PHARMACEUTICAL CHEMISTRY

LABORATORY MANUAL

Determination of boric acid in boracic water by potentiometric titration

Version: visual standardization of sodium hydroxide solution against potassium hydrogen phthalate

Organisation	Stredná odborná škola chemická, Vlčie hrdlo 50, 821 07 Bratislava
Author	Judita Dömötörová
Revised by	Judita Dömötörová
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1.1 Introduction

1.1.1 Boric water

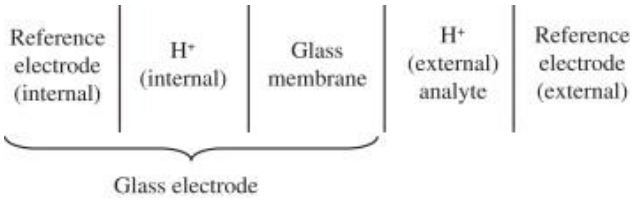
Boric acid is a **very weak acid** with a dissociation constant pK_a = 9.24, which behaves as a **monosaturated acid in aqueous solutions**. Its dilute aqueous solutions are mainly used in dermatology. Solutio acidi borici is a 3 % aqueous solution of boric acid, which is used as a gentle surface disinfectant of the skin and has a weak anti-inflammatory effect. Boric acid salts have found application in the impregnation of wood against mould. It is also known as E 284 as a weak preservative, which in the EU can only be used to preserve caviar as it is considered hazardous.

1.1.2 Potentiometry

Principle of potentiometry
Potentiometry is based on measuring the electromotive force (EMF) of a galvanic cell. The cell consists of an **indicator electrode** and a **reference electrode**. The potential of the indicator electrode depends on the concentration (activity) of the monitored substance, the potential of the reference electrode is constant. The EMN (the difference between the potentials of the measuring and reference electrodes) is a measure of the concentration of the monitored substance.

Glass electrode
The essential feature of an ideal ion-selective electrode is a **thin membrane** across which only the target ion can migrate. The most important ion-selective electrodes for pH determination are glass electrodes, liquid membrane electrodes, and ion-sensitive field-effect transistors (ISFETs).

The glass pH electrode is the most common example of an ion-selective electrode. The overall galvanic cell of a typical (combination) glass electrode incorporating both **glass** and **reference electrodes** can be represented by



The key to electrode selectivity lies in its glass membrane. The surface layers of the latter consist of fixed silicate groups associated with sodium ions ($-OSiO_2^-Na^+$). When this electrode is dipped in water, the sodium ions exchange with the solvated protons in water and the surface is then described as 'hydrated'. The glass membrane has an inner and outer hydrated layer. In these hydrated layers, the anion sites are covalently bound to the bulk of the glass and are fixed. However, the H^+ cations are mobile, being free to exchange with the external solution or with sodium ions in the body of the glass. When the electrode is placed in an aqueous solution of unknown pH, the activity of the H^+ ions in the test solution is likely to be different from the activity of the H^+ ions in the hydrated layer. This sets up a potential difference between the solution and the surface of the membrane. This boundary potential is determined by this difference in the activities.

The glass pH electrode system used nowadays consists of a **pH-sensitive measurement glass electrode** and a **separate reference electrode in a potassium chloride (KCl) gel-conducting solution** (Figure 1). These electrodes are usually housed in the combination sensor, containing both electrodes, which is connected to an electronic meter with a signal amplifier and temperature compensation. The meter displays the pH reading, which may be uploaded to a computer or controller. A silver wire enclosed in the measurement electrode forwards a signal indicating the difference in acidity between the solutions inside and outside the glass membrane. The reference electrode has a stable potential, which is independent of the measuring solution and must be calibrated outside the system in a reference solution. The most commonly used reference is a silver/silver chloride electrode in a buffer. The measurement and reference electrodes complete a circuit through the water sample (via a permeable porous junction built in the glass wall, Figure 1) allowing measurements of the voltage generated by the glass electrode.

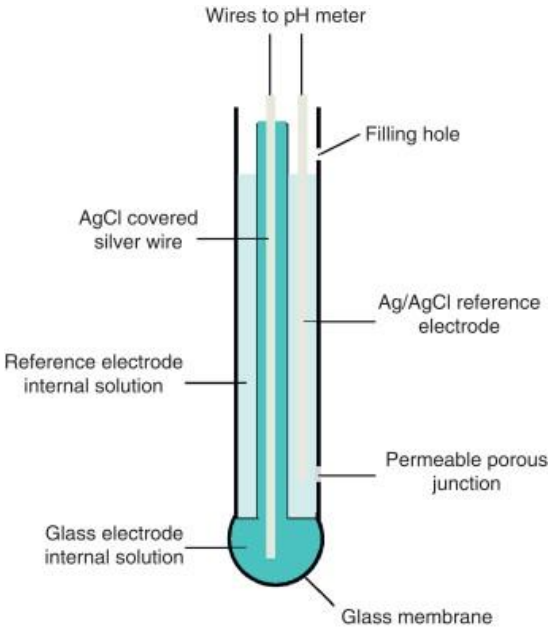


Fig. 1 Glass electrode

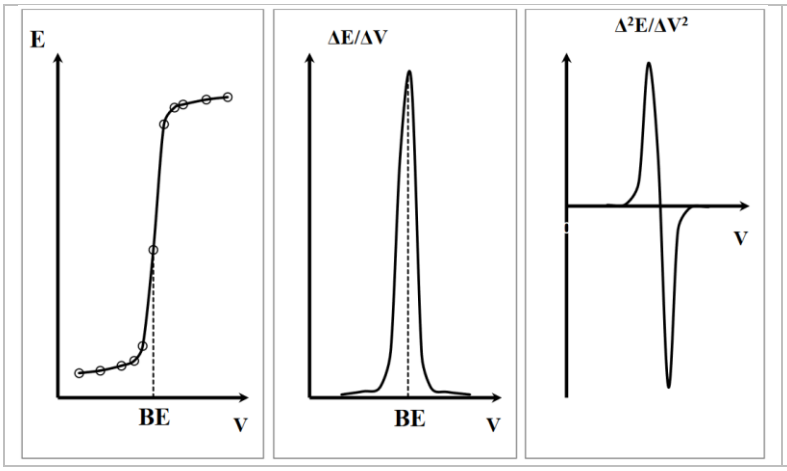
Common glass pH electrodes are extraordinary sensors in that they operate within a typical temperature range of 0–90 °C over the full pH range of 0–14 (14 orders of magnitude of the H^+ concentration), although they require accurate temperature measurement and compensation. The pH signal generated by a glass electrode can drift, or lose accuracy, over time due to a number of factors including fouling, sensor instability, and interference from external equipment. Therefore, accurate pH measurements require an **external recalibration procedure using standard solutions of known pH**. Other potential pitfalls of the pH glass electrodes include fragility, difficult miniaturization, leakage of the reference electrode buffer into the sample solution, poor response in low ionic strength solutions, high background noise, and moderate signal-to-noise ratio.

1.1.2.1 Potentiometric titration curves

Potentiometric titration curves typically have a sigmoidal shape. They represent the graphical relationship between the cell voltage (E) and the volume of the added titrant (V). In the case of neutralization titrations, the potentiometric titration curve specifically shows the relationship between pH and the volume of the added titrant (V).

The endpoint of the titration can be determined using either graphical methods or calculations.
Derivative curves:

1. The first derivative curve represents the dependence of $\frac{\Delta pH}{\Delta V}$ on V. The volume at the equivalence point is identified by the maximum or minimum of this curve. Here, ΔpH and ΔV are the differences between two consecutive values.
2. The second derivative curve represents the dependence of $\frac{\Delta^2 pH}{\Delta V^2}$ on V. The volume at the equivalence point is determined by the point where this curve crosses the x-axis, indicating the inflection point.



a. Potentiometric titration curve
b. First Derivative Curve
c. Second Derivative Curve

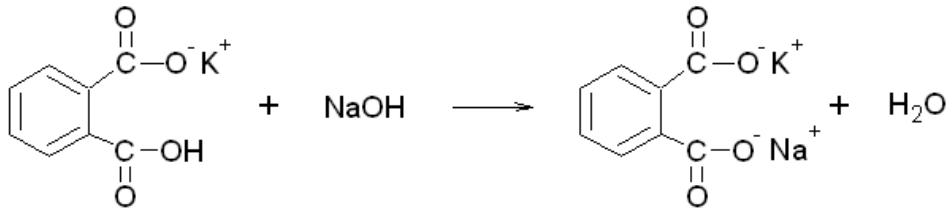
Calculation of the volume of the titration solution corresponding to the inflection point:

$$V_x = V^+ + \Delta V \frac{\Delta^2 pH^+}{\Delta^2 pH^+ + |\Delta^2 pH^-|}$$

where
V_x – is the volume of the titration solution corresponding to the inflection point
V^+ – is the volume at which the second pH difference is positive for the last time
ΔV – is the constant addition of the titration solution around the inflection point
$\Delta^2 pH^+$ – is the value of the last positive pH difference
$\Delta^2 pH^-$ – is the value of the first negative pH difference

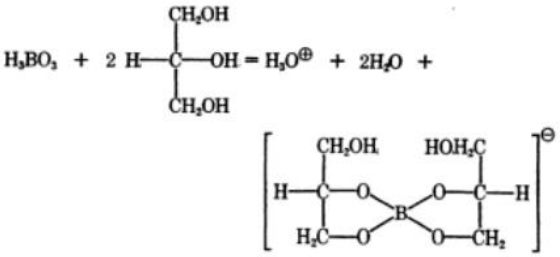
1.1.3 Standardisation of a volumetric solution of sodium hydroxide

Sodium hydroxide is titrated against potassium hydrogen phthalate (KHP) using phenolphthalein as the indicator.
Reaction equation: $C_6H_4.COOK.COOH + NaOH \rightarrow C_6H_4.COOK.COONa + H_2O$



1.1.4 Determination of boric acid

The determination of boric acid is possible alkalimetrically after the addition of polysaturated alcohols or sugars, such as glycerol or sorbitol, with which it forms stronger complex acids. In this case, boric acid reacts with sodium hydroxide in a 1:1 ratio.
Reaction to form a complex acid:



(5)

$H_3BO_3 + 2\ C_3H_8O_3 \rightarrow H[X]^- + H_3O^+ + 2\ H_2O$

The titration reaction of glyceroboric acid with sodium hydroxide (simplified notation of the acid is H[X]):
$H[X] + NaOH \rightarrow Na[X] + H_2O$ (6)

1.2 Assignment

1. Calculate the quantities of substances needed to prepare the solutions.
2. Familiarise yourself with the hazardous properties of the substances you will be working with.
3. Prepare the solutions and reagents.
4. Record the sample information.
5. Determine the concentration of sodium hydroxide by visual titration.

6. Determine the concentration of boric acid by potentiometric titration.
7. Complete the worksheet.
8. Answer the questions on the worksheet.

1.3 Chemicals

Potassium hydrogen phthalate (c = 0,1 mol.dm^{-3})
Sodium hydroxide (c = 0,1 mol.dm^{-3})
Phenolphthalein (0,1%)
Deionised water
pH calibration solutions

1.4 Materials and equipment

Analytical balance, weighting boat, pH meter, beakers, volumetric flasks with stoppers, funnel, stirring rod, laboratory stand, burette holder, burette, magnetic stirrer, stir bar, pipettes, pipetting bulb, permanent marker, paper towel

1.5 Procedure

1.5.1 Preparing of solutions

1. Calculate the quantities of substances needed to prepare the solutions below.
2. Prepare:
    a. 0,1 mol.dm^{-3} solution of potassium hydrogen phthalate
    b. 0,1 mol.dm^{-3} solution of sodium hydroxide

1.5.2 Working with the pH meter

1. Read the pH meter manual.
2. Standardize the pH meter with glass electrode using two standard buffers, as described in the instrument manual.

1.5.3 Standardisation of the sodium hydroxide volumetric solution

Note: Solutions of alkali hydroxides absorb carbon dioxide when exposed to air. Therefore, they should be stored in bottles with suitable non-glass, well-fitting stoppers and equipped with a tube filled with soda lime.

1. Pipet 20,0 ml of the potassium hydrogen phthalate standard solution into a 250 ml titration flask and dilute with deionized water to 100 ml. Add 5-10 drops of phenolphthalein.

2. Titrate with the sodium hydroxide solution (0,1 mol.dm^{-3}) until a pinkish violet color persists. Record the volume of titrant used.
3. Perform the required number of parallel determinations.
4. Calculate the exact concentration of the sodium hydroxide solution.

1.5.4 Determination of boric acid in boric water

1.5.4.1 Potentiometric titration

1. Prepare the apparatus for the pH metric titration according to the instructions for the pH meter provided and set up the pH meter.
2. Pipette 10,0 cm^3 of the stock solution you prepared in step 1 into the taller beaker, add 20 cm^3 of 20% glycerol and enough demineralised water so that the electrode(s) of the apparatus is immersed in the solution to be measured, according to the instructions. Place the beaker on the magnetic stirrer and fix the burette containing the sodium hydroxide volumetric solution over the sample.
3. Gradually add a known volume (e.g. 0,5 cm^3) of sodium hydroxide to the sample with constant stirring. After each addition, measure the pH of the reaction mixture. Record the volume and pH data in a table after the apparatus has settled. Continue the titration in this way until the entire titration curve has been measured (or up to twice the volume of consumption obtained in step 2).
4. Perform the required number of parallel determinations.
5. Construct the titration curve by plotting pH versus the volume of sodium hydroxide. Determine the equivalent titration volume from the titration curve.
6. Calculate the mass and mass fraction of boric acid in the boracic water.

1.6 Safety information

Chemical	Safety information
Glycerol	Not a hazardous substance or mixture according to Regulation (EC) No 1272/2008. Web: https://www.merckmillipore.com/GB/en/product/Glycerol,MDA_CHEM-137028
	CAS #: 56-81-5 EC Number: 200-289-5 Molar Mass: 92.09 g/mol Chemical Formula: $(HOCH_2)_2CHOH$ Hill Formula: $C_3H_8O_3$ Grade: Ph Eur,BP,JP,USP,ACS
Oxalic acid dihydrate	Hazard Statement(s) H302 + H312: Harmful if swallowed or in contact with skin. H318: Causes serious eye damage. Precautionary Statement(s) P264: Wash skin thoroughly after handling. P270: Do not eat, drink or smoke when using this product. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. P301 + P312: IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. P302 + P352 + P312: IF ON SKIN: Wash with plenty of water. Call a POISON CENTER/doctor if you feel unwell. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

<table>
<tr><th>Chemical</th><th>Safety information</th></tr>
<tr><td></td><td>Web: https://www.merckmillipore.com/GB/en/product/Oxalic-acid-dihydrate,MDA_CHEM-100489</td></tr>
<tr><td></td><td>CAS #: 6153-56-6 EC Number: 205-634-3 Molar Mass: 126.07 g/mol Chemical Formula: $(COOH)_2 * 2 H_2O$ Hill Formula: $C_2H_2O_4 * 2 H_2O$</td></tr>
<tr><td>Phenolphthalein</td><td>Phenolphthalein solution 1% in ethanol
Hazard Statement(s)
H350: May cause cancer.
H226: Flammable liquid and vapour.
H319: Causes serious eye irritation.
H341: Suspected of causing genetic defects.
Precautionary Statement(s)
P201: Obtain special instructions before use.
P210: Keep away from heat.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P308 + P313: IF exposed or concerned: Get medical advice/ attention.
Web: http://www.merckmillipore.com/GB/en/product/Phenolphthalein-solution-1%25-in-ethanol,MDA_CHEM-107227

Phenolphthalein indicator ACS,Reag. Ph Eur.
Hazard Statement(s)
H350: May cause cancer.
H341: Suspected of causing genetic defects.
H361f: Suspected of damaging fertility.
Precautionary Statement(s)
P201: Obtain special instructions before use.
P260: Do not breathe dust.
P308 + P313: IF exposed or concerned: Get medical advice/ attention.
Web: http://www.merckmillipore.com/GB/en/product/Phenolphthalein,MDA_CHEM-107233</td></tr>
<tr><td></td><td><table><tr><th>CAS #</th><th>EC Number</th><th>Hill Formula</th><th>Molar Mass</th><th>Grade Value</th></tr><tr><td>77-09-8</td><td>201-004-7</td><td>$C_{20}H_{14}O_4$</td><td>318.32 g/mol</td><td>ACS,Reag. Ph Eur</td></tr></table></td></tr>
<tr><td>Potassium hydrogen phthalate</td><td>Not a hazardous substance or mixture according to Regulation (EC) No 1272/2008.
Web: http://www.merckmillipore.com/GB/en/product/Potassium-hydrogen-phthalate,MDA_CHEM-102400</td></tr>
<tr><td></td><td><table><tr><th>CAS #</th><th>EC Number</th><th>Hill Formula</th><th>Molar Mass</th><th>Grade Value</th></tr><tr><td>877-24-7</td><td>212-889-4</td><td>$C_8H_5KO_4$</td><td>204.22 g/mol</td><td>Reag. Ph Eur,Reag. USP</td></tr></table></td></tr>
<tr><td>Sodium hydroxide</td><td>Hazard Statement(s)
H290: May be corrosive to metals.
H314: Causes severe skin burns and eye damage.
Precautionary Statement(s)
P234: Keep only in original packaging.
P260: Do not breathe dust.
P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.
P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.
P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.</td></tr>
</table>

Chemical	Safety information
	Web: https://www.merckmillipore.com/GB/en/product/Sodium-hydroxide,MDA_CHEM-106469
	CAS #: 1310-73-2 EC Number: 215-185-5 Molar Mass: 40 g/mol Chemical Formula: NaOH Hill Formula: HNaO
Sodium hydroxide solution 0,1 mol/L	Hazard Statement(s) H290: May be corrosive to metals. H318: Causes serious eye damage. Precautionary Statement(s) P234: Keep only in original packaging. P280: Wear eye protection/ face protection. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P390: Absorb spillage to prevent material damage. Web: https://www.merckmillipore.com/GB/en/product/Sodium-hydroxide-solution-01-mol-L,MDA_CHEM-137058

1.7 References

1. *SLOVENSKÁ KOMISIA CHEMICKEJ OLYMPIÁDY CHEMICKÁ OLYMPIÁDA Domáce kolo ÚLOHY Z PRAXE*, 2022. Online. [Accessed 9 August 2024]. Available from: https://www.iuventa.sk/wp-content/uploads/2022/10/CH59dkEFprul23.pdf
2. Merck | Life Science | Industrial & Lab Chemicals | eShop, 2023. *Merckmillipore.com*. Online. [Accessed 8 August 2024]. Available from: https://www.merckmillipore.com/SK/sk

WORKSHEET

Determination of boric acid in boracic water by potentiometric titration

Version: visual standardization of sodium hydroxide solution against potassium hydrogen phthalate

Title	**Determination of boric acid in boracic water by potentiometric titration**
Category	Food analysis Electrochemical methods, Potentiometry
Sending organisation	
Student	
Group of students	
Accompanying person	
Hosting organisation	Stredná odborná škola chemická, Vlčie hrdlo 50, 821 07 Bratislava
Author	Judita Dömötörová
Revised by	Judita Dömötörová
Instructor	
Date	
Save date	28/09/2025 19:50:00
Print date	28/09/2025 19:50
Notes	

1.1 Calculations

1. Read the procedure. Consider the quantities of solutions needed for individual and group work. From an environmental perspective, prepare only the volumes of solutions you will consume, including an adequate reserve (for repeating titrations, rinsing pipettes, burettes, etc.).
2. Consult the teacher about the planned quantities of substances to determine if the solutions will be used for further analyses.

TABLE 1 THE VOLUMES OF SOLUTIONS AND REAGENTS

Solution	Individual work		Group work	
	Amount*	Notes	Amount*	Notes
Potassium hydrogen phthalate	100 ml	3 x 10 ml = 30 ml		
Sodium hydroxide	200 ml	Standardization: 3 x 20 ml = 60 ml		

Solution	Individual work		Group work	
	Amount*	Notes	Amount*	Notes
		Determination: 3 x 10 ml = 30 ml		
Glycerol	100 g	2 x 20 ml = 60 ml		
Boric acid	10 g			

* Volume of solution to be prepared covers also a "reserve". Take into consideration the following volumetric flasks – 25 ml, 50 ml, 100 ml, 200 ml, 250 ml, 500 ml, 1000 ml, and 2000 ml.

3. Prepare 100 cm^3 potassium hydrogen phthalate solution with a concentration of 0,1 $mol.dm^{-3}$. Calculate the mass of potassium hydrogen phthalate needed.
   M(KHP) = ... $g.mol^{-1}$
   $m(KHP) = cVM$

4. Prepare 200 cm^3 of sodium hydroxide solution with a concentration of 0,1 $mol.dm^{-3}$. Calculate the mass of sodium hydroxide needed. Plan the amount of the solution according to the teacher's instructions, as you may use this solution for other analyses.
   M(NaOH) = ... $g.mol^{-1}$
   $m(NaOH) = cVM$

5. Prepare 100 g of a 10% (w/w) glycerol solution. Calculate the mass of glycerol and the volume of water needed.
   $m(glycerol) = m(solution)w(glycerol)$
   $m(H_2O) = m(solution) - m(glycerol)$
   $V(H_2O) = \frac{m(H_2O)}{\rho(H_2O)}$

1.2 Data

1. Fill in the information about boracic water.

TABLE 2 SAMPLE INFORMATION

Information	Description
Name	
Producer	
Chemical composition	
Boracic acid content	

2. Record the mass of the potassium hydrogen phthalate that was weighted.
   m(KHP) = ... g

3. Record the volume of the sodium hydroxide solution used for standardizing the sodium hydroxide solution by visual titration in the table.

TABLE 3 VOLUME OF SODIUM HYDROXIDE SOLUTION USED FOR STANDARDIZING (VISUAL TITRATION)

Trial	V(NaOH) (ml)
1	
2	
3	
Average	

4. Record the mass of boric water sample.
m(sample) = …… g

5. Record the pH and volume of the sodium hydroxide solution used for the determination of boric acid in the table.

TABLE 4 THE VOLUME AND PH OF THE SODIUM HYDROXIDE SOLUTION IN THE DETERMINATION OF BORIC ACID

Determination N°1			**Determination N°2**			**Determination N°3**		
N°	**V(NaOH) (ml)**	**pH**	**N°**	**V(NaOH) (ml)**	**pH**	**N°**	**V(NaOH) (ml)**	**pH**
1	0,0		1	0,0		1	0,0	
2	0,5		2	0,5		2	0,5	
3	1,0		3	1,0		3	1,0	
4	1,5		4	1,5		4	1,5	
5	2,0		5	2,0		5	2,0	
6	2,5		6	2,5		6	2,5	
7	3,0		7	3,0		7	3,0	
8	3,5		8	3,5		8	3,5	
9	4,0		9	4,0		9	4,0	
10	4,5		10	4,5		10	4,5	
11	5,0		11	5,0		11	5,0	
12	5,5		12	5,5		12	5,5	
13	6,0		13	6,0		13	6,0	
14	6,5		14	6,5		14	6,5	
15	7,0		15	7,0		15	7,0	
16	7,5		16	7,5		16	7,5	
17	8,0		17	8,0		17	8,0	
18	8,5		18	8,5		18	8,5	
19	9,0		19	9,0		19	9,0	

Determination N°1			Determination N°2			Determination N°3		
N°	V(NaOH) (ml)	pH	N°	V(NaOH) (ml)	pH	N°	V(NaOH) (ml)	pH
20	9,5		20	9,5		20	9,5	
21	10,0		21	10,0		21	10,0	
22	10,5		22	10,5		22	10,5	
23	11,0		23	11,0		23	11,0	
24	11,5		24	11,5		24	11,5	
25	12,0		25	12,0		25	12,0	
26	12,5		26	12,5		26	12,5	
27	13,0		27	13,0		27	13,0	
28			28			28		
29			29			29		
30			30			30		

1.3 Results

1.3.1 Standardization of sodium hydroxide solution

1. Calculate the exact concentration of potassium hydrogen phthalate using mass of KHP you have weighted.
   $c(\mathrm{KHP}) = \frac{m(\mathrm{KHP})}{V(\mathrm{KHP})M(\mathrm{KHP})}$

2. Calculate the exact concentration of the sodium hydroxide solution.
   Titration reaction equation: $C_6H_4.COOK.COOH + NaOH \rightarrow C_6H_4.COOK.COONa + H_2O$
   c(KHP) = ... mol.dm^{-3}, V(KHP) = ... cm^3
   V(NaOH) = ... cm^3

   Moles of potassium hydrogen phthalate: $n(\mathrm{KHP}) = c(\mathrm{KHP})V(\mathrm{KHP})$

   Moles of sodium hydroxide: $n(NaOH) = n(\mathrm{KHP})$

   Concentration of sodium hydroxide: $c(NaOH) = \frac{n(NaOH)}{V(NaOH)}$

   To check your result obtained by step-by-step calculation, you can use the following formula:
   $c_{NaOH} = \frac{c_{KHP}V_{KHP}}{V_{NaOH}}$
   where
   c(NaOH) is the concentration of sodium hydroxide, in mol.dm^{-3}
   V(NaOH) is the volume of sodium hydroxide, in dm^3
   c(KHP) is the concentration of potassium hydrogen phthalate, in mol.dm^{-3}
   V(KHP) is the volume of potassium hydrogen phthalate, in dm^3

1.3.2 Determination of boric acid

1. Calculate $\frac{\Delta pH}{\Delta V}$ and $\frac{\Delta^2 pH}{\Delta V^2}$ for each pair of data points when determining the boracic acid. Perform the calculations in Microsoft Excel.

$\Delta V = V_n - V_{n-1}$ $\Delta pH = pH_n - pH_{n-1}$ $\Delta V^2 = V^2{}_{n+1} - V^2{}_n$ $\Delta^2 pH = \Delta pH_{n+1} - \Delta pH_n$	$\Delta V = (V_2 - V_1)$ $\Delta pH = (pH_2 - pH_1)$ $\Delta V^2 = (\Delta V_2 - \Delta V_1)$ $\Delta^2 pH = (\Delta pH_2 - \Delta pH_1)$

N°	V(NaOH) (ml)	pH	ΔV (ml)	ΔpH	$\frac{\Delta pH}{\Delta V}$	ΔV^2	$\Delta^2 pH$	$\frac{\Delta^2 pH}{\Delta V^2}$
1								
…								

2. Plot the following graphs for each measurement when determining the boracic acid:
   a. pH vs. volume of sodium hydroxide, pH = f(V(NaOH))
   b. first derivative vs. volume of sodium hydroxide, $\frac{\Delta pH}{\Delta V}$ = f(V(NaOH))
   c. second derivative vs. volume of sodium hydroxide, $\frac{\Delta^2 PH}{\Delta V^2}$ = f(V(NaOH))

3. Explain the shape of titration curve.
4. Find the maximum of the first derivative and read the corresponding volume.
5. Calculate the volume of sodium hydroxide at the equivalence point from the second derivative.

$$V_x = V^+ + \Delta V \frac{\Delta^2 pH^+}{\Delta^2 pH^+ + |\Delta^2 pH^-|}$$

V_x – volume of the titrant corresponding to the inflection point
V^+ – volume at which the second difference of pH is positive for the last time
ΔV – constant addition of titrant in the vicinity of the inflection point
$\Delta^2 pH^+$ – value of the last positive difference of pH
$\Delta^2 pH^-$ – value of the first negative difference of pH

6. Calculate the mass fraction of boric acid in boric water.
   Reaction equations:
   $H_3BO_3 + 2\ C_3H_8O_3 \rightarrow H[X]^- + H_3O^+ + 2\ H_2O$
   $H[X] + NaOH \rightarrow Na[X] + H_2O$

   Moles of sodium hydroxide:
   $n(NaOH) = c(NaOH)V(NaOH)$
   Moles of boric acid:
   $$\frac{n(H_3BO_3)}{n(NaOH)} = \frac{1}{1} \Longrightarrow n(H_3BO_3) = n(NaOH)$$
   Mass of boric acid in 20 ml of titrated solution and in 100 ml of stock solution:
   $$m(H_3BO_3, 20\ ml) = n(H_3BO_3)M(H_3BO_3)$$

$$D_f = \frac{V(stock\ solution)}{V(titrated)} = \frac{100\ ml}{20\ ml} = 5$$

$$\mathrm{m}(H_3BO_3, 100\ ml) = \mathrm{m}(H_3BO_3, 20\ ml)D_f$$

Mass concentration of boric acid:

$M(H_3BO_3)$ = ... $g.mol^{-1}$

$$c_m(H_3BO_3) = \frac{m(H_3BO_3)}{V(H_3BO_3,\ stock\ solution)}$$

$c_m(H_3BO_3)$ = ... $g.dm^{-3}$ = ... g/100 ml

Mass fraction of boric acid:

$$w(H_3BO_3) = \frac{m(H_3BO_3)}{m(sample)}$$

Derived formula for calculating the mass fraction of boric acid in one step:

$$w(H_3BO_3) = \frac{c(NaOH)V(NaOH)M(H_3BO_3)D_f}{m(sample)}$$

7. Calculate the average concentration of boric acid from the three measurements.

$$w(H_3BO_3) = \frac{w_1(H_3BO_3) + w_2(H_3BO_3) + w_3(H_3BO_3)}{3}$$

Tasks

1. Write the sample calculation for determination N°1 on the workseet.
2. Perform calculations for determination N°1 to N°3 in MS Excel.
3. Calculate the average mass fraction of boric acid.

TABLE 5 AVERAGE MASS FRACTION OF BORIC ACID OF THE THREE MEASUREMENTS

N°	**$w(H_3BO_3)$ (%)**
1	
2	
3	
Average	

1.4 Conclusion

.....

.....

.....

1.5 Questions

Pharmaceutical chemistry

1. Describe the uses of boric water.
2. Find the toxicological properties of boric acid. Include a bibliographic reference for each source of information used. You can use free bibliography and citation generator CitacePro (STN ISO 690 style), https://www.citacepro.com or MyBib (ISO-690 style) https://www.mybib.com.
3. Look up information about Pharmacodynamics and Mechanism of action of boric acid in the DRUGBANK online database.

Analytical chemistry

1. Characterise the principle of potentiometry.
2. Characterize the principle of potentiometric titration.
3. Define pH.
4. Describe the parts of a glass electrode.
5. Describe the measurement using a pH meter.
6. Describe the principle of standardizing a measured solution of sodium hydroxide by visual titration.
7. Describe the principle of determining boric acid by potentiometric titration.
8. In your final evaluation, state the errors that affected the result you obtained.

Applied informatics

1. The Zincoren Eye/Ear Drops contain boric acid (1.9% w/v), sodium chloride (1% w/v), sulphacetamide (12% w/v) and zinc sulfate (0.1% w/v). Draw the structure of sulphacetamide in the ChemSketch program.
2. Sulfacetamide is a sulfonamide used to treat inflammatory ocular conditions and acne vulgaris. To find the properties and structure of a sulfacetamide you can use the ChemSpider or PubChem database.
   ChemSpider: https://www.chemspider.com/
   PubChem: https://pubchem.ncbi.nlm.nih.gov/
   a. Find the IUPAC name, formula and CAS number of sulfacetamide.
   b. Find the melting point of sulfacetamide.
   c. Download the structure of sulfacetamide in .mol format.
   d. Open the mol file in the ChemSketch program.
3. Below is the Abstract of a scientific paper.
   a. Search for the title, authors and journal the journal in which the paper was published.
   b. How can you access the full-text of this paper?
   c. Insert a bibliographic link to this paper. You can use free bibliography and citation generator CitacePro (STN ISO 690 style), https://www.citacepro.com or MyBib (ISO-690 style) https://www.mybib.com.

   Problem statement: Boric acid is a pesticide usually used to kill mites, fungi, plants and insect including fleas, termites, cockroaches and wood decay fungi. Besides, it was also used in many fields such as food preservative, in newborn baby's nurseries and

antiseptic. Many reports indicated that boric acid poisoning occurred due to the misuse of household product and illegal use of boric acid in food product. In this study, the concern issue was the usage of boric acid that may lead to boric acid poisoning.

Approach: This review had shown some information for boric acid such as its usage, the existent method for detection of boric acid in food. Besides, this review also discussed about the toxicology and pharmacokinetic of boric acid and the health impact of boric acid on human and animal.

Result: Previous studies showed that food products such as yellow noodles contain boric acid. The boric acid level in most foods was different among the factory and the production period. It is due to the lack of standard measurement during the processing.

Conclusion: Since boric acid was harmful to human health and may cause poisoning, hence, the control and the awareness of the usage of boric acid especially in food should be increased. There are numerous methods available for quantification of boric acid such as mannitol titration technique, colorimetric method. Accordingly, the analysis of boric acid is essential.

4. In the article “A fatal case of acute boric acid poisoning” a case of a 77-year-old man who ingested boric acid by mistake is described. What analytical method was used to determine boric acid?
5. Look up information about the pH meter you worked with today. Include a bibliographic reference for each source of information used. You can use free bibliography and citation generator CitacePro (STN ISO 690 style), https://www.citacepro.com or MyBib (ISO-690 style) https://www.mybib.com.
    a) Measurement range: pH value, temperature
    b) The instrument manual in English
    c) The price of the device at the seller in your country in EUR and in the currency of your country.
6. What functions did you use in your spreadsheet program to find the minimum of first derivative and find the related volume of titrant?

Laboratory manual

Determination of ethanol by refractometry

Organisation	Stredná odborná škola chemická, Vlčie hrdlo 50, 821 07 Bratislava
Author	Nils Stamberg, Vyšší odborná škola zdravotnická a Střední zdravotnická škola, Alšovo nábř. 6, 110 00 Praha (Czech version) Judita Dömötörová, Stredná odborná škola chemická, Vlčie hrdlo 50, Bratislava
Revised by	Judita Dömötörová
Save date	28/09/2025 19:50:00
Print date	28/09/2025 19:50:00

Introduction

Ethanol

Ethanol and isopropranol are found as an **active ingredient in oral, parenteral, and topical (including inhalational) prescription and nonprescription drug products**. Although it is primarily used because of its solvent properties to help solubilize many drugs, it also possesses several concentration-dependent pharmacological actions, including **sedative**, **carminative**, **cooling**, **antipyretic**, **rubefacient**, **cleansing**, and **antiseptic properties**. Concentrations of 40% or more may be found in some oral preparations, thus resulting in patients consuming a significant amount of alcohol during the course of the day. Various topical preparations may also result in appreciable amounts of alcohol absorption, particularly when the preparations are applied to large body surface areas; mucous membranes; hairy, inflamed, or denuded areas; or when used in conjunction with occlusive dressings, all factors which may enhance percutaneous absorption.

Refractometry

Refractometry is a method for measuring the **refractive index**, which is one of the characteristics of a substance. The refractive index allows for the determination of other characteristics with significant practical applications, such as **the density of the medium**, **the content of impurities**, **the concentration of the solution**, and **the quality**.

The method is based on the phenomenon of light refraction at the boundary between two media where light propagates at different rates. The principle of the device involves **measuring the critical angle (the angle of total internal reflection)** and calculating the refractive index using Snell's law.

Refractive index *n* is a physical quantity that that measures the optical density of a medium. **As the optical density of the medium increases, the speed of light decreases and the refractive index increases.** If the substance is a pure chemical compound, the refractive index is a physical constant (under constant conditions).

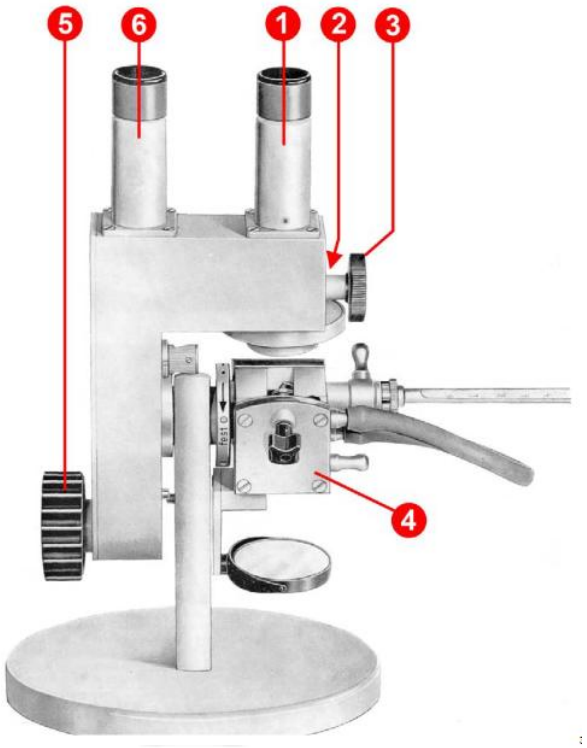
$n_{1,2} = \frac{v_1}{v_2} = \frac{\sin \alpha}{\sin \beta}$	$n_{1,2}$ – relative refractive index [1] α – angle of incidence [°] β – angle of refraction [°]

	v_1, v_2 – speed of light in the 1st and 2nd optical environments [m s^{-1}]

In refractometry, the critical angle of refraction β_m is determined. The critical angle β is the angle under which the light incident on the optical interface is refracted at the maximum angle α_{max} = 90°.

$$n = \frac{\sin \alpha_{max}}{\sin \beta} = \frac{\sin 90}{\sin \beta} = \frac{1}{\sin \beta}$$

The refractive index depends on the **optical environment** (character of the substance), the **wavelength** of the incident light (as the speed of light depends on the wavelength), **temperature** (it decreases with increasing temperature), **density**, and **concentration of the substance**.
The tables show the refractive index for a given temperature (20 °C) and wavelength (the wavelength of the D line of the sodium emission spectrum, λ = 589.3 nm, the sodium doublet). This refractive index is referred to as $\boldsymbol{n_D^{20}}$.



1 focusing telescope,
2 colour compensator with graduated circle,
3 setting knob for colour compensator,
4 standard prism body,
5 setting knob for prism and graduated circle turn,
6 reading microscope

Fig. 1. Abbe Refraktometer with standard prism body

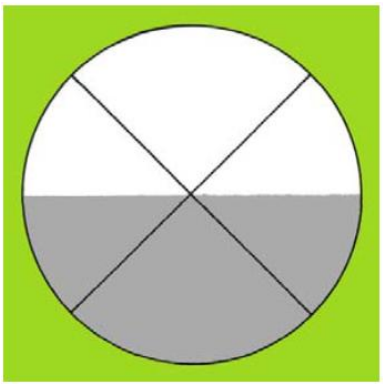


Fig. 2. Field of vision in the focusing telescope

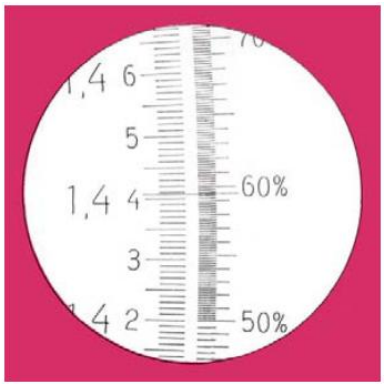


Fig. 3. Field of vision in the reading microscope

Measuring the refractive index is a simple and useful method for determining the concentration of substances and their characteristics. It is often used in research and industrial laboratories for quality control and product development. Additionally, it is employed in the study of pharmaceuticals, food analysis (including quality control of honey), assessment of perfumes and fragrances, and online monitoring of food, beverages, and medicines.

Assignment

1. Calculate the volume of ethanol needed to prepare the calibration solutions.
2. Familiarise yourself with the hazardous properties of the substances you will be working with.
3. Prepare the necessary solutions.
4. Determine the percentage of ethanol using refractometry.
5. Complete the worksheet.
6. Answer the questions on the worksheet.

Chemicals

Ethanol (anhydrous), demineralised water

Equipment

Volumetric flask, pipette, beaker, water bottle, permanent marker, paper towel, refractometer, disposable pipette

Procedure

1. Prepare a set of ethanol calibration solutions:
    a. Decide whether you will construct a polynomial calibration curve or work only in the low volume fraction region and then linearize the calibration curve. Accordingly, prepare a set of ethanol calibration solutions in 10 ml volumetric flasks:
        i. For a **polynomial calibration curve**: concentrations of 0 vol. % (demineralised water only), 20 %, 40 %, 60 %, 80 % and 100 % (anhydrous ethanol only)

ii. For a **linear calibration**: concentrations of 0 vol. % (demineralised water only), 10 %, 20 %, 30 % and 40 %.

2. Measure the refractive index of the calibration solutions:
   a. Open the prism chamber and clean both parts of the prism with cellulose cotton wool moistened with demineralised water, then wipe dry. Drip the measuring solution onto the horizontal part of the prism and close the prism chamber (excess liquid will flow out through the side groove, if necessary).
   b. Slowly turn the screw of the instrument and observe the thread-like cross, centering the light interface on it.

Fig. 3. Field of vision in the focusing telescope: a), c) incorrect, b) correct

   c. Repeat the measurement of refractive index at least twice and use the mean value. Start with the least concentrated solutions and proceed to the most concentrated.
3. Construct a calibration curve (i.e., the dependence of the refractive index of the solution on the volume fraction of ethanol) using MS Excel from the measured values.
4. Measure the refractive index of the sample. Repeat the measurement at least twice and use the mean value.
   a. If you are using a polynomial calibration dependence, measure the refractive index of the sample directly. If two different values of ethanol volume fraction could correspond to the measured refractive index (refer to the calibration curve), perform the measurement again with the sample diluted 1:1 with demineralized water. Depending on whether the refractive index value decreases or increases after dilution, determine which part of the calibration curve should be used for evaluation.
   b. If you are using a linear calibration curve, dilute the sample 2 to 4 times with demineralized water before measuring (so that the measured refractive index is approximately in the middle of the calibration line). Multiply the result by the appropriate dilution factor.
5. Determine the ethanol content of the sample:
   a. Read the percentage of ethanol from the polynomial calibration dependence.
   b. Calculate percentage of ethanol from the linear trend line.
6. Express the result as the volume percentage (V/V) of ethanol in the sample.

Safety Information

Chemical	Safety Information
Ethanol	Hazard Statement(s) H225: Highly flammable liquid and vapour.

Chemical	Safety Information
	H319: Causes serious eye irritation. Precautionary Statement(s) P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233: Keep container tightly closed. P240: Ground and bond container and receiving equipment. P241: Use explosion-proof electrical/ ventilating/ lighting equipment. P242: Use non-sparking tools. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P403 + P233: Store in a well-ventilated place. Keep container tightly closed. Web: https://www.merckmillipore.com/GB/en/product/Ethanol,MDA_CHEM-818760
	CAS #: 64-17-5 EC Number: 200-578-6 Molar Mass: 46.07 g/mol Chemical Formula: C_2H_5OH Hill Formula: C_2H_6O

References

1. *Abbe Refractometer*, [no date]. Online. [Accessed 8 August 2024]. Available from: https://www.mikroskop-online.de/Mikroskop%20BDA%20Gruppe%2032/32-110f-2%20%20Abbe%20Refractometer.pdf
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3. Merck | Life Science | Industrial & Lab Chemicals | eShop, 2023. *Merckmillipore.com*. Online. [Accessed 8 August 2024]. Available from: https://www.merckmillipore.com/SK/sk

Worksheet

Determination of ethanol by refractometry

Title	**Determination of ethanol by refractometry**
Category	Pharmaceutical analysis Optical methods, refractometry
Sending organisation	
Student	
Group of students	
Accompanying person	
Hosting organisation	Stredná odborná škola chemická, Vlčie hrdlo 50, 821 07 Bratislava
Author	Judita Dömötörová, Stredná odborná škola chemická, Vlčie hrdlo 50, Bratislava
Revised by	Judita Dömötörová, Stredná odborná škola chemická, Vlčie hrdlo 50, Bratislava
Instructor	
Date	
Save date	28/09/2025 19:50:00
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Notes	

Chemical calculations

1. Prepare a series of 10 ml of ethanol calibration solutions with volume fractions according to the table below by diluting 100% ethanol.

   Calculation for $\varphi(etOH) = 20\%$:

   $$\varphi(etOH) = \frac{V(etOH)}{V(solution)}$$

   $$V(etOH) = \varphi(etOH)\, V(solution)$$

TABLE 1 VOLUME OF 100% ETHANOL NEEDED TO PREPARE CALIBRATION SOLUTIONS

Cal. solution N°	ϕ(etOH) (%)	V(etOH) (ml)
1	0	
2	10	
3	20	
4	30	
5	40	

2. Prepare 10 ml of diluted sample solution according to the table below.

Dilution ratio refers to a simple dilution, in which a unit volume of a solute is combined with a desired volume of solvent. For example, in a solution with a 1:5 dilution ratio, entails combining 1 unit volume of solute (the material to be diluted) with 5 unit volumes of the solvent to give 6 total units of total volume.
Dilution factor (F) on the other hand refers to the ratio of the volume of the initial concentrated solute to the total volume of the final diluted solution.

$$F = \frac{V(final\ solution)}{V(stock\ solution)}$$

Example for preparation of 10 ml diluted sample with dilution factor 1:4:
Dilution ratio = 1:4
Total volume: 1 unit of sample + 4 units of solute = 5 total units
Dilution factor: $F = \frac{V(final\ solution)}{V(stock\ solution)} = \frac{5\ units}{1\ unit} = 5$
Volume of sample stock solution needed to prepare 10 ml if diluted sample:
$$V(stock\ solution) = \frac{V(final\ solution)}{F} = \frac{10\ ml}{5} = 2$$

Preparation of 10 ml diluted sample with dilution factor 1:1:
Total volume: 1 unit of sample + 1 units of solute = 2 total units
Dilution factor: $F = \frac{V(final\ solution)}{V(stock\ solution)}$
Volume of sample stock solution needed to prepare 10 ml if diluted sample:
$$V(stock\ solution) = \frac{V(final\ solution)}{F}$$

TABLE 2 DILUTION OF SAMPLE

Sample N°	Dilution ratio	Dilution factor	V(stock solution) (ml)
1	1:1		

Data

1. Record the refractive indices of the ethanol calibration solutions. Calculate the mean refractive index.

TABLE 3 REFRACTIVE INDEX OF CALIBRATION SOLUTIONS

Cal. solution N°	ϕ(etOH) (%)	n_D^{20}(1)	n_D^{20}(2)	n_D^{20}(mean)
1	0			
2	10			
3	20			
4	30			
5	40			

2. Record the refractive index of the sample and the dilution factor. Calculate the mean refractive index.

TABLE 4 REFRACTIVE INDEX OF SAMPLE

Sample N°	n_D^{20}(1)	n_D^{20}(2)	n_D^{20}(mean)	F
1				

Results

1. Plot the graph of the refractive index vs. the volume fraction of ethanol, n_D^{20} = f(ϕ(etOH)).
   a. Add a chart title and axis titles.
   b. Insert a trend line (linear).
   c. Display the equation and the R-squared value on the chart.
   d. Insert the graph into this document.

Insert the graph here.

GRAPH 1 CALIBRATION CURVE

2. Calculate parameters *a*, *b* of a regression line using functions in MS Excel.

   Microsoft Excel has three built-in functions that allow determining the slope, y-intercept, correlation coefficient, and R-squared values of a set of data. The functions are SLOPE(), INTERCEPT(), CORREL() and RSQ().
   The syntax for each are as follows:
   Slope, a: = SLOPE(known_y's, known_x's)
   y-intercept, b: = INTERCEPT(known_y's, known_x's)
   Correlation Coefficient, r: = CORREL(known_y's, known_x's)
   R-squared, r^2: = RSQ(known_y's, known_x's)

   TABLE 5 LINEAR REGRESSION

a	b	R^2

3. Calculate the volume fraction of ethanol in the sample.

   Trendline equation: $y = ax + b$
   Equation applied to determination: $n_D^{20} = a\varphi + b$
   Volume fraction of ethanol in sample: $\varphi = \frac{n_D^{20} - b}{a}$

   Calculation of the volume fraction of ethanol in diluted sample: $\varphi = \frac{n_D^{20} - b}{a}$

   Calculation of the volume fraction of ethanol in original sample: $\varphi(original) = \varphi(diluted)\ F$

4. Calculate the absolute and relative error.

   X – true value, x – measured value
   Absolute error: $\varepsilon = |x - X|$
   Relative error: $\varepsilon_r = \frac{|x-X|}{X}$

TABLE 6 ABSOLUTE AND RELATIVE ERROR

X	
x	
ε	
ε_r	
ε_r (%)	

Conclusion

.....

.....

.....

.....

Questions

Pharmaceutical chemistry

1. What are the main reasons for using ethanol as a solvent in pharmaceutical formulations, and how does it affect the solubility and stability of active ingredients?
2. Discuss the potential health risks and benefits of using ethanol in pharmaceutical formulations. How do regulatory guidelines ensure the safe use of ethanol in medications?
3. What type of alcohol is used in rubbing alcohol, and what is its concentration in the mixture?
4. Ethanol is a widely used solvent in the pharmaceutical industry. Bioethanol, produced by the fermentation of lignocellulosic waste, has a highly favorable carbon footprint. Discuss how bioethanol helps pharmaceutical companies reduce emissions.
5. Concentrations of 40% or more may be found in some oral preparations, thus resulting in patients consuming a significant amount of alcohol during the course of the day. Various topical preparations may also result in appreciable amounts of alcohol absorption, particularly when the preparations are applied to large body surface areas; mucous membranes; hairy, inflamed, or denuded areas; or when used in conjunction with occlusive dressings, all factors which may enhance percutaneous absorption. State the consequences of ethanol abuse.

Analytical chemistry

1. Describe the principle of refractometry.
2. Define the refractive index.
3. Describe the parameters on which the refractive index of a liquid depends.
4. Explain what the "D" and "20" in the designation n_D^{20} mean.
5. List the parts of an Abbe refractometer.
6. Briefly describe the procedure for measuring the refractive index using an Abbe refractometer.
7. Describe the principle of refractometric determination of ethanol in an ethanol-water mixture.
8. Explain the significance of tempering the solution to 20°C.

9. State another simple and cost-effective method for measuring the ethanol content in ethanol-water mixtures.
10. Explain the calibration curve method.

Applied informatics

1. Suggest a source from which refractive index information can be obtained for ethanol solutions of different concentrations. Insert a bibliographic link to the source of the information. You can use free bibliography and citation generator CitacePro (STN ISO 690 style), https://www.citacepro.com or MyBib (ISO-690 style) https://www.mybib.com.
2. Look up the density of the ethanol calibration solutions you worked with at the given temperature in the tables (or another appropriate source of chemical information). Insert a bibliographic link to the source of the information.
3. Typical uses of solvents in the synthesis of an API are solubilization (reaction medium), extraction and crystallization (purification). They may also participate in reactions as reactants or catalysts and be involved in azeotropic or extractive distillations as entrainers. The most commonly used alcohols in synthesis include ethanol, butanol, 2-ethylhexanol, isobutanol, methanol, and propanol. Find the properties of 2-ethylhexanol. To find the properties and structure of 2-ethylhexanol, you can use the ChemSpider or PubChem databases.
   ChemSpider: https://www.chemspider.com/
   PubChem: https://pubchem.ncbi.nlm.nih.gov/
   a. Find the IUPAC name, formula and CAS number of 2-ethylhexanol.
   b. Find the boiling point of 2-ethylhexanol.
   c. Download the structure of 2-ethylhexanol in .mol format.
   d. Open the .mol file in the ChemSketch program.
4. Draw the structure of 2-ethylhexanol in the ChemSketch program.
5. Below is the Abstract of a scientific paper.
   a. Search for the title, authors and journal the journal in which the paper was published.
   b. How can you access the full text of this paper?
   c. Insert a bibliographic reference for this paper. You can use free bibliography and citation generator CitacePro (STN ISO 690 style), https://www.citacepro.com or MyBib (ISO-690 style) https://www.mybib.com.

   Abstract: Organic solvents are commonly used in the pharmaceutical industry as reaction media, in separation and purification of synthesis products and also for cleaning of equipment. This paper presents some aspects of organic solvents utilization in an active pharmaceutical ingredient and a drug product manufacturing process.
   As residual solvents are not desirable substances in a final product, different methods for their removal may be used, provided they fulfill safety criteria. After the drying process, analyses need to be performed to check if amounts of solvents used at any step of the production do not exceed acceptable limits (taken from ICH Guideline or from pharmacopoeias). Also new solvents like supercritical fluids or ionic liquids are developed to replace "traditional" organic solvents in the pharmaceutical production processes.

   Keywords: organic solvents, residual solvents, acceptable limits

6. Find information about the refractometer you worked with today. Insert a bibliographic reference for each source of information used. You can use free bibliography and citation generator CitacePro (STN ISO 690 style), https://www.citacepro.com or MyBib (ISO-690 style) https://www.mybib.com.
   a. Unit of measure
   b. Range
   c. Resolution
   d. Prism material
   e. Min. sample volume

f. Device manual in English
g. The price of the device at the seller in your country in EUR and in the currency of your country.

Laboratory manual

Determination of Ibuprofen in BRUFEN 400 tablet

Organisation	Stredná odborná škola chemická, Vlčie hrdlo 50, 821 07 Bratislava
Author	Judita Dömötörová
Revised by	Judita Dömötörová
Save date	28/09/2025 19:50:00
Print date	28/09/2025 19:50:00

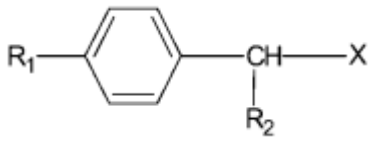
Introduction

Ibuprofen

Chemical name: **2-(4-isobutylphenyl)propanoic acid**
Molecular formula: $C_{13}H_{18}O_2$
CAS Number: 15687-27-1
Ibuprofen occurs as a **white to off-white crystalline powder**. Ibuprofen is **practically insoluble in water**. It is very **soluble in most alcohols**. Ibuprofen has a pKa of 4,4.

Ibuprofen is a white, crystalline anti-inflammatory drug used in numerous medications. It is the active ingredient marketed under various trade names including **Ibuprofen, Brufen, Dolgit, Ibalgin, Ibumax, Nurofen, Advil, Motrin**.
Ibuprofen is a **nonsterodial anti-inflammatory drug (NSAID) used as a pain reliever (analgesic), fever reducer (antipyretic) and inflammation reducer**. Inflammation is a general physiological response to tissue damage characterized by swelling, pain and heat.

Ibuprofen was developed while searching for an alternative pain reliever to aspirin in the 1950s. It and related compounds were synthesized in 1961 by Stewart Adams, John Nicholson, and Collin Burrows who were working for the Boots Pure Drug Company in Great Britain. Adams and Nicholson filed for a British patent on ibuprofen in 1962 and obtained the patent in 1964; subsequent patents were obtained in Unites States. The patent of Adams and Nicholson was for the invention of phenylalkane derivatives of the form shown in figure, where R1 could be various alkyl groups, R2 was hydrogen or methyl, and X was COOH or COOR, with R being alkyl or aminoalkyl groups.



The first clinical trials for ibuprofen were started in 1966. Ibuprofen was introduced under trade name Brufen in 1969 in Great Britain. It was introduced in the United States in 1974. Ibuprofen was initially offered by prescription, but it became available in over-the-counter medications in 1980s.

Ibuprofen works by **inhibiting the enzyme cyclooxygenase (COX), which in turn interferes with the synthesis of prostaglandins.** COX exists as several coenzyme forms that are similar in structure COX-1,

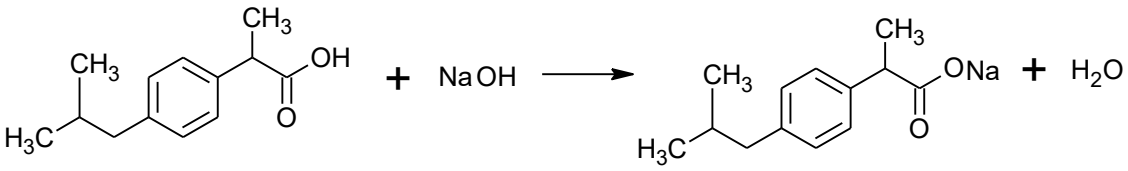
COX-2, COX-3; inhibitor is a nonselective inhibitor for both COX-1 and COX-2. COX-1 is continually produces in mammalian cells throughout the body in response to physiological stimuli. It is responsible for the production of prostaglandins, which get their name because it was originally believed they were synthesized in the prostate gland. In fact, prostaglandins are synthesized throughout the body and act like hormones by stimulating action in target cells. Prostaglandins, which are fatty acid compounds consisting of a 20-carbon chain including a 5-carbon ring, are involved in numerous physiological processes including renal function, blood clotting, and stomach mucus productions. COX-2 is synthesized only in specific parts of the body (kidneys, brain, trachea) as needed and is therefore called an induced enzyme. COX-2 produces prostaglandins in response to tissue damage and inflammation. Inflammatory prostaglandins produce swelling, pain, and fever.

A common goal in the development of pain and inflammation medicines has been the creation of compounds that have **ability to treat inflammation, fever and pain without disrupting other physical functions**. General pain relievers, such as aspirin and ibuprofen, **inhibit both COX-1 and COX-2.** A medication`s specific action toward COX-1 versus COX-2 determines the potential adverse side effects. Medications with greater specifity toward COX-1 will have greater potential for producing adverse side effects. By deactivating COX-1, nonselective pain relievers increase the chance of undesirable side effets, especially digestive problems such as stomach ulcers and gastrointestinal bleeding. COX-2 inhibitors are widely prescribed for arthritis and pain relief.

Ibuprofen is commonly used for **headaches (migraines), muscle pain, dental pain, cold, flu, fever and menstrual cramps**. Ibuprofen is most often sold **as tablets, but is also sold as liquid capsules, effervescents, chewable tablets, and liquid suspensions**. Tablets commonly contain 200 mg of ibuprofen and a 1200 mg per day limit taken over several dosage is recommended for adults. Common side effects associated with ibuprofen are **stomach pains, heartburn, constipation, headache, blurry vision, and ear-ringing**. More serious side effects often associated with high prolonged doses are stomach ulcers, gastrointestinal bleeding, and renal malfunction.

Determination of Ibuprofen

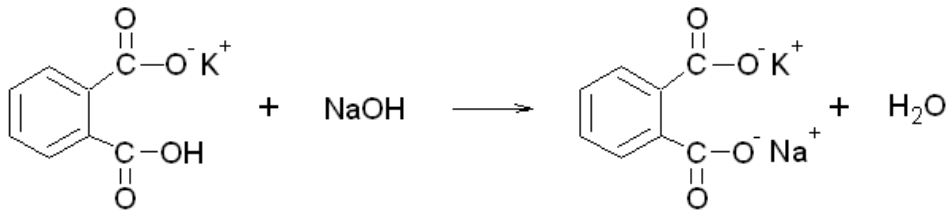
Ibuprofen dissolved in ethanol (methanol), is titrated with sodium hydroxide using phenolphthalein as the indicator.



Standardization of potassium hydroxide solution

Sodium hydroxide is titrated against potassium hydrogen phthalate (KHP) using phenolphthalein as the indicator.

Reaction equation: $C_6H_4.COOK.COOH + NaOH \rightarrow C_6H_4.COOK.COONa + H_2O$



Assignment

1. Calculate the quantities of substances needed to prepare the solutions.
2. Familiarise yourself with the hazardous properties of the substances you will be working with.
3. Prepare the solutions and reagents.
4. Record the sample information.
5. Standardize sodium hydroxide solution.
6. Determine the ibuprofen content in *Brufen 400*.
7. Complete the worksheet.
8. Answer the questions on the worksheet.

Materials

Mortar with pestle, analytical balance, weighting boat, chemical spoon, burette (25 ml), analytical funnel, titration flask (250 ml), beakers (150 ml, 2 pcs), volumetric flasks (50 ml, 100 ml), pipette (10 ml, 2 pcs), graduated cylinder (50 ml), glass marker pen, glassware labels

Chemicals

Sodium hydroxide (c = 0,1 mol.dm^{-3})
Potassium hydrogen phthalate (c = 0,1 mol.dm^{-3})
Phenolphthalein (0,1%)
Ethanol (96%)
Deionised water

Procedure

Preparing of solutions

1. Calculate the quantities of chemicals needed to prepare following solutions:
    a. 0,1 mol.dm^{-3} solution of potassium hydrogen phthalate
    b. 0,1 mol.dm^{-3} solution of sodium hydroxide
    c. 0,1% solution of phenolphthalein
2. Prepare the solutions mentioned above.

Standardization of sodium hydroxide solution

Note: Solutions of alkali hydroxides absorb carbon dioxide when exposed to air. Therefore, they should be stored in bottles with suitable non-glass, well-fitting stoppers and equipped with a tube filled with soda lime.

1. Pipet 20,0 ml of the potassium hydrogen phthalate standard solution into a 250 ml titration flask and dilute with deionized water to 100 ml. Add 5-10 drops of phenolphthalein.

2. Titrate with the sodium hydroxide solution (0,1 mol.dm^{-3}) until a pinkish violet color persists. Record the volume of titrant used.
3. Perform the required number of parallel determinations.
4. Calculate the exact concentration of the sodium hydroxide solution.

Determination of Ibalgin in BRUFEN 400 tablet

1. Weight one tablet of *Brufen 400*. Record the weight of tablet.
2. Grind the tablet to a fine powder in a mortar. It is important to prepare a homogeneous powder.
3. Accurately weight 0,2000 g of powder to the titration flask. Keep the rest of powder for parallel assay. Record the weight of powder.
4. Add 50 ml of 96% ethanol. Swirl the solution vigorously until the sample is completely dissolved.
5. Add a few drops of phenolphthalein and titrate with sodium hydroxide solution (0,1 mol.dm^{-3}) until a pinkish violet color persists. Record the volume of sodium hydroxide used.
6. Repeat assay with the rest of powder according to procedure outlined above.
7. Carry out a blank titration. Repeat the procedure from steps 1 to 5, omitting the addition of 0,2000 g of powder.
8. Calculate how many mg of ibuprofen contains one tablet of *Brufen 400*.

Safety information

Chemical	Safety Information
Ethanol	**Hazard Statement(s)** H225: Highly flammable liquid and vapour. H319: Causes serious eye irritation. **Precautionary Statement(s)** P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P240: Ground/bond container and receiving equipment. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P403 + P233: Store in a well-ventilated place. Keep container tightly closed. Web: http://www.merckmillipore.com/GB/en/product/Ethanol,MDA_CHEM-100983
Ibalgin	**Hazard Statement(s)** H302 Harmful if swallowed. Web: https://www.merckmillipore.com/SK/sk/product/-Ibuprofen-CAS-15687-27-1-Calbiochem,EMD_BIO-401003
Phenolphthalein	**Phenolphthalein solution 1% in ethanol** **Hazard Statement(s)** H350: May cause cancer. H226: Flammable liquid and vapour. H319: Causes serious eye irritation. H341: Suspected of causing genetic defects. **Precautionary Statement(s)** P201: Obtain special instructions before use. P210: Keep away from heat. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

<table>
<tr><th>Chemical</th><th>Safety Information</th></tr>
<tr><td></td><td>P308 + P313: IF exposed or concerned: Get medical advice/ attention.
Web: http://www.merckmillipore.com/GB/en/product/Phenolphthalein-solution-1%25-in-ethanol,MDA_CHEM-107227

Phenolphthalein indicator ACS,Reag. Ph Eur.
Hazard Statement(s)
H350: May cause cancer.
H341: Suspected of causing genetic defects.
H361f: Suspected of damaging fertility.
Precautionary Statement(s)
P201: Obtain special instructions before use.
P260: Do not breathe dust.
P308 + P313: IF exposed or concerned: Get medical advice/ attention.
Web: http://www.merckmillipore.com/GB/en/product/Phenolphthalein,MDA_CHEM-107233</td></tr>
<tr><td></td><td>
<table>
<tr><th>CAS #</th><th>EC Number</th><th>Hill Formula</th><th>Molar Mass</th><th>Grade Value</th></tr>
<tr><td>77-09-8</td><td>201-004-7</td><td>$C_{20}H_{14}O_4$</td><td>318.32 g/mol</td><td>ACS,Reag. Ph Eur</td></tr>
</table>
</td></tr>
<tr><td>Potassium hydrogen phthalate</td><td>Not a hazardous substance or mixture according to Regulation (EC) No 1272/2008.
Web: http://www.merckmillipore.com/GB/en/product/Potassium-hydrogen-phthalate,MDA_CHEM-102400</td></tr>
<tr><td></td><td>
<table>
<tr><th>CAS #</th><th>EC Number</th><th>Hill Formula</th><th>Molar Mass</th><th>Grade Value</th></tr>
<tr><td>877-24-7</td><td>212-889-4</td><td>$C_8H_5KO_4$</td><td>204.22 g/mol</td><td>Reag. Ph Eur,Reag. USP</td></tr>
</table>
</td></tr>
<tr><td>Sodium hydroxide</td><td>Hazard Statement(s)
H290: May be corrosive to metals.
H314: Causes severe skin burns and eye damage.
Precautionary Statement(s)
P280: Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301 + P330 + P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P308 + P310: IF exposed or concerned: immediately call a POISON CENTER or doctor/ physician.
Web: http://www.merckmillipore.com/GB/en/product/Sodium-hydroxide,MDA_CHEM-106469</td></tr>
<tr><td></td><td>
<table>
<tr><th>CAS #</th><th>EC Number</th><th>Hill Formula</th><th>Chemical Formula</th><th>Molar Mass</th><th>Grade Value</th></tr>
<tr><td>1310-73-2</td><td>215-185-5</td><td>HNaO</td><td>NaOH</td><td>40.00 g/mol</td><td>ACS,Reag. Ph Eur,ISO</td></tr>
</table>
</td></tr>
</table>

References

1. MYERS, Richard L, 2007. *The 100 Most Important Chemical Compounds: A Reference Guide*. ABC-CLIO. ISBN 9780313080579.
2. Q. ALAN XU and MADDEN, Timothy L, 2011. *Analytical Methods for Therapeutic Drug Monitoring and Toxicology*. John Wiley & Sons. ISBN 9780470922798.
3. PAVIA, Donald L, LAMPMAN, Gary M, KRIZ, George S and ENGEL, Randall G, 2018. *A microscale approach to organic laboratory techniques*. 6. Belmont, Ca: Brooks/Cole, Cengage Learning. ISBN 9781305968349.

4. BRITTAIN, Harry G, 2016. *Profiles of drug substances, excipients, and related methodology.*Elsevier Academic Press. ISBN 9780128047842.
5. BENKOVÁ, Marta , SOKOLÍK, Jozef , GRANČAI, Daniel and LEHKÝ, Milan , 2003. *Slovenský liekopis*. 1. Herba. ISBN 80-967994-7-9.
6. Merck | Life Science | Industrial & Lab Chemicals | eShop, 2023. *Merckmillipore.com*. Online. [Accessed 8 August 2024]. Available from: https://www.merckmillipore.com/SK/sk

Worksheet

Determination of Ibuprofen in BRUFEN 400 tablet

Title	**Determination of Ibuprofen in BRUFEN 400 tablet**
Category	Pharmaceutical analysis Neutralisation titration
Sending organisation	
Student	
Group of students	
Accompanying person	
Hosting organisation	Stredná odborná škola chemická, Vlčie hrdlo 50, 821 07 Bratislava
Author	Judita Dömötörová
Revised by	Judita Dömötörová
Instructor	
Date	
Save date	28/09/2025 19:50:00
Print date	28/09/2025 19:50
Notes	

Calculations

1. Read the procedure. Consider the quantities of solutions needed for individual work and for group work. From an environmental point of view, prepare only the volumes of solutions that you will consume, including an adequate reserve (to repeat titrations, rinse pipettes, burettes, etc.).
2. Consult the teacher about the planned quantities of substances for possible use of the solutions in further determinations.

TABLE 1 THE VOLUMES OF SOLUTIONS AND REAGENTS

Solution	Alternative 1 Individual work		Alternative 2 Work in groups	
	Volume*	**Notes**	**Volume***	**Notes**
$C_8H_5KO_4$	100 ml	20 ml/titration x 3 titrations		
NaOH**	200 ml	10 ml/standardization x 3 titrations 10 ml/assay x 3 titrations		
Ibalgin 400 or BRUFEN 600	1 tablet	We need 0,200 g for assay, 1 tablet weight approx. 0,500 g.		

* Volume of solution to be prepared covers also a "reserve". Take into consideration the following volumetric flasks – 25 ml, 50 ml, 100 ml, 200 ml, 250 ml, 500 ml, 1000 ml, and 2000 ml.

** Estimated consumption of NaOH solution in standardization is 10 ml, in assay it is max 10 ml.

3. Prepare 100 ml of potassium hydrogen phthalate (KHP) standard solution with a concentration of c = 0,1 mol.dm^{-3}. Calculate the mass of potassium hydrogen phthalate needed.
   M($C_8H_5KO_4$) = 204,2212 g.mol^{-1}

$m(\mathrm{KHP}) = c(\mathrm{KHP})V(\mathrm{KHP})M(\mathrm{KHP})$
$m(\mathrm{KHP}) =$ _____ g

4. Prepare 200 ml of sodium hydroxide solution with a concentration of c = 0,1 mol.dm^{-3}. Calculate the mass of sodium hydroxide need.
   M(NaOH)= 39,9971 g.mol^{-1}
   $m(\mathrm{NaOH}) = c(\mathrm{NaOH})V(\mathrm{NaOH})M(\mathrm{NaOH})$
   $m(\mathrm{NaOH}) =$ _____ g

Data

Standardisation of sodium hydroxide solution

1. Record the mass of the weighed potassium hydrogen phthalate.
   M(KHP) = ... g

2. Record the volume of sodium hydroxide used for standardisation.

TABLE 2 VOLUME OF SODIUM HYDROXIDE

Trial	V(NaOH) (ml)
1	
2	
3	
Average	

Determination of ibuprofen

1. Find the basic information about drug.

Brand name	
Manufacturer	
Dosage form	
Ingredients	
Active ingredient	
Quantity of active ingredient	

2. Write a bibliographic reference to the source of the information. You can use free bibliography and citation generator MyBib: https://www.mybib.com.

3. Record the volume of sodium hydroxide used to determine the ibuprofen content.

TABLE 3 DETERMINATION OF IBUPROFEN

Trial	m(tablet) (g)	m(powder) (g)	V(NaOH) (ml)
1			
2			

4. Record the volume of sodium hydroxide used in the blank titration.

TABLE 4 BLANK TITRATION

Trial	V(NaOH) (ml)
1	
2	
3	
Average	

Results

Standardization of sodium hydroxide solution

1. Calculate the exact concentration of potassium hydrogen phthalate using mass of KHP you have weighted.

   $c(\mathrm{KHP}) = \frac{m(\mathrm{KHP})}{V(\mathrm{KHP})M(\mathrm{KHP})}$

2. Calculate the exact concentration of the sodium hydroxide solution.
   Titration reaction equation: $C_6H_4.COOK.COOH + NaOH \rightarrow C_6H_4.COOK.COONa + H_2O$
   c(KHP) = ... mol.dm^{-3}, V(KHP) = ... cm^3
   V(NaOH) = ... cm^3

   Moles of potassium hydrogen phthalate: $n(\mathrm{KHP}) = c(\mathrm{KHP})V(\mathrm{KHP})$

   Moles of sodium hydroxide: $n(NaOH) = n(\mathrm{KHP})$

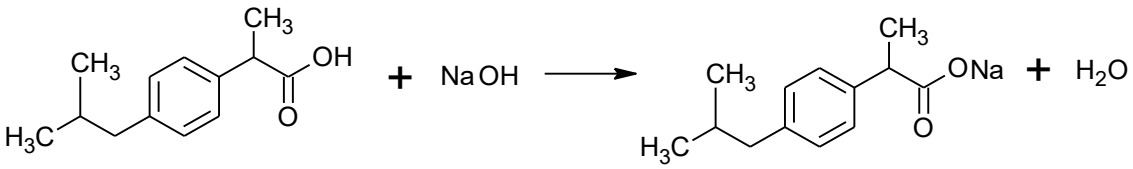
   Concentration of sodium hydroxide: $c(NaOH) = \frac{n(NaOH)}{V(NaOH)}$

   To check your result obtained by step-by-step calculation, you can use the following formula:
   $c_{NaOH} = \frac{c_{KHP}V_{KHP}}{V_{NaOH}}$

where
c(NaOH) is the concentration of sodium hydroxide, in mol.dm^{-3}
V(NaOH) is the volume of sodium hydroxide, in dm^3
c(KHP) is the concentration of potassium hydrogen phthalate, in mol.dm^{-3}
V(KHP) is the volume of potassium hydrogen phthalate, in dm^3

3. Calculate the amount of ibuprofen (in milligrams) present in the entire tablet.
Note: You have weighted approximately 0,200 g of powder into the titration flask, amount of ibuprofen must be calculated based on the entire tablet, which is approximately 500 mg.

Titration reaction equation:



Moles of sodium hydroxide: $n(NaOH) = c(NaOH)V(NaOH)$

Moles of ibuprofen: $n(ibuprofen) = n(NaOH)$

Weight of ibuprofen in the powder (in a portion of a tablet, approximately 0,2000 g):

$m(ibuprofen, portion\ of\ a\ tablet) = n(ibuprofen)M(ibuprofen)$

To calculate the weight of ibuprofen in whole tablet, apply the **rule of proportion**. Here is an example of calculation.

184,6 mg of ibuprofen	0,2000 g tablet (portion of a tablet, powder weighted to assay)
x mg of ibuprofen	0,5000 g tablet (the whole tablet)

$$m(ibuprofen, complete\ tablet) = \frac{m(complete\ tablet)}{m(part\ of\ a\ tablet)} m(ibuprofen, part\ of\ tablet)$$

$$m(ibuprofen, complete\ tablet) = \frac{0{,}5000\ g}{0{,}2000\ g} 184{,}6\ \text{g}$$

Using the actual weight of the powder $m(ibuprofen, part\ of\ tablet)$ a and the actual weight of the complete tablet $m(ibuprofen, complete\ tablet)$, , calculate the ibuprofen content for both trials.

Trial 1

..... mg of ibuprofen	..... g tablet (portion of a tablet)
x mg of ibuprofen	..... tablet (the whole tablet)

$$m(ibuprofen, the\ whole\ tablet) = \frac{m(the\ whole\ tablet)}{m(portion\ of\ a\ tablet)} m(ibuprofen, portion\ of\ tablet)$$

Trial 2

..... mg of ibuprofen	..... g tablet (portion of a tablet)
x mg of ibuprofen	..... tablet (the whole tablet)

$$m(ibuprofen, the\ whole\ tablet) = \frac{m(the\ whole\ tablet)}{m(portion\ of\ a\ tablet)} m(ibuprofen, portion\ of\ tablet)$$

To check your result obtained by step-by-step calculation, you can use the following formula:

$$m(ibuprofen, the\ whole\ tablet) = c(NaOH).V(NaOH)M(\text{ibuprofen})\frac{m(the\ whole\ tablet)}{m(portion\ of\ a\ tablet)}$$

where
m(ibuprofen, complete tablet) is the weight of ibuprofen in tablet used for assay, in mg
c(NaOH) is the concentration of sodium hydroxide, in mol.dm^{-3}
V(NaOH) is the volume of sodium hydroxide, in ml
M(ibuprofen) is the molar mass of ibuprofen, in g.mol^{-1} (M= 206,28 *g.mol^{-1})*
m(ibuprofen, portion of a tablet) is the weight of powder taken to assay, in g
m(the whole tablet) is the weight of complete tablet, in g

4. Calculate the average amount of ibuprofen in the whole tablet based on the results from both trials.

$$m(ibuprofen) = \frac{m(ibuprofen, trial\ 1) + m(ibuprofen, trial\ 2)}{2}$$

5. Record the results in the table below.

TABLE 5 RESULTS

Trial	m(tablet) (g)	m(powder) (g)	V(NaOH) (ml)	m(ibuprofen) (mg)
1				
2				
Average	-	-	-	

6. Compare the amount of the active substance obtained through analysis with the amount declared on the packaging.

Conclusion

.....

.....

.....

.....

Questions

Pharmaceutical chemistry

1. Define the antipyretic and antiphlogistic.
2. Describe the mechanism of action of ibuprofen using the DrugBank database.
   DrugBank: https://go.drugbank.com
3. What is the maximum daily dose of ibuprofen?
4. What are the common side effects associated with Ibalgin?
5. Describe the synthesis of Ibalgin. Books can be found on Google Books: http://books.google.com/.
   Recommended sources:
   a. Sittig, M. (1988). Pharmaceutical Manufacturing Encyclopedia, Volumes 1-2 (2nd Edition). William Andrew Publishing/Noyes. (p. 796-798)
   b. Pharmaceutical substances. 4th edition. (1999) (p. 1038-1041)
   c. Kirk-Othmer Chemical Technology and the Environment, Volume 1 (p. 12-14)
   d. Kleeman, Axel; Engel, Jürgen; Kutscher, Bernhard; Reichert, Dietmar (2009). Pharmaceutical Substances - Syntheses, Patents and Applications of the Most Relevant AIPs (5th Edition, Completely Revised). Thieme Medical Publishers Inc. (p. 681-684)
   e. Pharmaceutical Manufacturing Encyclopedia (3rd edition). (2007). William Andrew Publishing. (p. 1878-1881)

   Some books are available via remote access to e-resources of SLOVAK CENTRE OF SCIENTIFIC AND TECHNICAL INFORMATION. Ask the teacher for name and password for remote access. You can find useful information in Knovel database.
   Accessible via: http://www.cvtisr.sk/en/scientific-library/remote-access-to-e-resources.html?page_id=3273
6. Describe the manufacturing process of Ibalgin tablets.
   Recommended source: *Handbook of Pharmaceutical Manufacturing Formulations. Volume 5 Over-the-Counter Products.*
   Accessible via:
   https://archive.org/details/HANDBOOKOFPharmaceuticalManufacturingFormulationsVol5.OverTheCounterProducts2004

Analytical chemistry

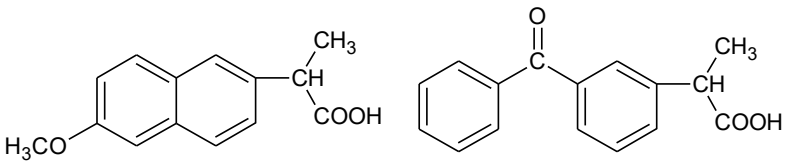
1. Describe the principle of the alkalimetric assay for ibuprofen.
2. Write the equation of standardization of sodium hydroxide against potassium hydrogen phthalate.
3. Specify other methods of Ibuprofen assay.
4. Calculate how many mg of ibalgin is equivalent to 1 ml of 0,1 M sodium hydroxide.
   ….. *mg of ibalgin is equivalent to 1 ml of 0,1 M sodium hydroxide*
   Algorithm of calculation: Moles of sodium hydroxide → Moles of ibalgin → Mass of ibalgin

Applied informatics

1. Find properties of ibuprofen. To find the properties and structure of a chemical you can usethe ChemSpider or PubChem database.
   ChemSpider: https://www.chemspider.com/
   PubChem: https://pubchem.ncbi.nlm.nih.gov/

a. Find the IUPAC name, formula and CAS number of ibuprofen.
b. Find the dissociation constant of ibuprofen.
c. Download the structure of ibuprofen in .mol format.
d. Open the mol file in the ChemSketch program.

2. Draw ibuprofen structure using chemical drawing software such as the ChemSketch.
3. Find the solubility of ibuprofen in ethanol at 298 K in research paper "Solubilities of Ibuprofen in Different Pure Solvents".
4. The Food and Drug Administration has also approved two other drugs with similar structures to ibuprofen for over-the-counter use as pain relievers. These new drugs are known by their generic names, X and Y. X is often administered in the form of its sodium salt. X and Y can be used to alleviate the pain of headaches, toothaches, muscle aches, backaches, arthritis, and menstrual cramps, and also be used to reduce fever. They appear to have a longer duration of action than older analgesics. What are the generic names of X and Y?
   You can use the Chemsketch program which has function "Search PubChem", or you can use the PubChem database - http://pubchem.ncbi.nlm.nih.gov.
   Note: Both structures, X and X, are inserted as objects. Click on them to open them in ChemSketch.



X Y

5. Analyze the metabolic pathways of ibuprofen in humans, focusing on the bioactivation and biotransformation processes. Discuss the major metabolites identified and their chemical structures. Include a bibliographic reference for the source of information used. You can use free bibliography and citation generator CitacePro (STN ISO 690 style), https://www.citacepro.com or MyBib (ISO-690 style) https://www.mybib.com.
6. In the paper "Photosensitization Caused by Ibuprofen," the photosensitization reaction of a patient treated with an oral ibuprofen preparation was studied. What wavelength of radiation was used for photoprovocation testing?

Preparation of reagents

Iron nitrate

	Unit	Value
$c_m(Fe(NO_3)_3)$	$(g.l^{-1})$	10
$V(Fe(NO_3)_3)$	ml	100
$M(Fe(NO_3)_3)$	$(g.mol^{-1})$	241,88
$M(Fe(NO_3)_3.9H_2O)$	$(g.mol^{-1})$	403,88
$m(Fe(NO_3)_3)$	g	**1,0000**
$m(Fe(NO_3)_3.9H_2O)$	g	**1,6698**

Calibration solutions

Calibration curve method (CCM)

Table 2 Preparing calibration solutions (Calibration curve method)

Standard	V(SA, ws) (ml)	c_m(SA) (mg.ml^{-1})	m(SA) (mg)
0	0	0,000	0,000
1	5	0,008	0,400
2	10	0,016	0,800
3	15	0,024	1,200
4	20	0,032	1,600
5	25	0,040	2,000
6	30	0,048	2,400

Quantity	Unit	Value
$c_m(SA)_1$	(mg.ml^{-1})	0,08
$V(SA)_2$	(ml)	50
M(SA)	(g.mol^{-1})	138,12

Calculation of c(SA) in mg/ml

SA solution	Dilution	m(SA) (g)	V(SA) (ml)	c_m(SA) (g.l^{-1})	c_m(SA) (mg.ml^{-1})
Stock solution	-	0,2	25	8	8
Working solution	100	-	-	0,08	0,08

Determination of salicylic acid

Calibration curve method

Table 5 Absorbance of standard solutions (Calibration curve method)

Standard	c_m(SA) (mg.l^{-1})	m(SA) (mg)	A
0	0,000	0	
1	0,008	0,4	
2	0,016	0,8	
3	0,024	1,2	
4	0,032	1,6	
5	0,040	2	
6	0,048	2,4	

Table 6 Absorbance of samples (Calibration curve method)

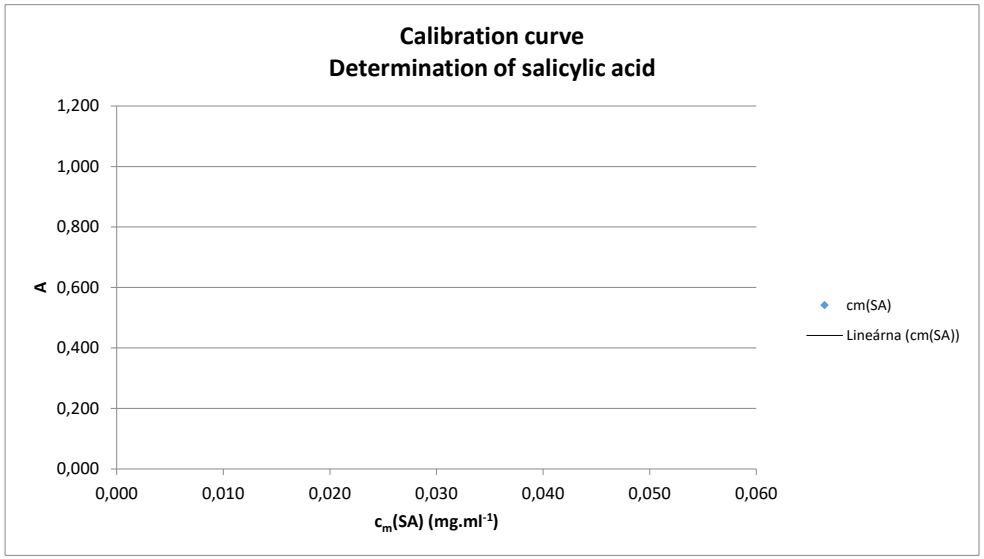
Sample	m(sample) (g)	V(sample) (ml)	A
S1			
S2			
S3			

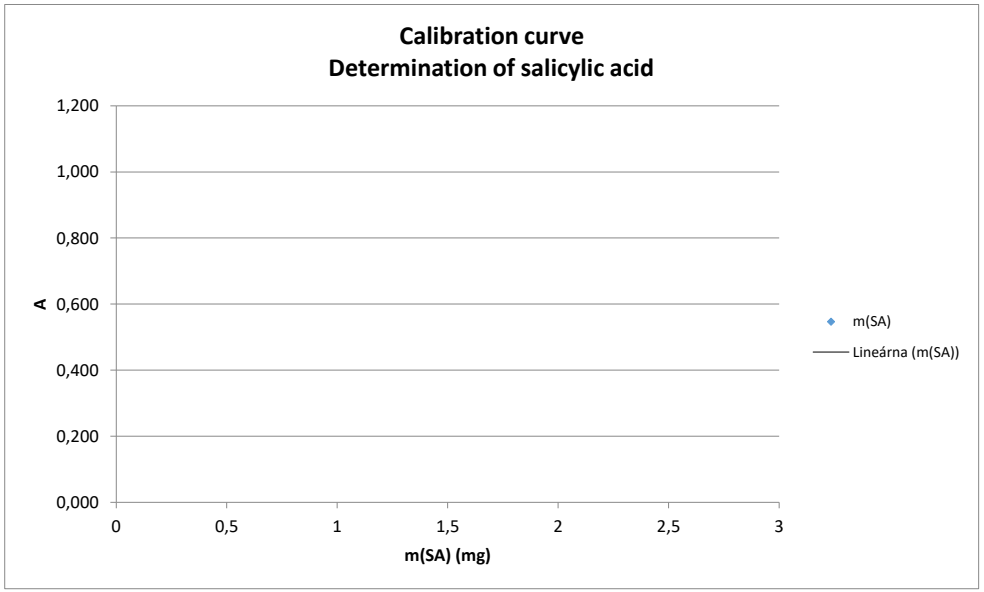
Table 9 Parameters of regression Line (Calibration curve method)

Curve	a	b	r^2
c(SA)	#DELENIENULOU!	#DELENIENULOU!	#DELENIENULOU!
m(SA)	#DELENIENULOU!	#DELENIENULOU!	#DELENIENULOU!

Table 10 Concentration of salicylic acid in samples (Calibration curve method)

Sample	m(sample) (g)	V(sample) (ml)	A	c_m(SA) (mg.ml^{-1})	m(SA) (mg)	w(SA) (mg.g^{-1})	w(SA) (%)
S1				##########	###########	############	############
S2							
S3							


Calibration curve
Determination of salicylic acid
1,200
1,000
0,800
0,600
0,400
0,200
0,000
A
0,000
0,010
0,020
0,030
0,040
0,050
0,060
cm(SA)
Lineárna (cm(SA))


Calibration curve
Determination of salicylic acid
1,200
1,000
0,800
0,600
0,400
0,200
0,000
A
0
0,5
1
1,5
2
2,5
3
m(SA) (mg)
m(SA)
Lineárna (m(SA))

LABORATORY MANUAL

Determination of salicylic acid in Acylpirin® by spectrophotometry

Organisation	Stredná odborná škola chemická, Vlčie hrdlo 50, 821 07 Bratislava
Author	Judita Dömötörová
Revised by	Judita Dömötörová
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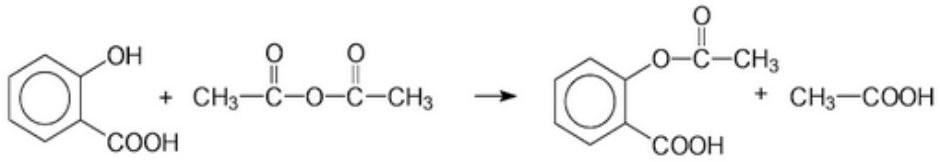
1.1 Introduction

1.1.1 Aspirin (Acylpyrin)

The prototypical analgesic used in the treatment of mild to moderate pain. It has anti-inflammatory and antipyretic properties and acts as an inhibitor of cyclooxygenase which results in the inhibition of the biosynthesis of prostaglandins. Acetylsalicylic acid also inhibits platelet aggregation and is used in the prevention of arterial and venous thrombosis.

1.1.2 Synthesis of acetylsalicylic acid

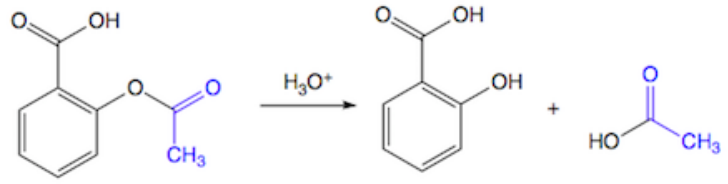
The synthesis of aspirin is classified as an esterification reaction. Salicylic acid is treated with acetic anhydride, an acid derivative, causing a chemical reaction that turns salicylic acid's hydroxyl group into an ester group. This process yields aspirin and acetic acid, which is considered a by-product of this reaction. Small amounts of sulfuric acid (and occasionally phosphoric acid) are almost always used as a catalyst.
Formulations containing high concentrations of aspirin often smell like vinegar because aspirin can decompose through hydrolysis in moist conditions, yielding salicylic and acetic acids.



salicylic acid acetic anhydride acetylsalicylic acid acetic acid

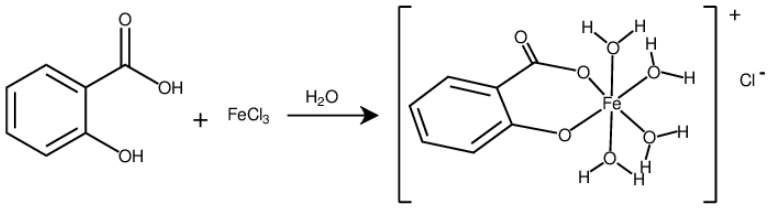
1.1.3 Decomposition of acetylsalicylic acid

Salicylic acid is a major hydrolytic degradation product of aspirin, responsible especially for gastric irritation during oral aspirin administration. More infromation about impurities of salicylic acid you can find at https://www.pharmaffiliates.com/impurities/86/acetylsalicylic%20acid.



1.1.4 Determination of salicylic acid in Acylpirin (Aspirin)

Colorless acetylsalicylic acid in an aspirin tablet will be converted to the reddish-purple salicylatoiron(III) complex to determine the percent acetylsalicylic acid in the tablet spectrophotometrically.



1.1.5 Spectrophotometry

Spectrophotometry is a technique to measure light absorption. It uses a light beam which passes through the sample, and each compound in the solution absorbs or transmits light over a certain wavelength.

Spectrophotometers consist of a light source, a monochromator, a sample chamber containing a cuvette, a detector (such as a photomultiplier tube or photodiode) to detect the transmitted light, a digital display and a data analysis software package.

There are generally two types of spectrophotometers: a single beam, and double beam. **Single beam spectrophotometers** use a single beam of light – visible or UV – which passes through a sample in a cuvette. Light intensity is measured before and after the light passes through the sample, and using Beer-Lambert's Law, the concentration of the analyte can be calculated. **Double beam spectrophotometers** work in a similar way to single beam spectrophotometers but with a key difference. The initial light source is split into two; one beam passes through the sample, and the other through a reference solution or the solvent. The ratio of the two light beams then corresponds to the absorbance of the sample.

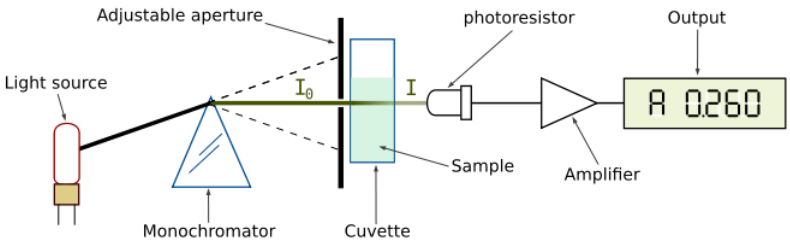


Fig. 3 Scheme of spectrophotometer

1.2 Assignment

1. Familiarize yourself with the dangerous properties of the substances you will be working with.
2. Prepare the solutions and reagents.
3. Determine salicylic acid in Acylpirin®.
4. Complete the worksheet.
5. Answer the questions on the worksheet.

1.3 Chemicals

Salicylic acid, p.a.
Iron nitrate, nonahydrate
Ethanol p.a.
D.I. water

1.4 Materials and Equipment

Calibration curve method: spectrophotometer, cuvettes (10 mm path length), analytical balance, weighting boat, stirring rod, chemical spoon, water bottle, beakers (50 ml, 2 pcs), volumetric flask (25 ml), volumetric flasks (50 ml, 8 pcs), volumetric flask (250 ml), mortar, pestle, filtration funnel, filtration paper, pipettes (5 ml, 20 ml), pipetting bulb, glass marker pen
Standard addition method: volumetric flask (100 ml), volumetric flasks (50 ml, 3 pcs)

1.5 Procedure

1.5.1 Calculations

1. Calculate the quantities of the above substances needed to prepare the volumes of solutions as given in the worksheet.
    a. Salicylic acid
    b. Iron nitrate

1.5.2 Preparation of solutions and reagents

1. Prepare a **stock solution of salicylic acid.** Accurately weight 0,2 g of salicylic acid, dissolve it in a small amount of ethanol, and fill up to the mark with ethanol in a 25 ml volumetric flask. *Note: Do not use Etaben®, use Vzduchol® instead.*
2. Prepare a **working solution of salicylic acid**. Pipette 2,5 ml of the stock solution into a 250 ml volumetric flask and fill up to the mark with distilled water.
3. Prepare 100 ml of **iron nitrate solution** with a concentration of 10 $g.l^{-1}$.

1.5.3 Construction of calibration curve

1. To seven 50 ml volumetric flasks pipette 0, 5, 10, 15, 20, 25, and 30 ml of the salicylic acid (working solution). Add 5 ml of the iron nitrate solution to each flask. Fill up to the mark with distilled water and mix the solution thoroughly by inverting and shaking the flask.
2. Measure the absorbance of the calibration solutions against the blank at 540 nm. Record the absorbance of calibration solutions in Table 5 of the worksheet.
3. Draw the calibration curve, plotting a graph of absorbance (A) versus the mass concentration of salicylic acid (c_m).

1.5.4 Determination of salicylic acid (Calibration curve method)

1. Accurately weight one tablet of Acylpirin® on an analytical balance. Record the mass of a tablet in the worksheet.
2. Grind the tablet in mortar and dissolve it in 20 ml of distilled water. Let the undissolved part to extract without mixing the solution for 20 minutes. Filter the solution to 50 ml volumetric flask. Rinse the undissolved portion on filter with small portions of distilled water and add the filtrate to the volumetric flask.
3. Add 5 ml of iron nitrate to the flask and fill up to the mark with distilled water. Cork the flask and mix the solution thoroughly.
4. Measure the absorbance of the solution against the blank at 540 nm. Record the absorbance in Table 6 of the worksheet. If the absorbance is too low, repeat the measurement using 2 tablets of Acylpirin.
5. Calculate the mass of salicylic acid in the tablet and express it in mg/g.

1.6 Safety Information

Chemical	Safety Information
Ethanol	**Hazard Statement(s)** H225: Highly flammable liquid and vapour. H319: Causes serious eye irritation. **Precautionary Statement(s)** P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P240: Ground/bond container and receiving equipment.

Chemical	Safety Information
	P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P403 + P233: Store in a well-ventilated place. Keep container tightly closed. Web: http://www.merckmillipore.com/GB/en/product/Ethanol,MDA_CHEM-818760
	CAS # 64-17-5 | EC Number 200-578-6 | Hill Formula C_2H_6O | Chemical Formula C_2H_5OH | Molar Mass 46.07 g/mol
Iron nitrate, nonahydrate	**Hazard Statement(s)** H314: Causes severe skin burns and eye damage. **Precautionary Statement(s)** P280: Wear protective gloves/ protective clothing/ eye protection/ face protection. P301 + P330 + P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P308 + P310: IF exposed or concerned: immediately call a POISON CENTER or doctor/ physician. Web: http://www.merckmillipore.com/GB/en/product/IronIII-nitrate-nonahydrate,MDA_CHEM-103883
	CAS # 7782-61-8 | EC Number 233-899-5 | Hill Formula FeN_3O_9 * 9 H_2O | Chemical Formula $Fe(NO_3)_3$ * 9 H_2O | Molar Mass 403.95 g/mol | Grade Value ACS,Reag. Ph Eur
Salicylic acid	**Hazard Statement(s)** H302: Harmful if swallowed. H318: Causes serious eye damage. **Precautionary Statement(s)** P280: Wear eye protection. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P313: Get medical advice/ attention. Web: http://www.merckmillipore.com/GB/en/product/Salicylic-acid,MDA_CHEM-100631
	CAS # 69-72-7 | EC Number 200-712-3 | Hill Formula $C_7H_6O_3$ | Chemical Formula HOC_6H_4COOH | Molar Mass 138.12 g/mol

1.7 References

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Further reading

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2. Kim D. Rainsford (19 April 2016). *Aspirin and Related Drugs*. CRC Press. *ISBN 978-0-203-64696-0*.
3. Gaurav Jain; Roop Krishen Khar; Farhan Jalees Ahmad (10 January 2013). *Theory and Practice of Physical Pharmacy - E-Book*. Elsevier Health Sciences. pp. 374–. *ISBN 81-312-3265-4*.