Chapter 4.12.2.

**Electrolytic preparation**
This preparation is a typical example of electrolytic oxidation (anodic oxidation). Anode is made of platinum sheet, the solution of potassium sulphate in 41% sulphuric acid is used as electrolyte. By passage of electric current, the reaction proceeds:

\[
2 \text{SO}_4^{2-} \rightarrow \text{S}_2\text{O}_8^{2-} + 2 e^-.
\]

**Electroplating**
This method is used for plating cathode – metal sheet – by layer of copper. Electroplating will be done for given time using known electric current, so, you will know total charge passed through system. After weighting of formed copper layer you will be able (using Faraday’s law) determine its atomic weight (see Chapter 4.24.1).

**Electrogravimetry**
Copper-coated sheet will be used as cathode for electrogravimetric determination of nickel. After weighting of nickel you can calculate its concentration in the given sample.

**Procedure**
*Preparation of potassium peroxodisulphate*
Build the apparatus according to Fig. 51. Add head of electrolyser (with electrodes) onto bottom part of apparatus. Surface of joint connection cannot be greased by usually used Ramsay’s grease or vaseline, but only by silicon grease (there is possibility of grease’s ignition during work with strong oxidizing agents). Attach the electrolyser by clamp (using the bottom part) to the stand and place it into Dewar vessel as low as possible. Be careful when manipulating with Dewar vessel – risk of implosion.

Polyethylene tube from bubble counter attach to glass pipe in electrolyser’s head (use the glass tube which ends above level of electrolyte). Attach tube connected with pipe in window’s frame (far from burners) to the other glass pipe in the head of electrolyser. It avoids a potential explosion, as mixture of hydrogen and oxygen is formed in side-reactions during electrolysis.

After assembly of apparatus and connection of wires ask teachers to check your apparatus and connection. Only after successful check ask for electrolyte, dry ice and ethanol for cooling (electrolyte and ethanol are kept in freezer; if you ask for them earlier, they warm up unnecessarily). Fill the bottom part of the electrolyser by electrolyte using hole in its head (by funnel) till level of electrolyte is about 25 mm below the end of pipe with air inlet. Switch on...
the bubbler – it will flow the air into your apparatus, and blow out all gaseous by-products
occurred during reaction.
Pour cool ethanol into Dewar vessel till level of ethanol will be slightly above a level of electrolyte inside the apparatus. Add crushed dry ice portion-wise into ethanol to adjust temperature of the bath in range from −20 °C to −25 °C. The electric source is still switched off. Only after reaching the required temperature start the electrolysis, and keep temperature in this range during whole electrolysis. Set optimal experimental conditions: current 1.5 A (exactly), the potential should be in range 6–10 V. *It is forbidden to manipulate with electric contacts and disassembling the apparatus during electrolysis (when electrical source is on).* Switch off the source after one hour of electrolysis, disconnect wires from head of electrolyser, and disconnect also both tubes. Place off the electrolyser from the Dewar vessel and disassemble the apparatus.

Scheme of electrolyser used for preparation
of potassium peroxodisulphate

\[ \text{Scheme of electrolyser used for preparation of potassium peroxodisulphate} \]

Isolate precipitated peroxodisulphate by vacuum filtration on a glass frit. Collect the filtrate into clean and dry filtration flask and pour it back to stock bottle. After this wash collected peroxodisulphate by ethanol; in this case discard the filtrate. *There must not be any ethanol in the stock bottle.*
Wash both parts of the electrolyser by water and let it dry on the plate. Dry washed product by air-flow on the frit. Weight the product and calculate (using Faraday’s law) theoretical yield of your preparation (change following equation with respect to real values of time of electrolysis and electrical current):

\[ m(K_2S_2O_8) = \frac{M(K_2S_2O_8) \cdot I \cdot t}{z \cdot F} = \frac{270.32 \text{ g mol}^{-1} \cdot 1.50 \text{ A} \cdot 3600 \text{ s}}{2 \cdot 96485 \text{ C mol}^{-1}}. \]
Calculate the yield of your preparation.

*Reactions of potassium peroxodisulphate*
Perform these reactions with your product:

1. Add ca. 3 ml of 5% solution of potassium iodide into a test tube. Add ca. 0.5 ml of 10% H_2SO_4. Add 0.1–0.2 g (one half of small lab-spoon) of prepared solid peroxodisulphate. Heat the mixture carefully on the gas burner.
2. Add 0.1–0.2 g of prepared solid peroxodisulphate into a test tube and add ca. 3 ml of
manganese(II) sulphate solution in 10% H$_2$SO$_4$. Add few drops of 5% silver(I) nitrate solution (catalyst). Heat the mixture carefully on the gas burner.

c. Repeat both reactions above, but now using ca. 0.5 ml of 10% solution of hydrogen peroxide instead of K$_2$S$_2$O$_8$. Make notices about your observation, balance corresponding equations and compare oxidation abilities of peroxodisulphate and hydrogen peroxide.

**Determination of relative atomic mass of copper**

Wash the metal sheet by immersing into 6M nitric acid for 5 s. Wash the sheet by water (firstly from water tap, after by distilled water and finally by ethanol) and let it dry on a clock glass in drying oven. Determine the weight of the metal sheet (after cooling to room temperature). You are going to do analytical experiment, so use analytical balances. Use tweezers when washing and manipulating with the sheet – do not touch it by fingers.

Assembly an apparatus according to **Fig. 52**. Clean both copper anodes mechanically (using emery paper) and hang them onto side conductors in the ring top. Hang clean metal sheet onto central conductor with hooks. Pour coppering solution into the vessel in such way that hooks will be above level of the solution. Switch on the coppering process using current 0.50 A for 20 min. Write down the value of potential, nevertheless, you need to know exact value of current and time for calculation of exact charge passed through during electrolysis. After 20 min switch off the source and get off the ring top from the bath. Take off the metal sheet, wash it by water and ethanol, dry it in drying box and (after cooling to room temperature) determine its weight. Pour coppering bath back into a stock bottle. Calculate mass of the copper. Determine relative atomic mass of copper using Faraday’s law:

$$ A_r = \frac{m \cdot z \cdot F}{I \cdot t} = \frac{m \cdot 2 \cdot 96485 \text{ C}}{0.50 \text{ A} \cdot 20 \cdot 60 \text{ s}}, $$

and discuss the difference between your value and tabulated value ($A_r = 63.54$).

**Electrogravimetric determination of concentration of Ni$^{2+}$ in unknown sample**

Wash the apparatus thoroughly by distilled water. Pipet 10.0 ml of the sample into the apparatus. Dilute the sample by distilled water to ca. 80 ml and add 5 g of solid (NH$_4$)$_2$SO$_4$ and 20 ml of 25% ammonia. The sulphate will play a role of basic electrolyte, which will ensure the flow of electric current. After dissolution of the ammonium sulphate hang two platinum anodes (ask the lab-assistant just before their use) on corresponding conducting wires in ring top, and hang the copper-coated metal sheet onto central hooks. Perform the electrolysis using potential ~10 V for 60–90 min. The end of electrolysis is indicated by complete electro-deposition of nickel, *i.e.* no nickel(II) is detectable in the solution using
diacetyldioxime: put one drop of electrolysed sample solution onto clock glass, add one drop of solution of diacetyldioxime and mix the mixture by a glass rod. Diacetyldioxime forms with nickel(II) ions intensively red precipitate according to scheme:

\[
\text{Ni}^{2+} + 2 \text{dimethylglyoxime} \rightarrow \text{Ni}^{2+} \text{dimethylglyoxime}_{2} + 2 \text{OH}^{-} - 2 \text{H}_{2}\text{O}
\]

Let the electrolysis until red colour is formed during such check. Chugaev reaction is very sensitive, so weak pink solution (not a precipitate!) is acceptable at end of the electrolysis. Switch off the electric source and get off the ring top from the bath. Take off the metal sheet, wash it by water and ethanol, dry it in drying box and (after cooling to room temperature) determine its weight. Platinum electrodes give back to the lab-assistant, electrolyte solution pour into waste sink.

From mass of formed nickel determine the concentration of nickel(II) ion in original sample solution:

\[
c = \frac{n}{V} = \frac{m}{M \cdot V} = \frac{m}{58,69 \text{ g mol}^{-1} \cdot 0,0100 \text{ dm}^{3}}.
\]

**Requirements for admitting of the task**

Prepared potassium peroxodisulphate with calculated yield.
Perform reactions with prepared peroxodisulphate and balanced equation of observed processes.
Calculated relative atomic mass of copper.
Electrogravimetrically determined nickel(II) concentration in the sample.
Task protocol.
5.7 Task 7 – Determination of distribution coefficient of iodine

Tasks
1. Determine distribution coefficient of iodine in chloroform : water mixture.
2. Determine distribution coefficient of iodine in toluene : water mixture.

Introduction
Separation of non-miscible liquids
For description of this technique see Chapter 4.13.

Titration
For details about titrations see Chapter 4.23.

Distribution coefficient
The ratio of concentration of compound dissolved in individual phases of non-miscible solvents is at given temperature and pressure constant and is called distribution coefficient, $K'$. Distribution coefficient describes relative affinity of given compound towards two mutually non-miscible solvents.

In this task you will determine distribution coefficient of iodine between organic solvent and water. You will use two organic solvents – chloroform and toluene, so you will determine two distribution coefficients:

$$K'(\text{CHCl}_3/\text{H}_2\text{O}) = \frac{[\text{I}_2(\text{CHCl}_3)]}{[\text{I}_2(\text{H}_2\text{O})]}$$

and

$$K'(\text{toluene}/\text{H}_2\text{O}) = \frac{[\text{I}_2(\text{toluene})]}{[\text{I}_2(\text{H}_2\text{O})]}.$$ 

The content of iodine will be determined by titration (see Chapter 4.23). In volumetric analysis, the process called iodometry employing redox half-reaction $\text{I}_2 + 2 \text{e}^- \rightarrow 2\text{I}^-$ is often used. Iodometry includes a lot of different determinations, employing selective reaction between iodine and sodium thiosulfate producing sodium iodide and sodium tetrathionate according to equation:

$$\text{I}_2 + 2 \text{Na}_2\text{S}_2\text{O}_3 \rightarrow 2\text{NaI} + \text{Na}_2\text{S}_4\text{O}_6.$$ 

Iodine forms with starch an intensively blue complex, which decolourization indicates quantitative course of the reaction. Therefore, the amount of iodine can be determined by titration with thiosulfate. In the equivalence point, following equation is valid:

$$n(\text{S}_2\text{O}_3^{2-}) = 2n(\text{I}_2).$$

After mixing of both layers in the separation funnel (see Chapter 4.13; thorough shaking is needed for establishing of equilibrium) and their separation, it is possible to determine concentration of iodine in individual solvents/phases by titration, and to calculate the distribution coefficient.

Procedure
Determination of distribution coefficient of iodine in chloroform : water mixture
Place approx. 0.3 g of iodine into 100ml Erlenmeyer flask and dissolve it in ca. 50 ml of chloroform. Filter the solution through a small cotton plug placed in the funnel into a 250ml separation funnel (you will remove potential remains of unsolved iodine or impurities). Add
170 ml of water, close the funnel and shake the mixture thoroughly. Allow separation of the layers by standing. Separate lower (chloroform) layer into clean and dry 100ml Erlenmeyer flask, and close it. Pipette 50.0 ml of aqueous (upper) layer from separation funnel into 250ml titration flask, add 10 ml of diluted aq. hydrochloric acid (1 : 1) and 0.5 g of potassium iodide (it forms triiodide anion with iodine, and triiodide forms with starch used in the end of the titration more intense colour than iodine itself, and the equivalence point is better visible). Titrate by stock solution of thiosulfate \((c = 0.002 \text{ M})\) to light yellow colour (almost to decolourization). Add 1 ml of starch solution and titrate the blue mixture to decolourization. Repeat the titration. If consumptions of both determinations differ more than by \(\pm 0.5 \text{ ml}\), perform the titration once more. Calculate concentration of iodine in aqueous layer based on average consumption. Due to a stoichiometry of the reaction, one can derive that:

\[
n(I_2, H_2O) = \frac{1}{2} \cdot n(S_2O_3^{2-}) = \frac{1}{2} \cdot c(S_2O_3^{2-}) \cdot V(S_2O_3^{2-}),
\]

and thus:

\[
c(I_2, H_2O) = \frac{n(I_2, H_2O)}{V(I_2, H_2O)} = \frac{c(S_2O_3^{2-}) \cdot V(S_2O_3^{2-})}{2 \cdot V(I_2, H_2O)}.
\]

Measure 1.00 ml of chloroform phase using the syringe into the titration flask containing ca. 50 ml of water, acidify by 10 ml of diluted aq. hydrochloric acid (1 : 1) and add 0.5 g of potassium iodide (besides of better visualization of the end of the titration (see above) it also improves the solubility of iodine in aqueous phase, in which will proceed the reaction with thiosulfate – therefore, the reaction between iodine and thiosulfate will proceed much faster). Titrate by analogous way as in the previous case until decolourization of iodine–starch colour. Repeat the determination once more time. If consumptions of both determinations differ more than by \(\pm 0.5 \text{ ml}\), perform third titration. Calculate concentration of iodine in the organic layer based on average consumption similarly as in the previous case. Remaining organic phase pour into to this purpose dedicated bottle. Finally calculate distribution coefficient of iodine between chloroform and water using equation mentioned above.

**Determination of distribution coefficient of iodine in toluene : water mixture**

Perform this determination analogously using 50 ml of toluene instead of chloroform. The difference will rise from lower density of toluene comparing to water – the organic layer will be the upper one. Pour aqueous layer into 250ml Erlenmeyer flask, and keep toluene layer in the separation funnel.

**Requirements for admitting of the task**

Calculated value of distribution coefficient of iodine in chloroform : water mixture.
Calculated value of distribution coefficient of iodine in toluene : water mixture.
Task protocol.
5.8 Task 8 – Rectification and work with gases

**Tasks**

1. Separate ethyl-acetate and toluene by rectification. Construct chart of time dependence of the boiling point. Determine refractive index of individual fractions.
2. Based on these data, estimate the ratio of ethyl-acetate and toluene in the sample.
3. Determine content of calcium(II) carbonate in the sample of marble.

**Introduction**

**Rectification**

Principle of rectification (fractionation) is described in Chapter 4.14.2.

**Refractive index determination**

Description of refractometer and how to work with is given in Chapter 3.5.8, principle of the measurement is described in Chapter 4.19. Ask the teacher for explanation how to operate the refractometer.

**Work with gases**

In a number of chemical reactions, some gases are evolved. In our case, we will measure amount of CO\(_2\) evolved during reaction of hydrochloric acid with marble:

\[
\text{CaCO}_3 + 2 \text{HCl} \rightarrow \text{CaCl}_2 + \text{CO}_2 + \text{H}_2\text{O}.
\]

Based on volume of evolved gas (CO\(_2\)) we will determine carbonate content in the sample for chemical analysis. It is based on equation of ideal gas:

\[
p \cdot V = n \cdot R \cdot T,
\]

where \(p\) is pressure (in Pascals, Pa), \(V\) is volume (in m\(^3\)), \(n\) is molar amount of the gas (in moles, mol), \(R\) is gas constant (8.314 J\(\cdot\)mol\(^{-1}\)\(\cdot\)K\(^{-1}\)) and \(T\) is thermodynamic temperature (in Kelvins, K).

From this equation, it is obvious, that at given temperature and pressure, 1 mol of each gas occupies the same volume, independently on the nature of the gas. The volume is extremely sensitive to temperature and pressure. Students often make errors by suggestion, that 1 mol of the gas occupies volume of 22.4 dm\(^3\). It is valid only for normal conditions, defined as normal pressure (101325 Pa) and temperature 0 °C (273.15 K), but not temperature 25 °C (298.15 K), usually suggested as laboratory/room/ambient temperature. At room temperature, 1 mol of the ideal gas occupies 24.5 dm\(^3\), as can be easily confirmed by a calculation. Therefore, before any simplification with using of „molar volume“, think, if all assumptions were fulfilled.

The simplest way how to determine volume of evolved gas is using of eudiometer. It is calibrated cylinder filled by a liquid, which is immersed in bottom-up fashion in a glass bath containing closing liquid (water is used in our case). The volume can be directly read on the cylinder as it is equal to volume of water pushed out from the cylinder. If heights of water in the cylinder and in the bath are at the same levels, the pressure of the gas in the cylinder corresponds to laboratory pressure. It levels in the cylinder and in the bath are in different heights, a difference in pressures between inside and outside of the apparatus is made. It is equal to hydrostatic pressure corresponding to difference in heights of water levels. As pressure of 1 bar corresponds (roughly) to 10 m of water column, the difference in water levels of 10 cm leads to pressure difference of ca. 1 kPa (i.e. make an error of \(\approx 1\)%). Therefore, it is right to move the cylinder in such way to reach the same altitudes of water levels inside and outside of the volumetric column, or to calculate appropriate correction for pressure inside the column. To a volume, there is also a contribution caused by a vapour pressure (tension) of the closing liquid (water), \(p_{aq}\). Tension of water vapour is at laboratory
temperature 2–3 kPa, so it counts at ambient pressure ~100 kPa by 2–3 %. The ideal gas equation should be corrected to this contribution, so:

\[(p - p_{aq}) \cdot V = n \cdot R \cdot T,\]

and from this equation the molar amount of the gas can be easily calculated. The dependence of water vapour pressure on temperature is given in **Tab. 6**.

**Tab. 6.** Water vapour tension \(p_{aq}\) at different temperatures.

<table>
<thead>
<tr>
<th>(t) (°C)</th>
<th>(p_{aq}) (kPa)</th>
<th>(t) (°C)</th>
<th>(p_{aq}) (kPa)</th>
<th>(t) (°C)</th>
<th>(p_{aq}) (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.6107</td>
<td>10</td>
<td>1.227</td>
<td>20</td>
<td>2.337</td>
</tr>
<tr>
<td>1</td>
<td>0.6567</td>
<td>11</td>
<td>1.312</td>
<td>21</td>
<td>2.486</td>
</tr>
<tr>
<td>2</td>
<td>0.7053</td>
<td>12</td>
<td>1.401</td>
<td>22</td>
<td>2.642</td>
</tr>
<tr>
<td>3</td>
<td>0.7579</td>
<td>13</td>
<td>1.497</td>
<td>23</td>
<td>2.809</td>
</tr>
<tr>
<td>4</td>
<td>0.8128</td>
<td>14</td>
<td>1.597</td>
<td>24</td>
<td>2.982</td>
</tr>
<tr>
<td>5</td>
<td>0.8723</td>
<td>15</td>
<td>1.705</td>
<td>25</td>
<td>3.167</td>
</tr>
<tr>
<td>6</td>
<td>0.9345</td>
<td>16</td>
<td>1.817</td>
<td>26</td>
<td>3.360</td>
</tr>
<tr>
<td>7</td>
<td>1.001</td>
<td>17</td>
<td>1.937</td>
<td>27</td>
<td>3.565</td>
</tr>
<tr>
<td>8</td>
<td>1.072</td>
<td>18</td>
<td>2.062</td>
<td>28</td>
<td>3.778</td>
</tr>
<tr>
<td>9</td>
<td>1.148</td>
<td>19</td>
<td>2.197</td>
<td>29</td>
<td>4.005</td>
</tr>
</tbody>
</table>

**Safety warning**
Concentrated hydrochloric acid is fuming and is very corrosive; work with gloves. Avoid its contact with a skin. Wash the skin immediately by water when spilled.

**Procedure**

*Separation of mixture of ethyl-acetate and toluene by rectification*

Distillation apparatus according to **Fig. 34** in **Chapter 4.1.4** is already arranged; do not disconnect individual parts of the apparatus.

Pour 100 ml of the sample into 250ml round-bottom flask. Add pieces of porcelain and connect the flask with distillation column. Move the heater up to fix the flask to apparatus.

Switch on the cooling water with such intensity that it will be possible to recognize individual blades of flowmeter (or it is possible to recognize colours of individual balls if ball flowmeter is used. Make sure, that valve of distillation head is closed (reflux valve) and arrange set of test tubes for collection of the fractions (label test tubes by numbers 1–8).

Switch on the heating nest and set it to maximal power. Make sure that warning light is lighting. Monitor starting of boiling and reaction of the thermometer in the distillation head. After first drops appear in the distillation head, let the column to equilibrate for 5 min.

After equilibration open the reflux valve to let distillate drop off from the head by 2 drops per 1 s. Collect 10 ml of individual fractions into the test tubes. Monitor the boiling temperature in 1 min intervals, and compile the temperatures in the table. Make a notice when changing the test tubes.

Switch off the heater when you collected 75 ml of the distillate, *i.e.* when there is 5 ml of liquid in the test tube no. 8. By thermal capacity it will fill some remaining liquid. In the round-bottom flask, there should be remaining ca. 20 ml of residue.

Determine refractive index of etalons (pure ethyl-acetate and toluene) and of each fraction. Show the results to teacher.

Disconnect the distillation flask from the column and pour remaining liquid into corresponding bottle. Wash the distillation flask with ethanol (washing can be poured into a sink). Employing suitable table editor construct chart of the distillation – *i.e.* dependence of boiling point on time and show areas where individual fractions were collected. Based on acquired data, estimate the ethyl-acetate/toluene ratio in the starting sample.
Eudiometric determination of CaCO₃ in sample of marble
Assembly an apparatus according to Fig. 53.

![Fig. 53. Apparatus for determination of CaCO₃ content in the sample.]

Weight 1.00 g of ground sample. Add it into Ostwald flask (using funnel). Make sure, that joints of the flask, dropping funnel and reduction tube are well lubricated by grease. Close the dropping funnel by a stopper with tubing connected to flask with CO₂. Open the valve of the funnel. With help of the teacher, open the valve of gas bomb (see Chapter 4.20) and allow a gentle stream of CO₂ passing through the apparatus to ensure saturation of water in the gas washing flask and in the glass bath. The reason is to avoid dissolution of CO₂ evolved during reaction in the water, which would distort the volume measurement. After ca. 5 min close the valve of the bomb. Fill the cylinder (eudiometer) by water. The best way is to lay the cylinder down into the bath in horizontal position to remove bubbles of air and flipping it to vertical direction and fix it by clamp. Place end of the tubing system under lower rim of the cylinder. Close the valve of the dropping funnel. Measure 10 ml of conc. HCl by a volumetric cylinder and pour it into dropping funnel. Close the apparatus by a regular stopper. Add the acid to the sample by a careful opening of the valve of the funnel. Measure the volume of CO₂ evolved using eudiometric cylinder.

Measure temperature (as the bath is long-time standing on the place dedicated to this task, the temperature of water is equilibrated with temperature of laboratory) and pressure in the laboratory, and find tension of water vapours at given temperature in Tab. 6. Calculate molar amount of evolved CO₂. It is obvious, that this molar amount is equal to molar amount of calcium(II) carbonate present in the sample. Calculate weight of CaCO₃ \( (M = 100.09 \text{ g mol}^{-1}) \) in your sample and mass ratio of CaCO₃ (%) in the marble.

Requirements for admitting of the task
Chart showing course of distillation – dependence of boiling point on time.
Refractive indexes of etalons and individual fractions.
Ratio of ethyl-acetate and toluene in the starting mixture.
Calculated percentage of CaCO₃ in the sample of marble.
Task protocol.
5.9 Task 9 – Vacuum distillation

**Tasks**
1. Purify given ester of acetic acid by vacuum distillation.
2. Construct chart of time dependence of the boiling point.
3. Determine refractive index of the product.
4. Determine density of the product.
5. Identify the isolated compound based on measured data.

**Introduction**

**Vacuum distillation**

As the isolated ester boils at normal pressure at a high temperature (>100 °C), vacuum distillation will be used. For principle and description of this technique see Chapter 4.14.1.

**Refractive index determination**

Description of refractometer and how to work with is given in Chapter 3.5.8, principle of the measurement is described in Chapter 4.19. Ask the teacher for explanation how to operate the refractometer.

**Procedure**

**Vacuum distillation**

At first you will remove moisture and mechanical impurities from the given sample. Add ca. 1 g of anhydrous sodium sulphate to 25 ml of the sample poured in Erlenmeyer flask, close the flask by a stopper and shake it. After clarification of the mixture, filter it through a folded (dry) filtration paper into 50ml Apollo-shape flask and add a stirring bar. Assembly an apparatus for vacuum distillation and let it check by the teacher. Contrary to Fig. 32 in Chapter 4.14.1 use a short distillation column – place it between the flask with distilled sample and distillation adapter with thermometer. Wrap the column by cotton (or aluminium foil) for thermal isolation. Use a distillation spider equipped with two 10ml and one 25ml tear-shaped flasks; weighted the 25ml flask in advance staying in a beaker together with a stopper. Adjust a gentle stream of water into a cooler (be careful, avoid dropping of water into an oil bath), set appropriate intensity of stirring (the stirring bar should move fast, but regularly without jumping), switch on the vacuum pump and start to evacuate the apparatus by closing the valve of safety flask. Open the valve of manometer – you can check tightness of apparatus by determination of pressure; if a move of mercury in the manometer is small, the apparatus is probably not tightly sealed and you have to fix it. If apparatus is tightly closed, start warming of the oil bath. Write down pressure and temperature during distillation in intervals of 1 min. Bath temperature should be in maximum higher by ca. 20 °C than is the boiling point of the product. When higher bath temperature is used, the product can boil too wildly. As you do not still know, what the boiling point will be, you should increase the bath temperature carefully and gradually to avoid sudden and extreme intensive boil. Therefore, set the stirring plate in the beginning to temperature 50 °C, and after tempering the bath increase the set temperature with step of 10 °C to assure a smooth boiling. Notice temperature of vapours in the apparatus – low-boiling impurities start to boil with gradual increase in temperature. Collect the first fraction into a 10ml flask until the boiling temperature reach constant value (i.e. boiling point of isolated ester). After this turn the distillation spider\(^6\) connected to collection adapter and change collection flask to in-advance-

\(^6\) If distillation spider (splitter) is absent, a change of collecting flasks should be performed in a following way: close the valve of manometer – it disconnects it from the apparatus, and avoids risk of broking manometer’s tube.
weighted 25ml flask and distil the product. When distillation is over, stop it – at first close the value of the manometer, let air in the apparatus by opening of valve of safety flask, switch off the pump and water and open the disconnected manometer to air carefully in such a way to reach slow return of the mercury column (it avoids broking of the manometer’s tube). Weight the flask with product (closed and standing in the beaker) and calculate the yield. Determine refractive index of the distillate.

Wash oil remaining from the outside wall of the flask by hot water and detergent, wash inside of the flask by ethanol. Wash all other vessels which were in contact with sample/product by ethanol.

**Calibration of automatic pipette**

During this Task, you do not use pycnometer for measurement of volume (pycnometers have usually too big volume, mostly 25 ml and more), but from the reason of small loss of the product you will measure required volume using a piston pipette (see Chapter 4.3). Sometimes the volume declared on the pipette differs slightly from the real pipetted volume, so, for the exact measurement you should do the calibration of the volume. You will use distilled water. Pipette water into the weighted vial (set the volume, which you will use for determination of density, *i.e.* 1.000 ml). **Attention, set volume corresponds to the first position of the piston!** Determine the weight of pipetted water (using analytical balances) and calculate exact volume of the pipette. Densities of water in dependence on temperature are outlined in **Tab. 7**. Determine the temperature of used water using thermometer (place thermometer into water in such a way, that mercury container will be under layer of water and does not contact with walls of the vessel). It the temperature will not be integer, interpolate a value of density from edge values listed in the table. Repeat the determination five times and average the values. If some result differs from others by more than ±0.5%, repeat the measurement. Use averaged volume for calculation of product’s density. If the volume you get differs from set volume of 1.000 ml by more than 2%, you probably do pipetting in a wrong way. In such case consult your technique with the teacher.

**Tab. 7.** Temperature dependence of water density.

<table>
<thead>
<tr>
<th>℃</th>
<th>ρ (g cm⁻³)</th>
<th>℃</th>
<th>ρ (g cm⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.999099</td>
<td>23</td>
<td>0.997536</td>
</tr>
<tr>
<td>16</td>
<td>0.998943</td>
<td>24</td>
<td>0.997299</td>
</tr>
<tr>
<td>17</td>
<td>0.998775</td>
<td>25</td>
<td>0.997047</td>
</tr>
<tr>
<td>18</td>
<td>0.998596</td>
<td>26</td>
<td>0.996786</td>
</tr>
<tr>
<td>19</td>
<td>0.998406</td>
<td>27</td>
<td>0.996515</td>
</tr>
<tr>
<td>20</td>
<td>0.998205</td>
<td>28</td>
<td>0.996235</td>
</tr>
<tr>
<td>21</td>
<td>0.997994</td>
<td>29</td>
<td>0.995946</td>
</tr>
<tr>
<td>22</td>
<td>0.997772</td>
<td>30</td>
<td>0.995649</td>
</tr>
</tbody>
</table>

**Determination of density of the product**

Pipette 1.000 ml of the product into weighted (using analytical balances) clean and dry vial using calibrated automatic pipette. Weight the vial again. Determine the weight three times, and average final values. When some result differs from others by more than ±1 %, repeat the measurement. Calculate density of the product.

by fast moving mercury column if loss of vacuum would be fast; fill the apparatus with air by opening of valve of safety flask or by disconnecting rubber tubing between the pump and the adapter, change the collecting flask and start evacuation again. A change of the flasks must be performed as quickly as possible to avoid overheating of the distilling mixture – in other case, too intensive boil can occur after repeated evacuation with spitting into a condenser. After evacuation connect the manometer.
Identification of the product
Identify the product by comparison of measured data with values outlined in Tab. 8.

Tab. 8. Values of refractive index $20n_D$ and density $\rho$ of acetic acid esters – possible samples.

<table>
<thead>
<tr>
<th>ester</th>
<th>$20n_D$</th>
<th>$\rho,(g\cdot cm^{-3})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>butyl-acetate</td>
<td>1.394</td>
<td>0.883</td>
</tr>
<tr>
<td>pentyl-acetate</td>
<td>1.402</td>
<td>0.876</td>
</tr>
<tr>
<td>hexyl-acetate</td>
<td>1.409</td>
<td>0.867</td>
</tr>
<tr>
<td>benzyl-acetate</td>
<td>1.502</td>
<td>1.054</td>
</tr>
</tbody>
</table>

Requirements for admitting of the task
Clean product with calculated yield of isolation.
Boiling point of the product at used low pressure.
Refractive index of the product.
Determined density of the product.
Identification of the product. **Pour the product into an appropriate bottle only after teacher’s agreement with product’s identification!**
Task protocol. Mandatory part of the protocol is a chart showing a course of distillation.
5.10 Task 10 – Determination of solubility of inorganic salt, and determination of content of water of crystallization in the solid sample

**Tasks**
1. Determine solubility of given sample of inorganic salt in water at laboratory temperature.
2. Identify the unknown sample based on its solubility.
3. Determine number of molecules of water of crystallization in given solid sample.

**Introduction**

**Solubility of ionic substances**

Equilibrium in closed system must fulfil special rule defined in end of 19th century by American mathematicians and physicist Josiah Willard Gibbs (1839–1903). Such rule is called *Gibbs law of phases (phase rule)*:

\[ P + F = C + 2, \]

where \( P \) is a number of *phases* in the system, \( F \) is a number of *degrees of freedom* and \( C \) is a number of chemical constituents in the system. A phase is each homogenous part (gas, liquid, solid phase) of the system and chemical constituent is each present chemical compound. Degree of freedom is any intensive physical variable (i.e., variable, which does not depend on total mass of the system; intensive variables are for example temperature, pressure, density, concentration etc.).

For pure liquid, \( P = 1 \) and \( C = 1 \) and, therefore, \( F = 2 \). You can choose two independent variables for given liquid (e.g., temperature and pressure), and values of other variables are conditionally given (e.g., density). During heating of the liquid to its boiling point, the second (gaseous) phase is formed, i.e., \( P = 2, C = 1, \) and such system has only one degree of freedom \( (F = 1) \). Therefore, at given pressure, the boiling point is exactly given by value of pressure (and oppositely, if we want to set boiling point to required temperature, the pressure must be changed to well-defined value). Similarly, for pure solid compound \((C = 1)\) is at given pressure its melting point constant (at melting point, two phases – solid and liquid – coexist, \( P = 2, \) and therefore, \( F = 1 \)).

In the case of solution of some compound in the solvent, \( C = 2 \) (constituents are dissolved compound as well as solvent) and \( P = 1 \) (there is only one homogenous phase – solution), and number of degrees of freedom is \( F = 3 \). At given temperature and pressure, the third parameter can be chosen – e.g., concentration. It is the case of for example two unlimitedly miscible liquids, e.g., water and ethanol.

Other situation occurs when dissolving compound is not unlimitedly miscible with the solvent. When e.g., salt will be gradually added to water, it dissolves until a critical concentration is reached. Further addition of salt does not dissolve and heterogeneous mixture of liquid and solid is obtained. For such a mixture, \( C = 2 \) and \( P = 2, \) and number of degrees of freedom is \( F = 2. \) At given temperature and pressure is therefore concentration of saturated solution conditionally defined and it is constant. Concentration of the saturated solution is called *solubility*, and saturated solution is such a solution, in which (at given temperature and pressure) equilibrium between solution and solid phase is reached. However, the equilibrium is not static, but some solid material still dissolves, and some ions from solution are deposited into a crystal lattice. At equilibrium, rates of both processes are equal. Therefore, for general salt \( X_y Y_z, \) following equation can be written:

\[ X_y Y_z (s) \xrightarrow{\text{eq}} x X (aq) + y Y (aq) \]

[charges of individual ions are omitted, and expressions (s) and (aq) mean, that given substance/ion are present in the solid phase or dissolved in aqueous solution].

The product \( P = [X]^x [Y]^y \) is (at given temperature and pressure) for a given substance constant. The variable \( P \) (sometimes labelled as \( K_s \)) is called *solubility product*.

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The solubility products are usually tabulated for poorly soluble compounds, so it is very low and therefore, it is usually presented in the form of negative logarithms, e.g. 
\[ P(\text{AgCl}) = K_s(\text{AgCl}) = 1.78 \cdot 10^{-10}, \quad -\log\{P(\text{AgCl})\} = pP(\text{AgCl}) = pK_s(\text{AgCl}) = 9.75. \]
The solubility can be expressed also by different ways. One other possibility is molar solubility \( S \), which is molar concentration of the saturated solution. The solubility of well soluble materials can be given also in molality (i.e. in moles of the compound dissolved in 1 kg of the solvent), or in weight fraction (grams of compound in 100 g of the solution), or in grams of compound in 100 ml of the solvent or solution, or in grams of compound in 100 g of the solvent. For conversion between different values it is sometimes needed to know a density of the saturated solution.

In this Task, you will determine solubility of well soluble inorganic salt.

*Water of crystallization*

In some cases, water molecules are incorporated into a lattice of the ionic compound during its crystallization from the solution. Such water molecules can be bounded in the lattice by different modes – e.g. by coordination to cation, by hydrogen bonds to anions, by hydrogen bonds between each other, etc. Sometimes, in dependence on temperature of crystallization process, different solid phases are crystallized out, differing in a number of water molecules per formula unit of the salt. For example, sodium carbonate (\( \text{Na}_2\text{CO}_3 \), washing soda) crystallizes (based on conditions) in the form of monohydrate, heptahydrate or decahydrate. Even table salt (sodium chloride, NaCl), which is well known and standardly used in its anhydrous form, crystallizes at temperature below 0 °C in form of dihydrate, \( \text{NaCl}\cdot2\text{H}_2\text{O} \).

In addition, crystalline hydrates can (at least partly) loose some of water of crystallization upon storage.

*Work with piston pipette*

For description of this technique see [Chapter 4.3](#).

*Annealing in flame of gas burner and annealing to the constant weight*

For description of this technique see [Chapter 4.7.1](#). After annealing till constant weight use the last weight obtained. Do not average last two values. Although this fact is obvious, related mistake is appearing rather frequently in the protocols.

*Procedure*

The sample is given as aqueous suspension in labelled 250ml Erlenmeyer flask and in the form of solid material in the flask with the same code as given for the suspension. Check, that there is also undissolved solid material beside liquid phase in the Erlenmeyer flask.

Plan the work on individual sub-tasks in such a way that you will reasonably use your time spent in the practicum during annealing/cooling of the crucibles.

*Determination of number of molecules of water of crystallization*

Anneal two small labelled (by marker or pencil) crucibles till constant weight in the oven set to 400 °C. Left individual annealing for 10 min, put crucibles into a desiccator using tongs and let them cool down to laboratory temperature. Be careful, after you will close the desiccator with hot content it will be inside slightly pressurized (\( p\cdot V = n\cdot R\cdot T \)) and viscosity of grease used for sealing of joints will be lowered due to slight warming and, therefore, the cover of the desiccator can slide down. Therefore, hold the desiccator’s cover by hand and after a short while (20 s) open it just for balancing the pressure. Use analytical balances for weighting. Carry out all weightings using the same balances.

Weigh ca. 0.25 g of the solid sample into the crucibles. The phrase “ca. ... as accurately as
possible" means about this value, but with maximal accuracy – in this case it means to **weigh the standard on analytical balances with accuracy on four decimal places**. Anneal the samples for 10 min at 400 °C in the oven. Put crucibles into a desiccator using tongs and let them cool down to laboratory temperature. Weight the crucibles using analytic balances and repeat annealing for next 10 min. Let the crucibles cool down in the desiccator and weight again. If the weight obtained after the second annealing differs from the first weighting by more than ±1 mg, anneal the crucibles again for next 10 min till constant weight will be reached.

From the weight lost determine content of water present in the solid sample per formula unit of the salt (i.e. number of water molecules of crystallization). To finish this calculation you need to know result of following sub-task (identity of the sample).

**Determination of solubility and identification of the sample**

Anneal three big **labelled** (by marker or pencil) crucibles to constant weight using Méker gas burner. Notice the remarks mentioned above in instructions for determination of content of water of crystallization.

Stir the suspension of the sample and measure its temperature by thermometer – thermometer attach to a stand via clamp and immerse it into suspension of the sample in such way, that mercury bulb will be under surface of the suspension but does not touch the walls of the flask. Leave the thermometer immersed at least for 5 min before reading of temperature.

Using piston pipette, measure 5.00 ml of liquid phase from the suspension sample into each of three crucibles annealed to the constant weight. Be careful to avoid sucking of solid material from the suspension into the pipette’s tip. **Pay attention, the volume set on the pipette corresponds to the first position of the pipette’s piston (push-button)!** Weight the crucibles with sample solution on the analytical balances. Place the crucibles onto ceramic net and heat them by non-luminous flame of the gas burner. Take care that solution will boil only gently and material does not be spitted around. Intensity of heating can be easily regulated by keeping the burner in the hand and moving it. After volatiles will be removed, put the heater directly below the net with crucibles and anneal the sample at highest possible temperature for 10 min.

Weight the cooled crucibles and anneal them again for next 5 min. Let them cool down in the desiccator and weight them again. If the weight obtained after the second annealing differs from the first weighting by more than 3 mg, anneal the crucibles again for next 5 min till constant weight will be reached.

Calculate weight of the solid remaining in the crucibles. In the case, that weight of one of the three samples differs significantly (>5 %) from two others, exclude such value from further calculations. If all weights significantly differ from each other, repeat all experiment with new three liquid samples.

Calculate solubility of the sample. Present results in grams of the dissolved material per 100 ml of solution, in grams of dissolved material per 100 g of solution, in grams of dissolved material per 100 g solvent (water), in molarity (mol⋅dm⁻³) and in molality (mol⋅kg⁻³). Calculate also density of the saturated solution at room temperature (in g⋅cm⁻³).

Wash the crucibles and let them dry out.

Using entries outlined in **Tab. 9** identify your unknown sample.
**Tab. 9.** Solubility of salts (calculated for anhydrous formulas). The values are given in grams of anhydrous salt dissolved in 100 g of water.

<table>
<thead>
<tr>
<th>Salt</th>
<th>$M_r$</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>24</th>
<th>25</th>
<th>26</th>
<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li$_2$SO$_4$</td>
<td>109.94</td>
<td>34.6</td>
<td>34.5</td>
<td>34.4</td>
<td>34.3</td>
<td>34.3</td>
<td>34.2</td>
<td>34.2</td>
<td>34.1</td>
<td>34.1</td>
<td></td>
</tr>
<tr>
<td>Na$_2$SO$_4$</td>
<td>142.04</td>
<td>16.4</td>
<td>17.7</td>
<td>19.1</td>
<td>20.6</td>
<td>22.2</td>
<td>23.9</td>
<td>25.8</td>
<td>27.9</td>
<td>30.1</td>
<td>32.5</td>
</tr>
<tr>
<td>MgSO$_4$</td>
<td>120.36</td>
<td>32.3</td>
<td>32.9</td>
<td>33.5</td>
<td>34.0</td>
<td>34.6</td>
<td>35.2</td>
<td>35.8</td>
<td>36.3</td>
<td>36.9</td>
<td>37.5</td>
</tr>
<tr>
<td>MgCl$_2$</td>
<td>95.21</td>
<td>54.2</td>
<td>54.3</td>
<td>54.4</td>
<td>54.6</td>
<td>54.7</td>
<td>54.8</td>
<td>54.9</td>
<td>55.0</td>
<td>55.2</td>
<td>55.3</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>110.98</td>
<td>72.6</td>
<td>74.0</td>
<td>75.5</td>
<td>77.2</td>
<td>78.9</td>
<td>80.7</td>
<td>82.7</td>
<td>84.8</td>
<td>87.0</td>
<td>89.3</td>
</tr>
<tr>
<td>Na$_2$CO$_3$</td>
<td>105.99</td>
<td>19.4</td>
<td>20.6</td>
<td>21.8</td>
<td>23.2</td>
<td>24.6</td>
<td>26.2</td>
<td>27.8</td>
<td>29.6</td>
<td>31.4</td>
<td>33.4</td>
</tr>
</tbody>
</table>

**Requirements for admitting of the task**

- Solubility of the sample presented in all required units.
- Density of the saturated solution at room temperature.
- Identity of the unknown sample.
- Calculated number of water molecules of crystallization.
- Task protocol.