FOOD
CHEMISTRY

LABORATORY MANUAL

Evaluating the buffering capacity of soft drinks, fruit juices and tea

1.1 Introduction

1.1.1 Titratable acidity

The purpose of this work is to measure the initial pH of various commonly used beverages and to determine their ability to maintain a low pH by measuring their buffering capacities.

Dental erosion
Dental erosion is defined as **loss of tooth structure by a chemical process not involving any bacteria**. Dental erosion is a prevalent problem everywhere. Soft drinks are one of the principal factors in the aetiology of extrinsic dental erosion. Drinks that are consumed frequently are fruit juices, carbonated drinks and tea. The drink which causes erosion has low pH intrinsically. **The erosive potential depends on low pH and buffering capacity of the drinks.** There are various acids that are present in beverages - carbonated drinks contain **carbonic acid**, packaged fruit juices contain **organic acids derived from the fruits** and the **preservatives** which are responsible for their erosive potential. It has been accepted that titratable acidity, which is a measurement of the total acid content, is a more important indicator than actual pH value in determining erosive potential of beverages. **The acid content of the drinks influences the buffering capacity and therefore the drinks that have low pH are strongly buffered with a potential erosive capability**.

Titratable acidity
Titratable acidity of a solution is measured by reacting the acids present with a base such as **sodium hydroxide** to a chosen **end point, close to neutrality**. The titratable acidity is kept at **5,5** (the 'so-called' critical pH for tooth mineral dissolution) and **7** (neutral).

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

$$\underbrace{\begin{array}{c}\text{Reference}\\\text{electrode}\\\text{(internal)}\end{array}\Bigg|\begin{array}{c}\text{H}^{+}\\\text{(internal)}\end{array}\Bigg|\begin{array}{c}\text{Glass}\\\text{membrane}\end{array}}_{\text{Glass electrode}}\Bigg|\begin{array}{c}\text{H}^{+}\\\text{(external)}\\\text{analyte}\end{array}\Bigg|\begin{array}{c}\text{Reference}\\\text{electrode}\\\text{(external)}\end{array}$$

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.

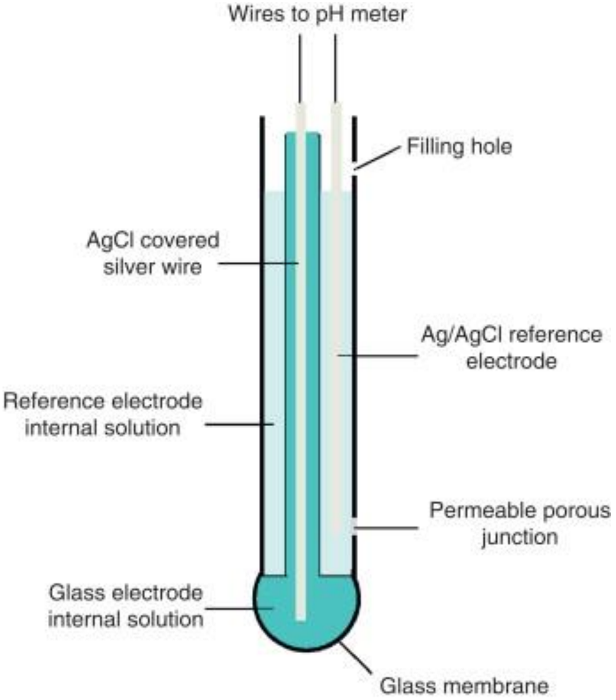


Fig. 1 Glass electrode

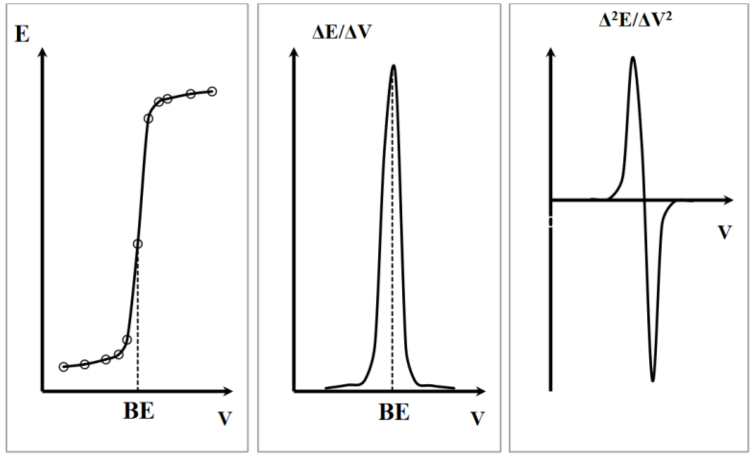
Common glass pH electrodes are extraordinary sensors in that they operate within a typical temperature range of 0–90 °C over the full pH range of 0–14 (14 orders of magnitude of the H^+ concentration), although they require accurate temperature measurement and compensation. The pH signal generated by 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.

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

Standardisation of a volumetric solution of sodium hydroxide

The exact concentration of sodium hydroxide can be determined by standardisation against potassium hydrogen phthalate.

$KHC_8H_4O_4 + NaOH \rightarrow KNaC_8H_4O_4 + H_2O$

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 potentiometric titration.
6. Determine the buffering capacity of soft drinks by potentiometric titration.
7. Complete the worksheet.
8. Answer the questions on the worksheet.

1.3 Chemicals

Potassium hydrogen phthalate
Sodium hydroxide (c = 1 mol.dm^{-3})

pH calibration solutions
Deionised water

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 and reagents

1. Calculate the mass of sodium hydroxide needed to prepare a 1 mol.dm^{-3} solution.
2. Prepare the sodium hydroxide solution.

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

1. Calculate how many grams of solid potassium hydrogen phthalate need to be so that approximately 10 mL of a 1 M sodium hydroxide solution is consumed during the titration of this amount of potassium hydrogen phthalate.
2. Transfer the potassium hydrogen phthalate quantitatively into a 250 ml beaker and dissolve in approximately 100 ml of demineralised water.
3. Calibrate a pH meter according to manual.
4. Place the stir bar in the beaker and place it on the magnetic stirrer. Immerse the electrode thoroughly rinsed with demineralised water in the solution. Switch on the pH meter and the stirring, and wait for the pH value to stabilise.
5. Add a precisely known volume (e.g. 0,5 cm^3) of sodium hydroxide to the sample while stirring constantly. After each addition, allow the pH to stabilize and record the obtained values (volume, pH) in the table. Continue the titration in this way until you have measured the entire titration curve (possibly up to pH 13).
6. Perform the required number of parallel determinations.
7. Construct the titration curve pH = f(V) from the measured values. Determine the equivalent titration volume from the titration curve.
8. Calculate the exact concentration of sodium hydroxide.

1.5.4 Determination of buffering capacity of soft drinks

1. Take preserved fruit juice, a carbonated beverage, and tea as samples.
2. Measure the *initial pH* of each drink using a pH meter. Place a 100 ml of freshly opened drink at room temperature into a beaker and stir using a magnetic stirrer until a stable reading is obtained. Take three readings of each drink from each group to give a mean measurement for that drink.
3. *Buffering capacity:* Titrate 100 ml of each drink with 1M sodium hydroxide added in 0,2 ml increments until the pH reaches 5,5 and 7.
4. Repeat the titrations in triplicate for all drinks to check for reproducibility and to provide a mean value for each drink.
5. Calculate the titratable acidity of all samples.
6. Compare the titratable acidity of the samples.

1.6 Safety information

Chemical	**Safety information**
Potassium hydrogen phthalate	Not a hazardous substance or mixture according to Regulation (EC) No 1272/2008. Web: https://www.sigmaaldrich.com/SK/en/product/sial/p1088
	CAS #: 877-24-7 EC Number: 212-889-4 Molar Mass: 204.22 g/mol Hill Formula: $C_8H_5KO_4$
Sodium hydroxide	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. 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.

Chemical	Safety information
	Web: https://www.merckmillipore.com/GB/en/product/Sodium-hydroxide-solution-01-mol-L,MDA_CHEM-137058

1.7 References

1. JINDAL, Rahul and SINGH, Smita, 2010. Evaluating the buffering capacity of various soft drinks, fruit juices and tea. *Journal of Conservative Dentistry*. 2010. Vol. 13, no. 3, p. 129. DOI https://doi.org/10.4103/0972-0707.71643.
2. Merck | Life Science | Industrial & Lab Chemicals | eShop, 2023. *Merckmillipore.com*. Online. [Accessed 8 August 2024]. Available from: https://www.merckmillipore.com/SK/sk

ANNEX

Dental erosion is the **partial demineralisation of the tooth surface** caused by **repeated exposure to acids**. The loss of surface tissue, which results from simultaneous and/or subsequent exposure to mechanical forces is known as erosive tooth wear (ETW). Excessive consumption of acidic beverages and foods has been the main focus of research into erosion. Enamel dissolution is significantly associated with chemical parameters: ***pH, buffer capacity***, ***titratable acidity***, ***viscosity*****, as well as** ***calcium*****,** ***phosphate*** **and** ***fluoride concentrations*** in the beverages and foods. Some of these parameters are used to calculate the degree of saturation of a given substance, which represents its driving force to demineralise dental hard tissues. **Undersaturated substances with low pH and high titratable acidity and high buffer capacity have greater erosive potential, while substances with high concentrations of Ca^{2+} and phosphate cause less demineralisation.** Other physical parameters also modulate the demineralisation processes. Swishing drinks in the mouth tends to cause more erosion, since the Nernst layer is continuously renewed and does not reach saturation. Recent systematic reviews confirm that **frequent consumption of carbonated/soft drinks are the main dietary factor associated with ETW**. **Vitamin C and frequent consumption of natural fruit juices and acidic snacks or sweets are also significantly associated with more ETW**; whereas **higher consumption of milk and yoghurt is a protecting factor**. Patients presenting with ETW should have their dietary habits assessed by recording their complete dietary intake in a diet record sheet. Dentists should assess the erosive potential of the different beverages and foods, as well as the frequency of ingestion, then elaborate specific preventive measures and dietary interventions individually tailored to each patient.

Saads Carvalho T, Lussi A. Chapter 9: Acidic Beverages and Foods Associated with Dental Erosion and Erosive Tooth Wear. Monogr Oral Sci. 2020;28:91-98. doi: 10.1159/000455376. Epub 2019 Nov 7. PMID: 31940633.

WORKSHEET

Evaluating the buffering capacity of soft drinks, fruit juices and tea

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	6,12 g	3*2,04 g		
Sodium hydroxide	200 ml	Standardization: 3 x 10 ml = 30 ml Determination in 1 drink: 3 x 7 ml = 35 ml 3 x drink = 3 x 35 = 105 ml		

* 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 200 cm^3 of sodium hydroxide solution with a concentration of 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 in other analyses.
   M(NaOH) = ... $g.mol^{-1}$
   $m(NaOH) = cVM$

4. Calculate how many grams of solid potassium hydrogen phthalate need to be weight so that approximately 10 mL of a 1 M sodium hydroxide solution is consumed during the titration of this amount of the acid.
   M(KHP) = ... $g{\cdot}mol^{-1}$

   Reaction equation:
   $KHC_8H_4O_4$ + NaOH → $KNaC_8H_4O_4 + H_2O$
   m(KHP) = ? g c(NaOH) = 1 $mol.dm^{-3}$

$M(KHP) = 204{,}2212\ g \cdot mol^{-1}$ $V(NaOH) = 10\ cm^3$

$$n(NaOH) = c(NaOH)\,V(NaOH)$$
$$n(KHP) = n(NaOH)$$
$$m(KHP) = n(KHP)\,M(KHP)$$

Data

Standardisation of sodium hydroxide solution

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

2. Record the volume and the pH of the sodium hydroxide solution when standardising in the table.

Table 2 Volume and pH of sodium hydroxide solution at standardisation

Standardization N°1			**Standardization N°2**			**Standardization 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	
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	13,5		28	13,5		28	13,5	
29	14,0		29	14,0		29	14,0	
30	14,5		30	14,5		30	14,5	
31	15,0		31	15,0		31	15,0	
32	15,5		32	15,5		32	15,5	
33	16,0		33	16,0		33	16,0	

Determination of buffering capacity

1. Fill in the information about the soft drink, juice and tea.

TABLE 3 SAMPLE INFORMATION

Drink	Information	Description
1	Name	
	Producer	
	Ingredients	
2	Name	
	Producer	
	Ingredients	
3	Name	
	Producer	
	Ingredients	

2. Record the volume of the sodium hydroxide solution used to titrate samples to pH 5,5 and 7 in the table. Calculate the mean volume of the sodium hydroxide solution.

TABLE 4 THE VOLUME AND PH OF THE SODIUM HYDROXIDE SOLUTION IN THE DETERMINATION OF TITRATABLE ACIDITY OF SAMPLES

Trial	**Drink N°1**			**Drink N°2**			**Drink N°3**		
	Initial pH	**pH 5,5**	**pH 7**	**Initial pH**	**pH 5,5**	**pH 7**	**Initial pH**	**pH 5,5**	**pH 7**
		V(NaOH) (ml)	**V(NaOH) (ml)**		**V(NaOH) (ml)**	**V(NaOH) (ml)**		**V(NaOH) (ml)**	**V(NaOH) (ml)**
1									
2									
3									
Average									

Results

Standardization of sodium hydroxide solution

1. Calculate $\frac{\Delta pH}{\Delta V}$ and $\frac{\Delta^2 pH}{\Delta V^2}$ for each pair of date when determining the sodium hydroxide. Perform 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**	$\boldsymbol{\Delta V}$ **(ml)**	$\boldsymbol{\Delta pH}$	$\boldsymbol{\frac{\Delta pH}{\Delta V}}$	$\boldsymbol{\Delta V^2}$	$\boldsymbol{\Delta^2 pH}$	$\boldsymbol{\frac{\Delta^2 pH}{\Delta V^2}}$
1								
2								
…								

2. Plot the following graphs for each measurement when determining the sodium hydroxide:
   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. Find the maximum of the first derivative and read the corresponding volume.
4. 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

5. Calculate the molar concentration of sodium hydroxide in volumetric solution.
Reaction equation: $C_8H_4O_4HK + \mathrm{NaOH} \rightarrow C_8H_4O_4HK\mathrm{Na} + \mathrm{H_2O}$

Moles of KHP:

$$n(KHP) = \frac{m(KHP)}{M(KHP)}$$

Moles of sodium hydroxide:

$$\frac{n(NaOH)}{n(KHP)} = \frac{1}{1} \Longrightarrow n(NaOH) = n(KHP)$$

Concentration of sodium hydroxide:

$$c(NaOH) = \frac{n(NaOH)}{V(NaOH)}$$

Derived formula for calculating the concentration of sodium hydroxide in one step:

$$c(NaOH) = \frac{c(KHP)V(KHP)}{V(NaOH)}$$

6. Perform a model calculation for measurement N°1 on the worksheet.
7. Perform calculation for measurement N°2 and N°3 in MS Excel.
8. Calculate the average concentration of sodium chloride of the three measurements.

$$c(NaOH) = \frac{c_1(NaOH) + c_2(NaOH) + c_3(NaOH)}{3}$$

Determination of buffering capacity of drinks

1. Calculate neutralisation capacity of samples in mmol.

$$TA = \frac{c(NaOH)V(NaOH, pH\ 7)}{V(sample)}.1000$$

TABLE 5 TITRATABLE ACIDITY OF SAMPLES

Sample	**V(NaOH) (ml)**		**TA (mmol)**
	pH 5,5	**pH 7**	
Drink 1			
Drink 2			
Drink 3			

2. Compare the titratable acidity of the different drinks.

3. Compare the erosive capability of the different drinks.

TABLE 6 EVALUATION OF EROSIVE CAPABILITY OF SAMPLES

Arranged by TA	**Sample**	**Erosive capability**
Highest TA		
Lowest TA		

Conclusion

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……

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Questions

Dental erosion

1. Explain erosive tooth wear.
2. Describe how beverages affect the pH in the mouth.
3. Define titratable acidity.
4. How does titratable acidity provide a better indication of the erosive potential of beverages compared to just measuring the pH? Provide examples of beverages with high and low titratable acidity and their likely impact on dental erosion.
5. Considering the principles of acid-base chemistry, why might a beverage with a low pH but low titratable acidity cause less dental erosion than one with high titratable acidity? Use general acid-base concepts to support your answer.
6. Based on your understanding of dental erosion and the properties of beverages, what recommendations would you provide to someone looking to minimize their risk of dental erosion while still enjoying their favorite drinks? Consider both the choice of beverages and consumption habits.

Analytical chemistry

1. Characterise the principle of potentiometry.
2. Characterize the principle of potentiometric titration.
3. Define the 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 potentiometrically.
7. Describe the principle of determining titratable acidity potentiometrically.
8. In your final evaluation, state the errors that affected the result you obtained.

Applied informatics

1. The main organic acids found in orange juice are citric, malic, and ascorbic acid. The major sugars found in orange juice are sucrose, glucose, and fructose. Draw the structure of these chemicals in the ChemSketch program.

   Tip: To insert pre-drawn templates of sugars use "Open Template Window" function .

2. Citric Acid is the preferred acidulant in the soft drink industry because its pleasant sour taste is characteristic of citrus beverages. To find the properties and structure of a citric acid you can use the ChemSpider or PubChem database.
   ChemSpider: https://www.chemspider.com/StructureSearch.aspx
   PubChem: https://pubchem.ncbi.nlm.nih.gov/
   a. Find the IUPAC name, formula and CAS number of citric acid.
   b. Find dissociation constants of citric acid.
   c. Download the structure of citric acid 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.

Aims and Objective:
The purpose of this study is to measure the initial pH of various commonly used beverages and to determine their ability to maintain a low pH by measuring their buffering capacities.

Materials and Methods:
Twelve commercially available drinks were taken and divided into four groups (preserved fruit juices, tea, mineral water and carbonated drinks. Each group comprised of three drinks. Their initial pH were measured with pH meter and their buffering capacities were measured by adding 1M NaOH in the increments of 0.2 ml into 100 ml of each drink till the pH raised to 5.5 and 7 respectively.

Statistical Analysis:
The volume of NaOH required to raise the pH to 5.5 and 7 were recorded in all the groups. This data was subjected to statistical analysis using Mann- Whitney tests.

Results:
Total titratable acidity measurement shows that among all the drinks, there was no significant difference between carbonated drinks and preserved fruit juices while a significant difference was present between carbonated drinks, preserved fruit juices and tea.

Conclusion:
In this in vitro study, it was found that packaged apple juice had the most buffering capacity with maximum erosive potential whereas green tea had the least.
Keywords: Buffering capacity, dental erosion, titratable acidity

4. In the paper “Measuring the buffering capacity of commercially available soft drinks in India: An in vitro study” the initial pH of various commonly used beverages was measured to determine their ability to maintain a low pH by measuring their buffering capacities. What is the initial pH, the volume of base needed to increase pH to 5,5 and the volume of base needed to increase pH to 7,0 for Sprite?
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 sodium chloride in brine by refractometry

1.1 Introduction

1.1.1 Sodium chloride in cheese

Salt in cheese serves two major roles—namely, it acts as a preservative and contributes directly to flavor and quality. The preservative action of NaCl is due to its depressing effect on the water activity of the cheese. Moreover, salt increases the osmotic pressure of the aqueous phase of foods, causing dehydration of bacterial cells, killing them or, at least, preventing their growth. NaCl contributes directly to saltiness in cheese, a flavor that is generally highly appreciated. It contributes indirectly to flavor of cheese by its controlling influence on microbial and enzymatic activities which, in turn, influence lactose metabolism, cheese pH, degradation of fats and casein, and the formation of flavor compounds, such as peptides, free amino acids, and free fatty acids. In addition to these functions, salt exerts a number of important effects on cheese. Salt, together with pH and calcium level, has a large effect on the extent of paracasein hydration or aggregation, which in turn affects the water binding.

1.1.2 Refractometry

Refractometry is a **non-spectral optical method**. The principle of method is the measurement of the **refractive index** of substances.

Refractive index *n* is a physical quantity that is a measure of the optical density of the environment. As the optical density of the medium increases, the speed of light decreases and the refractive index increases. If the substance is a chemical individual, the refractive index is a physical constant (at constant conditions).

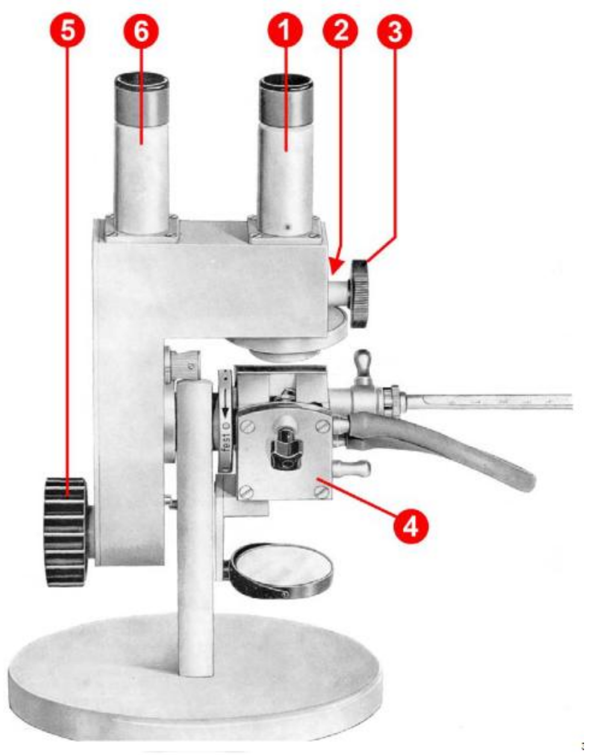
$n_{1,2} = \frac{v_1}{v_2} = \frac{\sin\alpha}{\sin\beta}$	$n_{1,2}$ – relative refractive index [1] α – angle of incidence [°] β – angle of refraction [°] v_1, v_2 – speed of light in the 1st and 2nd optical environments [m s^{-1}]

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 (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). Such a 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

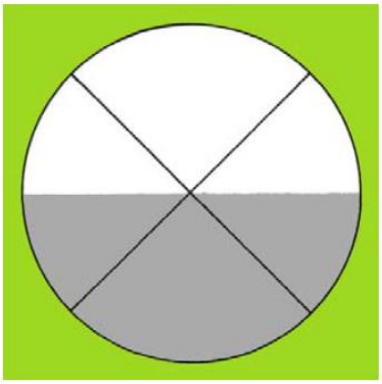


Fig. 2. Field of vision in the focusing telescope

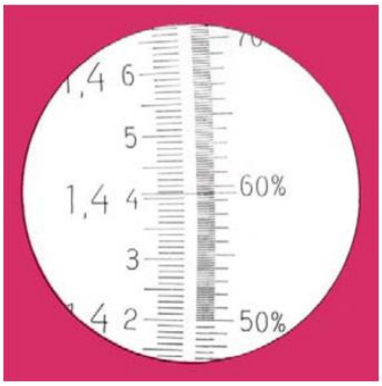


Fig. 3. Field of vision in the reading microscope

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.

1.2 Assignment

1. Calculate the quantities of substances needed to prepare the solutions.
2. Prepare the solutions and reagents.
3. Record the sample information.
4. Familiarize yourself with the dangerous properties of the substances you will be working with.
5. Determine the sodium chloride content of the brine.
6. Complete the worksheet.
7. Answer the questions on the worksheet.

1.3 Chemicals

Sodium chloride, deionised water

1.4 Materials and equipment

Analytical balance, beaker, weighing boat, water bottle, chemical spoon, volumetric flask with stopper (25 ml, 100 ml), pipette, *burette*, disposable pipette, thermometer, refractometer Carl Zeiss, paper towel, permanent marker

1.5 Procedure

1.5.1 Preparation of solutions and reagents

1. Calculate the quantities of substances needed to prepare the solutions below.
2. Prepare:
   a. 20 wt. % sodium chloride stock solution
   b. standard sodium chloride solutions with concentrations of 0%, 5%, 10%, 15%, and 20% in 25 ml flasks by diluting the stock solution.

1.5.2 Determination of refractive index of sodium chloride solutions

1. Temper the solutions to 20°C.
2. Measure the refractive index of the sodium chloride solutions. Repeat each measurement three times and calculate the arithmetic mean from the measured values.
3. Construct a calibration curve for the determination of sodium chloride.
4. Measure the refractive index of the sample solution. Repeat the measurement three times and calculate the arithmetic mean of the measured values.
5. Using the calibration curve, calculate the mass fraction of sodium chloride in the brine.

The most common measurement errors:
If there is an insufficient amount of sample in the refractometer and the device cannot be adjusted, a larger amount of sample must be dispensed with a pipette to ensure that the lower prism is covered. The sample must form a continuous film between the prisms. If there is excess sample after closing the prisms, it must be removed by drying with filter paper.

1.5.3 Working with a refractometer

See annex.

1.6 Safety information

Chemical	Safety Information
Sodium chloride	Not a hazardous substance or mixture according to Regulation (EC) No 1272/2008. Web: https://www.merckmillipore.com/GB/en/product/Sodium-chloride,MDA_CHEM-106406
	CAS #: 7647-14-5 EC Number: 231-598-3 Molar Mass: 58.44 g/mol Chemical Formula: NaCl Hill Formula: ClNa

1.7 Rereferences

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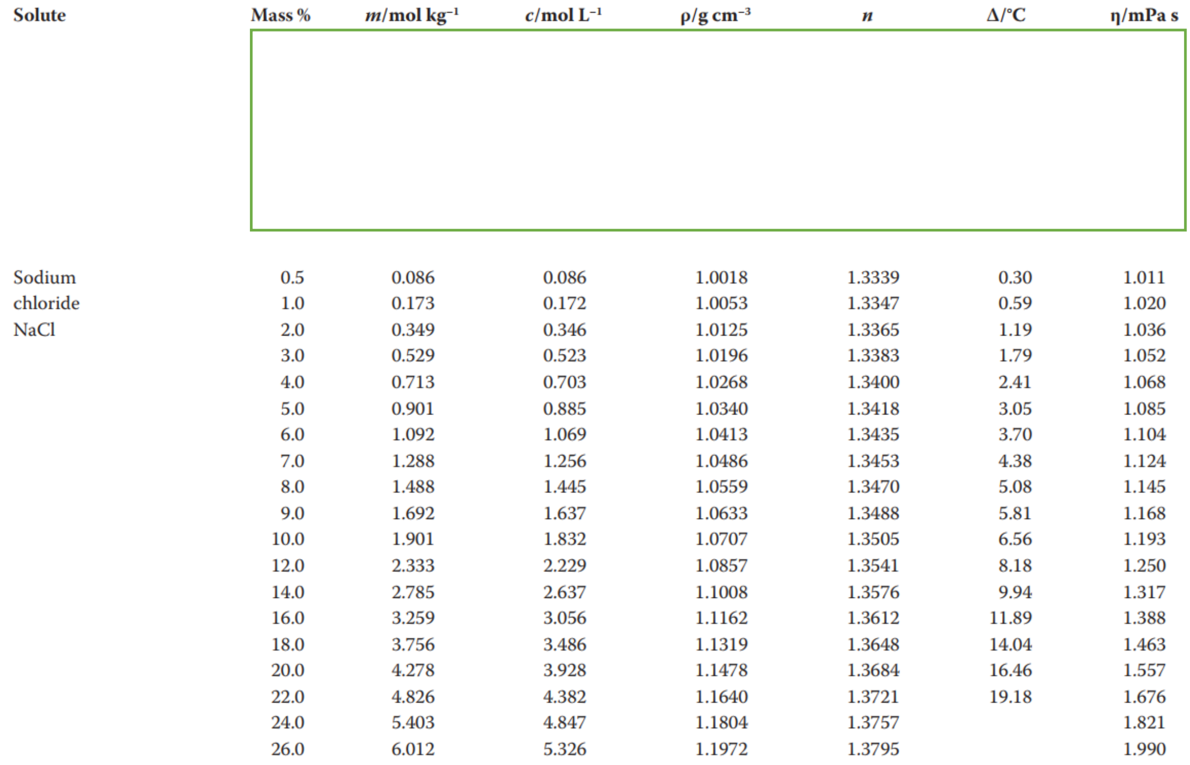
Annex

Composition of an aqueous solution of sodium chloride

TABLE 1 COMPOSITION OF AQUEOUS SODIUM CHLORIDE SOLUTION



Solute	Mass %	m/mol kg^{-1}	c/mol L^{-1}	ρ/g cm^{-3}	n	Δ/°C	η/mPa s
Sodium chloride NaCl	0.5	0.086	0.086	1.0018	1.3339	0.30	1.011
	1.0	0.173	0.172	1.0053	1.3347	0.59	1.020
	2.0	0.349	0.346	1.0125	1.3365	1.19	1.036
	3.0	0.529	0.523	1.0196	1.3383	1.79	1.052
	4.0	0.713	0.703	1.0268	1.3400	2.41	1.068
	5.0	0.901	0.885	1.0340	1.3418	3.05	1.085
	6.0	1.092	1.069	1.0413	1.3435	3.70	1.104
	7.0	1.288	1.256	1.0486	1.3453	4.38	1.124
	8.0	1.488	1.445	1.0559	1.3470	5.08	1.145
	9.0	1.692	1.637	1.0633	1.3488	5.81	1.168
	10.0	1.901	1.832	1.0707	1.3505	6.56	1.193
	12.0	2.333	2.229	1.0857	1.3541	8.18	1.250
	14.0	2.785	2.637	1.1008	1.3576	9.94	1.317
	16.0	3.259	3.056	1.1162	1.3612	11.89	1.388
	18.0	3.756	3.486	1.1319	1.3648	14.04	1.463
	20.0	4.278	3.928	1.1478	1.3684	16.46	1.557
	22.0	4.826	4.382	1.1640	1.3721	19.18	1.676
	24.0	5.403	4.847	1.1804	1.3757		1.821
	26.0	6.012	5.326	1.1972	1.3795		1.990

Salt solution for salting cheeses

For salting cheeses, a salt brine with a composition of 18–20% salt is most suitable. With this composition, the brine is also resistant to spoilage and potential attack by unsuitable microorganisms. If the salt content falls below 16%, the brine can become a source of contamination.

Proper salting of cheeses is essential for their quality. Too little salt can result in high moisture content, making the cheese soft and more acidic, and can lead to the formation of undesirable molds on the surface. Conversely, a high concentration of brine does not necessarily ensure good salting. Above 23% salt, there is an increased risk that moisture will be lost from the surface of the cheese too quickly, leading to the formation of a crust that can inhibit or reduce further salt absorption into the cheese. Consequently, the center of the cheese may remain insufficiently salted.

Source: https://syrar.cz/solny-roztok-na-nasoleni-syru/

WORKSHEET

Sodium chloride in brine by refractometry

1.1 Chemical calculations

1. Calculate mass of sodium chloride needed to prepare 100 ml of 20% (w/w) stock solution.
   M(NaCl) = ... $g.mol^{-1,}$ ρ(20% NaCl, 20°C) = ... $g.cm^{-3}$
   $w(NaCl) = \frac{m(NaCl)}{m(20\%\ NaCl)}, \rho(20\%\ NaCl) = \frac{m(20\%\ NaCl)}{V(25\%\ NaCl)}$
   $m(NaCl) = w(20\%\ NaCl).m(20\%\ NaCl) = w(20\%\ NaCl).\rho(20\%\ NaCl)V(20\%\ NaCl)$

2. Look up the density and molar concentration of the sodium chloride calibration solutions in the chemical tables See Annex). Record the data to the Table **1**.

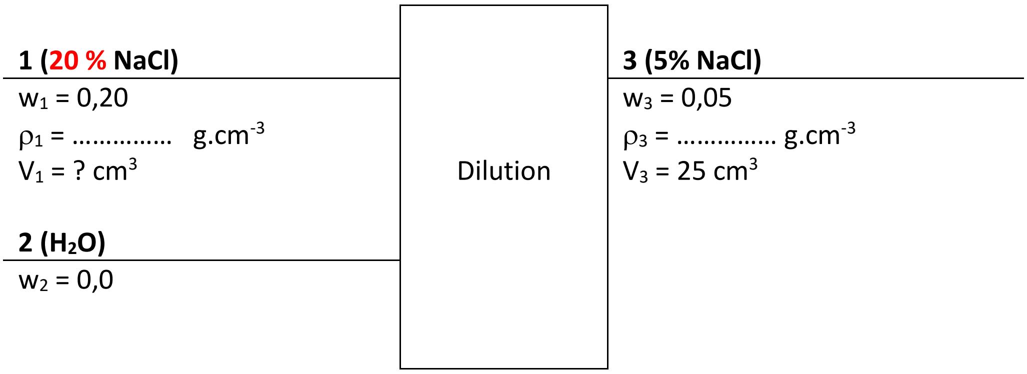
3. Calculate the volume of sodium chloride stock solution needed to prepare 25 ml of diluted solutions with the composition according to the table below.

   Sample calculation for calibration solution no. 1
   1 – 20% NaCl solution, **2** – H_2O, **3** – 5 % NaCl solution
   Mass of solution: $m_1 + m_2 = m_3$
   Mass of NaCl: $m_1w_1 + m_2w_2 = m_3w_3$

   $w_2 = 0$
   $m_1w_1 = m_3w_3$

   We substitute for $m_1 = \rho_1V_1$, and $m_3 = \rho_3V_3$ and we get $\rho_1V_1w_1 = \rho_3V_3w_3$.
   Then we can calculate the volume of 20% solution needed to prepare a 5% solution.
   $V_1 = \frac{\rho_3V_3w_3}{\rho_1w_1}$

   Balance:



1 (20 % NaCl)
w_1 = 0,20
ρ_1 = ... $g.cm^{-3}$
V_1 = ? cm^3

2 (H_2O)
w_2 = 0,0

Dilution

3 (5% NaCl)
w_3 = 0,05
ρ_3 = ... $g.cm^{-3}$
V_3 = 25 cm^3

4. Calculate the mass concentration of sodium chloride in the calibration solutions. Write the values in the Table **1**.
   Sample calculation for calibration solution no. 1
   $c_{m1} = c_1 M$

TABLE 1 CONCENTRATION OF SODIUM CHLORIDE CALIBRATION SOLUTIONS

Calibration solution	w (%)	ρ (g.cm^{-3})	c (mol.dm^{-3})	c_m (g.dm^{-3})	V(ss) (cm^3)
1	0				
2	5				
3	10				
4	15				
5	20				

Legend: V(ss) – volume of sodium chloride stock solution

1.2 Data

1. Complete the sample information.

TABLE 2 SAMPLE INFORMATION

Sample	Information	Description
S1	Name	Salt brine for salting cheeses
	Composition	
S2	Name	Salt brine for salting cheeses
	Composition	
S3	Name	Salt brine for salting cheeses
	Composition	

2. Record the refractive index of calibration solutions. Calculate the arithmetic mean of the three measurements.

TABLE 3 REFRACTIVE INDEX OF SODIUM CHLORIDE CALIBRATION SOLUTIONS

Calibration solutions	w (%)	$n_D^{20}(1)$	$n_D^{20}(2)$	$n_D^{20}(3)$	n_D^{20}(mean)
1	0				
2	5				
3	10				
4	15				
5	20				

3. Record the refractive index of the sample. Calculate the arithmetic mean of the three measurements.

TABLE 4 REFRACTIVE INDEX OF SAMPLE

Sample	$n_D^{20}(1)$	$n_D^{20}(2)$	$n_D^{20}(3)$	n_D^{20}(mean)
S1				
S2				
S3				

1.3 Results

1. Draw a calibration curve - graphical dependence of the refractive index on the mass fraction of sodium chloride $n_D^{20} = f(w)$.
    a. Mark the axes of the graph.
    b. Insert a trend line (linear).
    c. Display the regression equation on the graph and the value of R^2.
    d. Insert the graph in this document or in an attachment.

2. Evaluate the measured data using linear regression.
   Regression line equation (general):$y = ax + b$
   Regression line equation (sodium chloride analysis): $n_D^{20} = aw + b$

TABLE 5 PARAMETERS OF LINEAR REGRESSION

a	b	r^2

Perform sample calculation for sample V1. Do the calculation for other samples in a spreadsheet (e.g. MS Excel).

3. Calculate the mass fraction of sodium chloride in sample.
   The equation of the regression line: $n_D^{20} = aw + b$
   Mass fraction of sodium chloride in the sample: $w = \frac{n_D^{20} - b}{a}$

4. Find the molar concentration of sodium chloride in the sample using the chemical tables. If needed use linear interpolation to calculate the value.
   c = ... $mol.dm^{-3}$

   Linear interpolation
   x_1 = ..., y_1 = ...
   x = ..., y = ?
   x_2 = ..., y_2 = ...

$$y = y_1 + \frac{(x - x_1)(y_2 - y_1)}{(x_2 - x_1)}$$

5. Calculate the mass concentration of sodium chloride in the sample.

$$c_m = cM$$

c_m = ... $g.dm^{-3}$

6. Record the calculated values in the Table 6.

TABLE 6 SODIUM CHLORIDE CONTENT IN THE SAMPLE

Sample	w (%)	ρ ($g.cm^{-3}$)	c ($mol.dm^{-3}$)	c_m ($g.dm^{-3}$)
S1				
S2				
S3				

1.4 Conclusion

....

....

....

....

1.5 Questions

Analytical chemistry

1. Describe the principle of refractometry.
2. Define the refractive index.
3. Describe what parameters the refractive index of a liquid depends on.
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 on an Abbe refractometer.
7. Describe the principle of refractometric determination of sodium chloride in brine.
8. Explain the meaning of tempering the solution to 20°C.
9. State another simple and cost-effective method of measuring the salt content in brine.
10. Explain the calibration curve method.

Food chemistry

1. What is the optimal sodium chloride content in the brine for salting cheeses?
2. Explain the cause of the preservative effect of salt on cheese.
3. What is the effect of sodium chloride on the osmotic pressure of the aqueous phase of cheese? What effect does this have on bacterial cells?

4. Explain the functions of the optimal sodium chloride content in the brine for salting cheeses.

Applied informatics

1. Suggest a source from which refractive index information for chloride solutions of different concentrations can be obtained. 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 sodium chloride 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. Most people with lactose intolerance or lactase non-persistence can tolerate dairy products that are naturally low in lactose. Examples are hard cheeses such as cheddar, colby, Swiss, mozzarella and Monterey Jack and aged cheeses such as Parmigiano Reggiano. To find the properties and structure of lactose, you can use ChemSpider or PubChem database.
   ChemSpider: https://www.chemspider.com/StructureSearch.aspx
   PubChem: https://pubchem.ncbi.nlm.nih.gov/
   a. Find the IUPAC name, formula and CAS number of lactose.
   b. Download the structure of lactose in .mol format.
   c. Open the. mol file in the ChemSketch program.
4. Draw the structure of lactose in the ChemSketch program.
   Tip: To insert pre-drawn templates of sugars use "Open Template Window" function .
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 link to this paper. You can use free bibliography and citation generator MyBib: https://www.mybib.com.

   Salt levels in cheese range from ~0.7% (w/w) in Swiss-type to ~6% (w/w) in Domiati. Salt has three major functions in cheese: it acts as a preservative, contributes directly to flavour, and is a source of dietary sodium. Together with the desired pH, water activity and redox potential, salt assists in cheese preservation by minimizing spoilage and preventing the growth of pathogens. The dietary intake of sodium in the modern western diet is generally excessive, being two to three times the level recommended for desirable physiological function (2.4 g Na, or ~6 g NaCl per day). However, cheese generally makes a relatively small contribution to dietary sodium intake except if high quantities of high-salt cheeses such as Domiati and feta are consumed. In addition to these functions, salt level has a major effect on cheese composition, microbial growth, enzymatic activities and biochemical changes, such as glycolysis, proteolysis, lipolysis and para-casein hydration, that occur during ripening. Consequently, the salt level markedly influences cheese flavour and aroma, rheology and texture properties, cooking performance and, hence, overall quality. Many factors affect salt uptake and distribution in cheese and precise control of these factors is a vital part of the cheesemaking process to ensure consistent, optimum quality.

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.

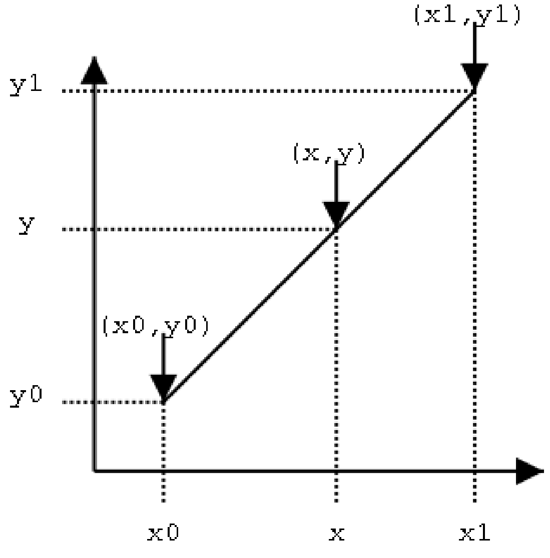
7. What functions did you use in your spreadsheet program to evaluate the data by least square method (linear regression)?

Annex

Linear interpolation

What is Linear Interpolation?

Interpolation is a method for estimating the value of a function between any two known values. Often some relationship is there, and with the help of experiments at a range of values to predict other values. Interpolation is useful to estimate the function of the un-tabulated points. Interpolation is useful to estimate any desired value at some specific known coordinate point.



Linear interpolation is useful while searching for a value between given data points. Therefore mathematician considers it as “filling in the gaps” for a given data values in tabular format. The strategy for linear interpolation is to use a straight line to connect the given data points on positive as well as the negative side of the unknown point.

Often, Linear interpolation is not accurate for non-linear data. If the points in the data set to change by a large value, then linear interpolation may not give a good estimate. Also, it involves estimating a new value by connecting two adjacent known values with a straight line.

Formula of Linear Interpolation

Its simplest formula is given below:

$$y = y_1 + \frac{(x - x_1)(y_2 - y_1)}{(x_2 - x_1)}$$

This formula is using coordinates of two given values to find the best fit curve as a straight line. Then this will give any required value of y at a known value of x.

In this formula, we are having terms as:

x_1 and y_1 are the first coordinates
x_2 and y_2 are the second coordinates
x is the point to perform the interpolation
y is the interpolated value.

LABORATORY MANUAL

Determination of sugar as glucose in a soft drink

Organisation	Stredná odborná škola chemická, Vlčie hrdlo 50, 821 07 Bratislava
Author	Judita Dömötörová
Revised by	Judita Dömötörová
Save date	28/09/2025 19:42:00
Print date	28/09/2025 19:42:00

1.1 Introduction

1.1.1 Determination of glucose

This method is based on the color which forms when sugar is reduced from 3,5-dinitrosalicylic acid (DNSA) to 3-amino-5-nitrosalicylic acid as shown in Figure 1. The sucrose does not react with DNSA, therefore it must be broken down into simple sugars like glucose first by boiling the sample with hydrochloric acid as shown in Figure 2. In addition, the sugar in soft drinks is too highly concentrated to be used for this method unless it is diluted. The red-brown color of the product is detected at 580 nm.

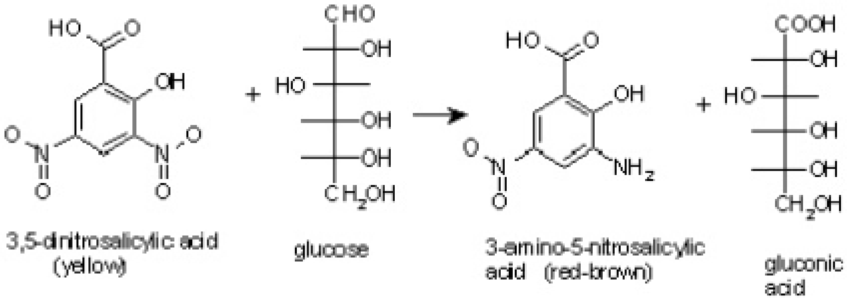


Fig. 1 The reaction of 3,5-dinitrosalicylic acid and glucose.

This method tests for the presence of free carbonyl group (C=O), the so-called reducing sugars. This involves the oxidation of the aldehyde functional group present in, for example, glucose and the ketone functional group in fructose. Simultaneously, 3,5-dinitrosalicylic acid (DNS) is reduced to 3-amino,5-nitrosalicylic acid under alkaline conditions

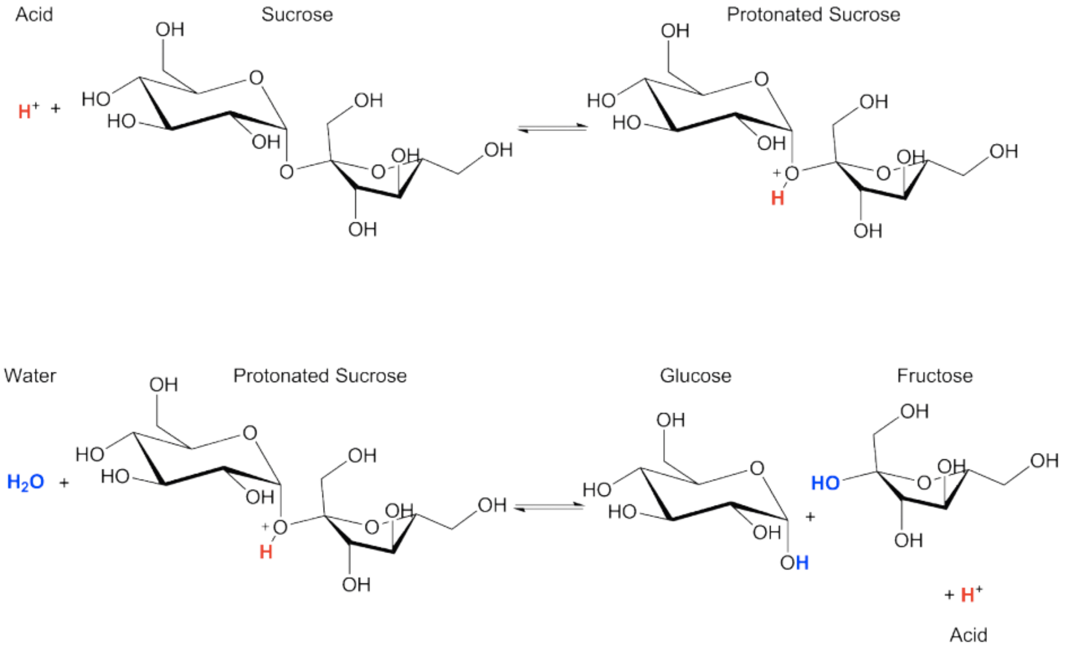


Fig. 2 Hydrolysis of sucrose.

1.1.2 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.

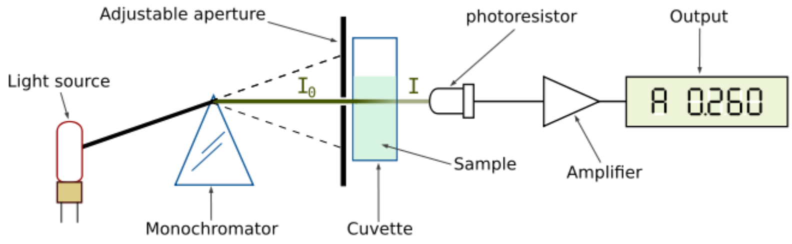


Fig. 3 Scheme of spectrophotometer

1.2 Assignment

1. Calculate the amount of chemicals needed to prepare solutions and reagents.
2. Familiarize yourself with the dangerous properties of the substances you will be working with.
3. Prepare the necessary solutions.
4. Determine the sucrose concentration in the soft drink.
5. Complete the worksheet.
6. Answer the questions on the worksheet.

1.3 Chemicals

1. Sucrose stock solution (1000 mg/dL = 1g/100ml)
2. 6 M hydrochloric solution
3. 2,5 M sodium hydroxide solution
4. 0,05 M 3,5-dinitrosalicylic acid (DNSA) solution
   Dissolve 10 g of DNSA in 200 ml water in 1000 ml beaker. Followed by continuous stirring slowly add a solution of 16 g of NaOH dissolved in 150 ml distilled water. Incubate mixture in 50°C with stirring to obtain a clear solution. ~~In small portions, add 403 g of potassium sodium tartrate tetrahydrate.~~ Filter mixture using paper filter and make up the volume to 1000 ml with water. Store in dark glass bottle at temperature below 20 °C. This has to be used within 20-30 min.
5. D.I. water
6. Soft drink (non-diet, not dark-colored)

1.4 Materials and Equipment

spectrophotometer, cuvettes (10 mm path length), analytical balance, weighting boat, beakers, volumetric flasks (25 ml, 9 pcs), funnel, test tubes with stoppers or screw caps (9 pcs), parafilm, test tube tack, pipettes (2 ml, 5 ml), pipetting bulb, glass marker pen

1.5 Procedure

1.5.1 Preparing of solutions and reagents

1. Calculate the quantities of the above substances needed to prepare the volumes of solutions as given in the worksheet.
   a. 3,5-dinitrosalicylic acid
   b. Hydrochloric acid
   c. Sodium hydroxide

   d. Sucrose

2. Prepare standard sucrose solution with concentration 1000 mg/dL.
3. Prepare five diluted sucrose stock solutions as standards.
4. Prepare diluted soft drinks as samples.

1.5.2 Determination of sucrose

1. Pipet 2 mL of each sucrose standard and samples into a test tube. Also pipet 2 mL of D.I water into a separate test tube for the blank solution.
2. Add 2 mL of 6 M hydrochloric acid solution.
3. Place in boiling water for 10 minutes.
4. Add 8 mL of 2,5 M sodium hydroxide solution.
5. Add 2 mL of 0,05 M DNSA solution then cover the test tube with parafilm and shake well to mix.
6. Place in boiling water for 5 minutes followed by ice water for 10 minutes. (The time between DNSA addition and measurements should be same for all test solutions.)
7. Measure the absorbance of the five standards and references at 580 nm.
8. Measure the absorbance of samples and calculate the concentration of glucose in each sample.

1.6 Safety Information

Chemical	Safety Information
3,5-dinitrosalicylic acid	**Hazard statement(s)** H302 Harmful if swallowed. **Precautionary statement(s)** P301 + P312 + P330 IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. Web: https://www.sigmaaldrich.com/catalog/product/aldrich/128848?lang=en&region=SK
	CAS Number 609-99-4 Linear Formula (O2N)2C6H2-2-(OH)CO2H Molecular Weight 228.12 Beilstein Registry Number 2220661 EC Number 210-204-3 MDL number MFCD00007104 PubChem Substance ID 24847877
Hydrochloric acid	**Hydrochloric acid fuming 37%** **Hazard Statement(s)** H290: May be corrosive to metals. H314: Causes severe skin burns and eye damage. H335: May cause respiratory irritation. **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/Hydrochloric-acid-fuming-370/0,MDA_CHEM-101834
Sodium hydroxide	**Hazard Statement(s)** H290: May be corrosive to metals.

<table>
<tr><th>Chemical</th><th>Safety Information</th></tr>
<tr><td></td><td>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></tr><tr><td>1310-73-2</td><td>215-185-5</td><td>HNaO</td><td>NaOH</td><td>40.00 g/mol</td></tr></table></td></tr>
<tr><td>Sucrose</td><td>Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.
This substance is not classified as dangerous according to Directive 67/548/EEC.
Web: http://www.merckmillipore.com/GB/en/product/Sucrose,MDA_CHEM-107653</td></tr>
<tr><td></td><td><table><tr><th>CAS #</th><th>EC Number</th><th>Hill Formula</th><th>Molar Mass</th></tr><tr><td>57-50-1</td><td>200-334-9</td><td>$C_{12}H_{22}O_{11}$</td><td>342.29 g/mol</td></tr></table></td></tr>
</table>

1.7 References

1. *Determination of Sugar as Glucose in a Soft Drink Using the LAMBDA PDA UV/Vis Spectrophotometer. APPLICATION NOTE*, [no date]. Online. Perkin Elmer. [Accessed 8 August 2024]. Available from: https://www.perkinelmer.com/lab-solutions/resources/docs/APP-Determination-of-Sugar-as-Glucose-in-Soft-Drink-using-LAMBDA-465-012356A_01.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 sugar as glucose in a soft drink

Learning unit	**Determination of sugar as glucose in a soft drink**
Category	Analytical chemistry, Food analysis Optical methods, Spectrophotometry
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 19:42:00
Print date	28/09/2025 19:42:00
Notes	

Chemical 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 PREPARING SOLUTIONS AND REAGENTS

Solution	**Alternative 1 Individual work**		**Alternative 2 Work in groups**	
	Volume*	**Notes**	**Volume***	**Notes**
Sucrose				
Hydrochloric acid	50 ml	2 ml x 9 = 18 ml		
Sodium hydroxide	100 ml	8 ml x 9 = 72 ml		
3,5-dinitrosalicylic acid	50 ml	2 ml x 9 = 18 ml		
Drink	2 ml			

he volume of solution to be prepared should cover both the volume needed for titration and an additional “reserve. Take into consideration the following volumetric flasks – 25, 50, 100, 200, 250, 500, 1000, and 2000 ml.
Take into account 9 reagents dosage: blank, 5 standards, and 3 samples.

3. Prepare 50 ml of a sucrose stock solution with a mass concentration of c_m = 1000 mg.dl^{-1}.
   $m = c_m V$

   m(sucrose)= …………………… mg = …………………… g

4. Prepare 25 ml of sucrose calibration solutions with concentrations of 20, 40, 60, 80, and 100 mg.dl^{-1} by diluting the stock solution (SS) with a concentration of 1000 mg.dl^{-1}.

TABLE 2 PREPARING CALIBRATION SOLUTIONS

Standard	**c_m (mg.dl^{-1})**	**V(ss) (ml)**
0	0	
1	20	
2	40	
3	60	
4	80	
5	100	

Model calculation for standard 1
Stock solution (1): c_{m1} = 1000 mg.dl^{-1}, V_1 = ... ml

Standard solution (2): c_{m2} = 20 mg.dl^{-1}, V_2 = 25 ml

The mass of sucrose transferred from stock solution (1) (c_1 = 1000 mg.dl^{-1}) and present in standard solution (2) (c_2 = 20 mg.dl^{-1}, 25 ml) is calculated as follows:

$$m_1 = m_2$$
$$c_{m1}V_1 = c_{m2}V_2$$
$$V_1 = \frac{c_{m2}V_2}{c_{m1}}$$

5. Prepare 50 ml of a sample solution diluted 200-times. Consult with your instructor regarding the dilution factor.
   Model calculation for sample S1
   Volume of the drink: $V(\text{sample}) = \frac{V(\text{dil. solution})}{D_f}$
   Volume of the sample: V(sample)= …………………… ml

TABLE 3 DILUTION OF SAMPLE

Sample	V(sample) (ml)	V(dil. Solution) (ml)	D_f
S1			
S2			
S3			

6. Prepare 50 ml of hydrochloric acid solution with a concentration of c = 6 $mol.dm^{-3}$ by diluting from a 36% solution.
   M(HCl) = 36,461 $g.mol^{-1}$, ρ(36% HCl) = 1,1791 $g.cm^{-3}$
   $$V(36\%\ HCl) = \frac{cMV}{w\rho}$$
   V(36% HCl) = ... ml

7. Prepare 100 ml of sodium hydroxide solution with a concentration of c = 2,5 $mol.dm^{-3}$.
   M(NaOH) = 39,997 $g.mol^{-1}$
   $$m(NaOH) = cMV$$
   m(NaOH) = ... g

Data

1. Record the information about the sample.

TABLE 4 INFORMATION ABOUT SAMPLE

Sample	Item	Information
S1	Name	
	Sugar content	
	Web of producer	
S2	Name	
	Sugar content	
	Web of producer	
S3	Name	
	Sugar content	
	Web of producer	

2. Record the absorbance of the glucose standard solutions.

TABLE 5 ABSORBANCE OF STANDARDS

Standard	**c_m (mg.dl^{-1})**	**A**
1	20	
2	40	
3	60	
4	80	
5	100	

3. Record the absorbance of the samples.

TABLE 6 ABSORBANCE OF STANDARDS

Sample	**Name**	**A**
S1		
S2		
S3		

Results

1. Plot the graph of absorbance *A* vs. concentration of sucrose c_m.
    a. Label the axes of the graph.
    b. Insert a trend line (linear).
    c. Display the regression equation on the graph and the value of R^2.
    d. Insert the graph into this document or as an attachment.

2. Evaluate the measured data using linear regression.
Regression line equation (general):$y = ax + b$
Regression line equation (sucrose analysis): $A = ac_m b$

TABLE 7 PARAMETERS OF LINEAR REGRESSION

a	**b**	**r^2**

2. Calculate the concentration of sucrose in the sample.
Model calculation for sample S1
Concentration of sucrose in the sample: $c_m = \frac{A-b}{a}$

c_m = …………………… mg.dl^{-1}

The original concentration of sugar in the soft drinks:

$$c_m(original\,) = c_m(diluted\,)D_f$$

c_m(original) = …………………… mg.dl^{-1} = …………………… g.dl^{-1}

TABLE 8 CONCENTRATIONS OF SUGAR IN SOFT DRINK SAMPLES

Sample	**Name**	**c_m(diluted) (mg.dl^{-1})**	**D_f**	**c_m(original) (mg.dl^{-1})**	**c_m(original) (g.dl^{-1})**
S1					
S2					
S3					

3. Calculate the absolute error, relative error, and the percent error. Ask the teacher for the exact value. This step is applicable only if you analyzed a model sample with a known concentration.
Model calculation for sample S1
Concentration of sucrose: c(sucrose, exact value) = …………………… mg/dL
Concentration of sucrose: c(sucrose, approximation) = …………………… mg/dL

Given some value *v* and its approximation *v*$_{\text{approx}}$, the **absolute error** is

$$\varepsilon = |v - v_{approx}|$$

where the vertical bars denote the absolute value. If $\nu \neq 0$ the **relative error** is

$$\eta = \frac{|v - v_{approx}|}{|v|}$$

and the **percent error** is

$$\eta = \frac{|v - v_{approx}|}{|v|}100$$

Conclusions

………

………

………

Questions

Analytical chemistry

1. Describe the principle of VIS spectrometry.
2. Describe the principle of spectrophotometric determination of sucrose using 3,5-dinitriosalicylic acid.
3. Explain why sodium hydroxide and sodium-potassium tartrate are added when preparing a solution of 3,5-dinitriosalicylic acid.
4. Explain why hydrochloric acid is added to the sucrose solution and the solution is boiled.
5. Explain why sodium hydroxide is added in the process of determination of sugars by 3,5-dinitrosalicylic acid.
6. Explain why it is necessary to cover the test tube with parafilm.
7. Propose a method to determine glucose and sucrose in a soft drink sample. The aim of the procedure is to determine the amount of glucose and sucrose in the sample.
8. The absorbance of the solution is measured at 580 nm. Explain how the red-brown colour of the solution is related to the selected wavelength.
9. Compare the DNSA and anthrone method for determining sugars.
10. List two commonly used tests for reducing sugars and explain the principle of each test.

Food chemistry

1. What are the three most frequently occurring soluble sugars in soft drinks? Which of these are monosaccharides and which are disaccharides?
2. Define reducing sugars.
3. Low-calorie sweeteners (LCS) are sweeteners that contain few to no calories but have a higher intensity of sweetness per gram than sweeteners with calories. These include artificial sweeteners, such as Aspartame and Sucralose, as well as extracts from plants like steviol glycosides and monk fruit. Beverages containing LCS sometimes carry the label “sugar-free” or “diet.” Discuss the health effects of LCS.

Applied informatics

1. Find the chemical and physical properties of 3,5-dinitriosalicylic acid. To find the properties and structure of a chemical, you can use the ChemSpider or PubChem databases.
   ChemSpider: https://www.chemspider.com/StructureSearch.aspx
   PubChem: https://pubchem.ncbi.nlm.nih.gov/
   a. Find the IUPAC name, formula and CAS registration number of 3,5-dinitriosalicylic acid.
   b. Find the dissociation constant of 3,5-dinitriosalicylic acid.
   c. Find the solubility of 3,5-dinitriosalicylic acid in water at 25°C (20°C).
   d. Download the structure of 3,5-dinitriosalicylic acid in .mol format.
   e. Open the .mol file in the ChemSketch program.
2. Draw the reaction of 3,5-dinitriosalicylic acid with glucose using chemical software, such as ChemSketch.

   Tip: To insert pre-drawn templates of sugars use “Open Template Window” function .
3. Melting point test, TLC, IR, NMR, or GC-MS can be used to identify the product. Find the IR spectrum and melting point of 3,5-dinitriosalicylic acid. Use PubChem database (https://pubchem.ncbi.nlm.nih.gov/) for melting point and NIST database for the IR spectrum (http://webbook.nist.gov/chemistry/). Search for information by CAS registration number.
4. Below is the Abstract of a scientific paper.

a. Search for the title, authors and 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.

Abstract
Transformation of renewable biomass into value-added chemicals and biofuels has evolved to be a vital field of research in recent years. Accurate estimation of reducing sugars post pretreatment of lignocellulosic biomass has been very inconsistent. For a few decades, 3,5-dinitrosalicylic acid (DNS) assay has been widely employed for the estimation of reducing sugars derived from pretreatment of lignocellulosic biomass. This assay tests for the presence of free carbonyl group (C=O), the so-called reducing sugars. This involves the oxidation of the aldehyde functional group present to the corresponding acid while DNS is simultaneously reduced to 3-amino-5-nitrosalicylic acid under alkaline conditions. However, the presence of other active carbonyl groups can potentially also react with DNS leading to incorrect yields of reducing sugars. Therefore, a detailed study has been carried out to evaluate the influence of active carbonyl compounds like furfural and 5-hydroxymethylfurfural (5-HMF) in the overall estimation of reducing sugars (glucose, xylose and arabinose) by DNS assay. In addition to this, reducing sugars estimation in the presence of furans were also investigated, it reveals that reducing sugars estimation was found to be 68% higher than actual sugars. Therefore, current findings strongly indicate that the employment of DNS assay for quantifying the reducing sugars in the presence of furans is not appropriate.

5. In the paper “Quantification of total sugars and reducing sugars of dragon fruit-derived sugar-samples by UV-Vis spectrophotometric method” the development of the phenol-sulfuric acid method and the 3,5-dinitrosalicylic acid (DNS) method is described. What is the amount of sample taken for analysis, the amount of DNSA reagent, the heating time, and the linearity range?
6. The application note “Determination of Sugar as Glucose in a Soft Drink Using the LAMBDA PDA UV/Vis Spectrophotometer” was used as a reference for procedure in this lab manual. Explain why the application note is a good source of chemical information.
7. Look up information about the spectrophotometer 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) Wavelength range
    b) Band width
    c) Reading
    d) Source of light
    e) The instrument manual in English
    f) The price of the device at the seller in your country in EUR and in the currency of your country.
8. What functions did you use in your spreadsheet program to evaluate the data by least square method (linear regression)?

Laboratory manual

Determination of sulfur dioxide in wine

Organisation	Stredná odborná škola chemická, Vlčie hrdlo 50, 821 07 Bratislava
Author	Judita Dömötörová
Revised by	Judita Dömötörová
Save date	28/09/2025 19:49:00
Print date	28/09/2025 19:49:00

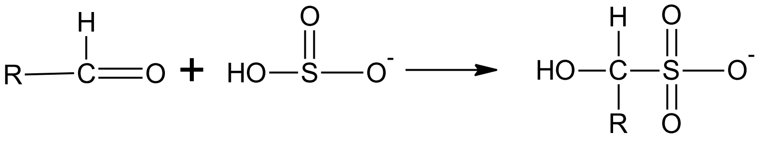
Introduction

Sulfur dioxide in wine

Sulfur dioxide and sulfites have long been used as **preservatives**, serving both as **antimicrobial substance and as antioxidant**. Their use as preservatives in wine dates back to Roman times. Sulfur dioxide is a gas that can be used in compressed form in cylinder. It is liquid under pressure of 3,4 atm and can be injected directly in liquids. It can also be used to prepare solutions in ice cold water. It dissolves to form sulfurous acid. Instead of sulfur dioxide solutions, a number of **sulfites** can be used because when dissolved in water, they all yield active SO_2.

SO_2 (gas) → SO_2 (aq)
SO_2 (aq) + H_2O → H_2SO_3 (aq)
$H_2SO_3 \rightarrow H^+ + HSO_3^-$ ($K_1=1{,}7.10^{-2}$)
$HSO_3^- \rightarrow H^+ + SO_3^{2-}$ ($K_2=5.10^{-6}$)
$2HSO_3^- \rightarrow S_2O_5^{2-} + H_2O$

All of these forms of sulfur are known as **free sulfur dioxide**. The bisulfite ion (HSO_3^-) can react with aldehydes, dextrins, pectic substances, proteins, ketones, and certain sugars from addition compounds.



$$R-CH=O + HO-S(=O)-O^- \longrightarrow HO-CH(R)-S(=O)_2-O^-$$

Table Sources of SO_2 and their content of active SO_2

Chemical	**Formula**	**Content of active SO_2**
Sulfur dioxide	SO_2	100,00%
Sodium sulfite, anhydrous	Na_2SO_3	50,82%
Sodium sulfite, heptahydrate	$Na_2SO_3.7\ H_2O$	25,41%
Sodium hydrogen sulfite	$NaHSO_3$	61,56%
Sodium metabisulfite (disulfite)	$Na_2S_2O_5$	67,39%
Potassium metabisulfite (disulfite)	$K_2S_2O_5$	57,63%
Calcium sulfite	$CaSO_3$	64,00%

Reference: Principles of Food Chemistry (John M. DeMan)

The addition compounds are known as **bound sulfur dioxide**. Sulfur dioxide is extensively used in wine making, and in wine acetaldehyde reacts preferentially with bisulfite. Excess bisulfite reacts with sugars.

It is possible to classify bound SO_2 into three forms: aldehyde sulfurous acid, glucose sulfurous acid, and rest sulfurous acid. The latter holds the SO_2 in less tightly bound form. Sulfites in wine serve a dual purpose: (1) antiseptic or bacteriostatic and (2) antioxidant. These activities are dependent on the form of SO_2 present. The various forms of SO_2 in wine are represented schematically in figure.

The free SO_2 includes the **water-soluble SO_2** and the **undisociated H_2SO_3 and constitutes** about 2,8% of the total. The **bisulfite** form constitutes 96,3% and the **sulfite** form 0,9% (all at pH 3,3 and 20°C). The bound SO_2 is mostly (80%) present as **acetaldehyde SO_2**, 1% as **glucose SO_2**, and 10 to 20 % as **rest SO_2**. The various forms of sulfite have different activities. The two free forms are the only ones with antiseptic activity. The antiseptic activity is limited to the SO_3^{2-} ion. **The antiseptic activity of SO_2 is highly dependent on the pH.** The lower the pH the greater the antiseptic action of SO_2. Sulfurous acid inhibits molds and bacteria and to a lesser extent yeasts. For this reason, SO_2 can be used to control undesirable bacteria and wild yeasts in fermentation without affecting the SO_2–tolerant cultured yeasts. The amount of SO_2 added to foods is self-limiting because at levels from 200 to 500 ppm the product may develop an unpleasant of-flavor.

Sulfur dioxide destroys thiamine (vitamin B_1) and is therefore not permitted for use in foods containing this vitamin. SO_2 is also widely used in **dried fruits**. Other applications are in **dried vegetables** and dried potato products.

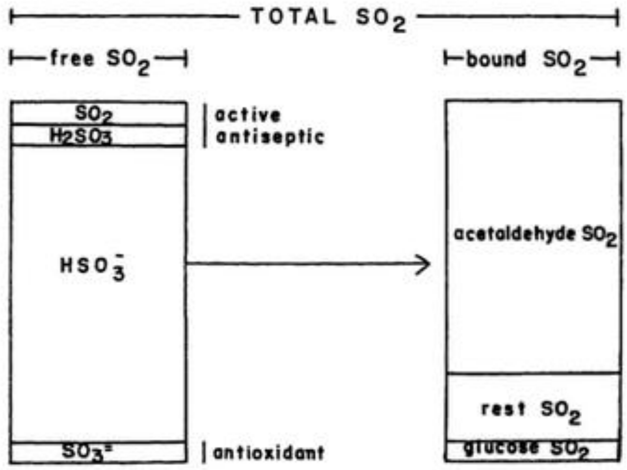


Figure 11–1 The Various Forms of SO_2 in Wine and Their Activity. *Source:* Reprinted with permission from J.M. deMan, 500 Years of Sulfite Use in Winemaking, *Am. Wine Soc. J.*, Vol. 20, pp. 44–46, © 1988, American Wine Society.

Determination of sulfur dioxide in wine

Free sulfur dioxide is determined by direct titration with iodine using starch indicator solution. The total sulfur dioxide is determined by iodimetric titration after alkaline hydrolysis.

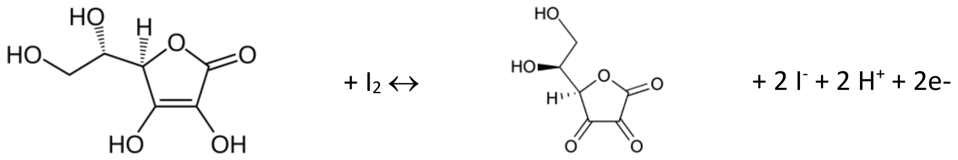
Reaction equation:

$H_2SO_3 + I_2 + H_2O \rightarrow H_2SO_4 + 2\ HI$

Standardization of iodine solution

Iodine solution is standardized by titrating of the standard ascorbic acid solution using starch indicator solution.

Reaction equation:



Preparing of iodine solution

Iodine is sparingly soluble in water but undergoes rapid dissolution in the presence of potassium iodide due to the formation of the corresponding triodide ion:

$I_2 + KI \rightarrow I_3^-$

Thus, potassium iodide plays dual role – to solubilize iodine in aqueous KI solution, as reducing agent, the excess KI helps in retaining liberated I_2 in solution through interaction with KI.

Assignment

1. Familiarize yourself with the dangerous properties of the substances you will be working with.
2. Prepare the solutions and reagents.
3. Standardize the iodine solution with ascorbic acid.
4. Determine free, total, and bounded sulfur dioxide in wine.
5. Complete the worksheet.
6. Answer the questions on the worksheet.

Materials

Analytical balance, weighting boat, chemical spoon, beakers, stirring rod, water bottle, volumetric flasks with stoppers (100 ml, 1000 ml), titration flask (250 ml), burette, stand, pipette (5 ml, 10 ml, 25 ml), glass marker pen, glassware labels

Chemicals

Standardization of iodine solution

1. Ascorbic acid solution, ($c(C_6H_8O_6) = 0{,}01\ mol.dm^{-3}$).
2. Iodine solution, ($c(I_2) = 0{,}01\ mol.dm^{-3}$): Dissolve 2,6 g of sublimated iodine in 100 ml of 25% KI solution a make up to 1000 ml with distilled water. Keep this solution in a dark-colored (or foil-wrapped) bottle and away from light.

Determination of sulfur dioxide

1. Sodium hydroxide solution, ($c(NaOH) = 1\ mol.dm^{-3}$)
2. Iodine solution, ($c(I_2) = 0{,}01\ mol.dm^{-3}$)
3. Sulfuric acid solution, 20% (w/w)
4. Starch solution: Mix 0,5 g of starch in 100 ml of cold water and boil for 2 minutes, let it cool to room temperature.

Procedure

Preparing of solutions

1. Calculate the quantities of the above substances needed to prepare the volumes of solutions as given in the worksheet.
    a. Ascorbic acid
    b. Iodine
    c. Starch
    d. Sodium hydroxide

Standardization of iodine solution

1. Pipette 10 ml of the ascorbic acid standard solution into a titration flask. Add 1 ml of starch solution.
2. Rinse the burette with iodine solution (use gloves) and fill the burette with iodine solution.
3. Titrate with iodine solution to blue color that remains at least 20 seconds. Record the volume of iodine $V(I_2)$ used.
4. Repeat the titration two more times. Results should agree within 0,1 ml.
5. Calculate the exact concentration of the iodine solution.

Determination of free sulfur dioxide

1. Introduce carbon dioxide into a 250 ml titration flask to displace oxygen.
   Note: If you start the analysis immediately after opening the bottle, you do not need to introduce carbn dioxide.
2. Pipette 50 ml of the wine sample; add 10 ml of 20% sulfuric acid solution and 5 ml of starch solution.
   Note: If you express the free sulfur dioxide in mg/kg (ppm), weight 50 ml of the sample.
3. Immediately titrate with iodine solution until blue color remains at least 30 seconds. Titrate quickly to minimize influence of interfering substances. Record the volume of iodine used, $\mathbf{V_a}$.
4. Repeat the titration two more times.

Determination of total sulfur dioxide

1. Weight accurately 50 g of the wine sample to 250 ml to titration flask, pipette 25 ml of 0,1 $mol.dm^{-3}$ sodium hydroxide solution. Mix the solution in flask and let it stand for 15 minutes.
2. Add 15 ml of 20% sulfuric acid solution and 5 ml of starch solution.
3. Titrate the solution using the method described above for the determination of free sulfur dioxide. Record the volume of iodine used, $\mathbf{V_b}$.
4. Repeat the titration two more times.

Determination of bounded sulfur dioxide

1. Bounded SO_2 is calculated from difference of total SO_2 and free SO_2.

Duration of analysis:

Three parallel determinations of free SO_2 (first is indicative) take about 30 minutes, while total SO_2 about 50 minutes.

Accuracy:
The difference between the results of the two parallel determinations should not exceed ± 0,005 g/100 ml.

Use of method:
Method is suitable for fruity and grape wines. Other wines give less accurate results. In colored and turbid samples sulfur dioxide is determined by potentiometric titration. The electromotive force (EMF, cell potential) is measured as a function of the amount of titrant added.

Safety Information

<table>
<tr><th>Chemical</th><th>Safety Information</th></tr>
<tr><td>Ascorbic acid</td><td>Web: http://www.merckmillipore.com/GB/en/product/L%28%2B%29-Ascorbic-Acid,MDA_CHEM-100468</td></tr>
<tr><td></td><td><table><tr><th>CAS #</th><th>EC Number</th><th>Hill Formula</th><th>Molar Mass</th></tr><tr><td>50-81-7</td><td>200-066-2</td><td>$C_6H_8O_6$</td><td>176.12 g/mol</td></tr></table></td></tr>
<tr><td>Iodine</td><td>Hazard Statement(s)
H312 + H332: Harmful in contact with skin or if inhaled
H315: Causes skin irritation.
H319: Causes serious eye irritation.
H335: May cause respiratory irritation.
H372: Causes damage to organs through prolonged or repeated exposure if swallowed.
H400: Very toxic to aquatic life.
Precautionary Statement(s)
P273: Avoid release to the environment.
P302 + P352: IF ON SKIN: Wash with plenty of soap and water.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P314: Get medical advice/ attention if you feel unwell.
Web: http://www.merckmillipore.com/GB/en/product/Iodine,MDA_CHEM-104761</td></tr>
<tr><td></td><td><table><tr><th>CAS #</th><th>EC Number</th><th>Hill Formula</th><th>Molar Mass</th></tr><tr><td>7553-56-2</td><td>231-442-4</td><td>I_2</td><td>253.8 g/mol</td></tr></table></td></tr>
<tr><td>Potassium iodide</td><td>Hazard Statement(s)
H372: Causes damage to organs through prolonged or repeated exposure if swallowed.
Precautionary Statement(s)
P314: Get medical advice/ attention if you feel unwell.
Web: http://www.merckmillipore.com/GB/en/product/Potassium-iodide,MDA_CHEM-105043</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></tr><tr><td>7681-11-0</td><td>231-659-4</td><td>IK</td><td>KI</td><td>166.00 g/mol</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.</td></tr>
</table>

<table>
<tr><th>Chemical</th><th>Safety Information</th></tr>
<tr><td></td><td>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></tr><tr><td>1310-73-2</td><td>215-185-5</td><td>$HNaO$</td><td>$NaOH$</td><td>40.00 g/mol</td></tr></table></td></tr>
<tr><td>Sodium hydroxide</td><td>Sodium hydroxide solution 1 mol/L
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-solution-1-mol/L,MDA_CHEM-137031</td></tr>
<tr><td>Starch</td><td>Web: http://www.merckmillipore.com/GB/en/product/Starch,MDA_CHEM-101252</td></tr>
<tr><td></td><td><table><tr><th>Empirical Formula</th><th>CAS #</th></tr><tr><td>$(C_6H_{10}O_5)_n$</td><td>9005-84-9</td></tr></table></td></tr>
<tr><td>Sulfuric acid</td><td>Sulfuric acid 96%
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/Sulfuric-acid-960/0,MDA_CHEM-100714</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></tr><tr><td>7664-93-9</td><td>231-639-5</td><td>H_2O_4S</td><td>H_2SO_4</td><td>98.08 g/mol</td></tr></table></td></tr>
<tr><td>Sulfuric acid</td><td>$c(H_2SO_4) = 0.25$ mol/l (0.5 N)
Hazard Statement(s)
H290: May be corrosive to metals.
Web: http://www.merckmillipore.com/GB/en/product/Sulfuric-acid,MDA_CHEM-109073</td></tr>
</table>

References

1. DeMan J. M.: *Principles of Food Chemistry*. Springer. 3rd edition. 1999. ISBN: 083421234X
2. Silva, C. R., Simoni, J. A., Collins, C. H., & Volpe, P. L. O. (1999). Ascorbic Acid as a Standard for Iodometric Titrations. An Analytical Experiment for General Chemistry. *Journal of Chemical Education*, *76*(10), 1421. doi: 10.1021/ed076p1421

3. Príbela A.: *Analýza potravín. Cvičenie.* Slovenská vysoká škola technická : Bratislava. 1. vyd. 1987.
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Worksheet

Determination of sulfur dioxide in wine

Learning unit	Determination of sulfur dioxide in wine
Category	Food analysis Volumetric analysis, Iodometry
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 19:49:00
Print date	28/09/2025 19:49:00
Notes	

Chemical 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 SOLUTIONS AND REAGENTS

Solution	Alternative 1 Individual work		Alternative 2 Work in groups	
	Volume*	**Notes**	**Volume***	**Notes**
Ascorbic acid	100 ml	10 ml/titration x 3 titrations = 30 ml		
Iodine	200 ml	*Standardization:* 10 ml/titration x 3 titrations = 30 ml *Determination of SO_2:* 10 ml/titration x 6 titrations = 60 ml *Total: 90 ml*		
Sulfuric acid (20%)	100 ml	*Determination of SO_2:* 10 ml/titration x 3 titrations + 15 ml/titration x 3 titrations = 75 ml		
Sodium hydroxide	100 ml	25 ml/titration x 3 titrations = 75 ml		

Starch indicator	50 ml	*Standardization:* 1 ml/titration x 3 titrations = 3 ml *Determination of SO_2:* 5 ml/titration x 6 titrations = 30 ml *Total: 33 ml*		
Wine sample	350 ml	50 ml/titration x 6 titrations = 300 ml		

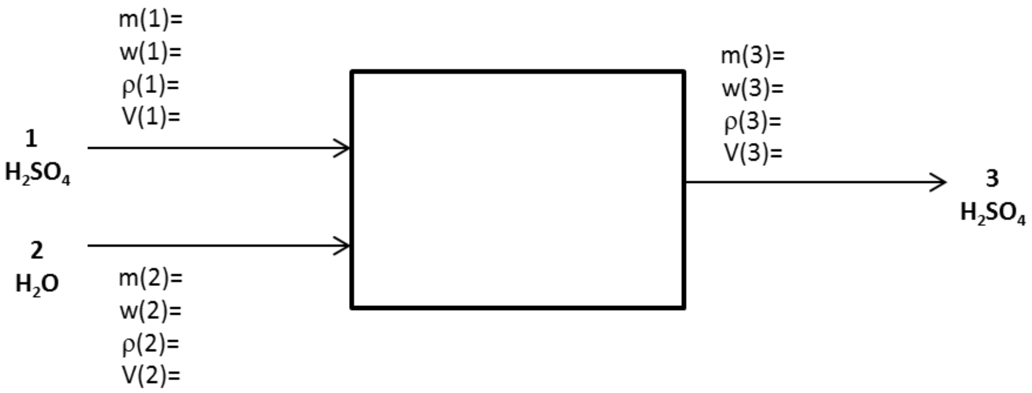
* Volume of solution to be prepared covers also a “reserve”. Take into consideration the following volumetric flasks – 25 ml, 50 ml, 100 ml, 200 ml, 250 ml, 500 ml, 1000 ml, and 2000 ml.

3. Prepare 100 ml of ascorbic acid standard solution at a concentration of 0,01 mol.dm^{-3}. Calculate the mass of ascorbic acid.
   $M(C_6H_8O_6)$= 176,1241 g.mol^{-1}
   $m(C_6H_8O_6) = c.M.V$
   $m(C_6H_8O_6)$= .... g

4. Prepare 200 ml of iodine solution at a concentration of 0,01 mol.dm^{-3}. The procedure is described in manual. Adjust the amounts of chemicals.

   $m(I_2)$ = …………………… g, m(KI) = …………………… g, $V(H_2O)$ = …………………… ml

5. **Prepare 100 ml of a 20% (w/w) sulfuric acid solution using 96% sulfuric acid.**
   $M(H_2SO_4)$= 98,078 g.mol^{-1}
   $\rho(H_2SO_4, 96\%)$= 1,8355 g.cm^{-3}
   $\rho(H_2SO_4, 20\%)$= 1,1398 g.cm^{-3}
   $\rho(H_2O)$= 0,9982 g.cm^{-3}



1) – original (concentrated) H_2SO_4 solution, **2)** - H_2O, **3)** – final (diluted) H_2SO_4 solution

Material balance:
Total material balance of a system: $m(1) + m(2) = m(3)$
Material balance of a component: H_2SO_4: $m(1).w(1) + m(2).w(2) = m(3).w(3)$
where w(2) = 0

a. Mass of diluted sulfuric acid solution (3): $m(3) = \rho(3).V(3)$

b. Mass of concentrated sulfuric acid solution (1): $m(1) = \frac{m(3).w(3)}{w(1)}$

c. Volume of concentrated sulfuric acid solution (1): $V(1) = \frac{m(1)}{\rho(1)}$

d. Mass of water (2): $m(2) = m(3) - m(1)$

e. Volume of water (2): $V(2) = \frac{m(2)}{\rho(2)}$

To prepare 100 ml of 20% H_2SO_4 solution we need: .... ml of 96% H_2SO_4 and .... ml of H_2O.

6. Prepare 50 ml of starch solution.
   Mix 0,5 g of starch in 100 ml of cold water and boil for 2 minutes. If you need different volume of starch solution as given above, recalculate the quantity of starch and water.

   m(starch) = ... g, m(H_2O) = ... ml

7. Prepare 100 ml of a 1 mol.dm^{-3}sodium hydroxide solution.
   M(NaOH)= 39,9971 g,mol^{-1}
   $m(NaOH) = c.M.V$
   m(NaOH)= .... g

Data

1. Record the information about the sample.

TABLE 2 SAMPLE INFORMATION

Sample	Parameter	Wine label
Sample 1	Appellation (name)	
	Producer	
	Bottler	
	Date of manufacture	
	Country of origin	
	Alcoholic degree	
Sample 2	Appellation (name)	
	Producer	
	Bottler	
	Date of manufacture	
	Country of origin	
	Alcoholic degree	

Chapter IV, Section 1, of Regulation 607/2009 sets out rules for the indication of compulsory information on wine labels. The mandatory information must appear in the same field of vision on the container, in such a way that all the information (except the lot number) is readable without having to turn the container. The mandatory information must be clearly distinguishable from surrounding text or graphics. Ingredients which may trigger an allergic reaction (see Annex IIIa to labeling directive 200/13/EC) must be indicated on the label preceded by the word “contains”.
For the indication of sulphites, the following terms may be used: “*sulphites*”, “*sulfites*”, “*sulphur dioxide*” or “*sulfur dioxide*”. The indication of sulphites may be accompanied by the pictogram included in Annex X to Regulation 607/2009.

2. Record the exact mass of ascorbic acid you have weighted.
   $m(C_6H_8O_6)$= …………………… g

3. Record the volume of iodine solution used for standardization.

Table 3 Volume of iodine solution

Trial	**$V(I_2)$ (ml)**
1	
2	
3	
Average	

4. Record the volume of iodine solution used for the determination of sulfur dioxide.

Table 4 Consumption of iodine solution

Sample	**Trial**	**Free SO_2**		**Total SO_2**	
		m (g)*	**V_a (ml)**	**m (g)***	**V_a (ml)**
Sample 1	1				
	2				
	3				
Sample 2	1				
	2				
	3				

* The mass of sample is to be determined when results are given in mg/kg (ppm), otherwise results are given in mg/l.

Results

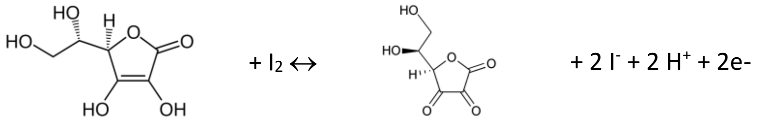
1. Calculate the exact concentration of ascorbic acid using mass you have weighted.

$$c(C_6H_8O_6) = \frac{m}{M.V}$$

$c(C_6H_8O_6)$ = …………………… mol.dm^{-3}

2. Calculate the exact concentration of the iodine solution.

Reaction equation:



Moles of ascorbic acid: $n(C_6H_8O_6) = c(C_6H_8O_6).V(C_6H_8O_6)$

Moles of iodine: $n(I_2) = n(C_6H_8O_6)$

Concentration of iodine: $c(I_2) = \frac{n(I_2)}{V(I_2)}$

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

$$c(I_2) = \frac{c(C_6H_8O_6).V(C_6H_8O_6)}{V(I_2)}$$

where
$c(C_6H_8O_6)$ is the concentration of ascorbic acid, in $mol.dm^{-3}$
$V(C_6H_8O_6)$ is the volume of ascorbic acid solution, in dm^3
$c(I_2)$ is the concentration of iodine, in $mol.dm^{-3}$
$V(I_2)$ is the volume of iodine, in dm^3

3. Calculate the content of free sulfur dioxide in mg/kg or mg/L using formula:

$$w(\mathrm{SO_2}) = \frac{V_a K.0{,}64.1000}{m}$$

where
$w(SO_2)$ is the sulfur dioxide content, in mg/kg (ppm)
V_a is the volume of I_2, ($c(I_2) = 0{,}01\ mol.dm^{-3}$), in ml ($cm^3$)
m is the mass of sample (50 ml of wine sample), in g
K is the factor for the iodine solution

$$K = \frac{c(I_2,\ actual)}{c(I_2,\ no\min a\,l)}$$

Factor K is the ratio of the actual concentration of a standard solution $c(X)_{ACTUAL}$ (actual value) and the desired concentration of the same solution $c(X)_{NOMINAL}$ (nominal value).
As an example, for an iodine solution with a nominal concentration $c(I_2)_{NOMINAL} = 0{,}01$ mol/l and an actual concentration $c(I_2)_{ACTUAL} = 0{,}0098$ mol/l, the factor K is calculated as follows:

$$K = \frac{c(I_2,\ actual)}{c(I_2,\ no\min a\,l)} = \frac{0{,}0098\ mol/l}{0{,}01\ mol/l} = 0{,}98$$

In order to obtain the consumption of a solution of concentration $c(I_2) = 0{,}01$ mol/l, the volume of the consumed iodine solution in the titration has to be multiplied by the factor K = 0,98.

$$c_m(\mathrm{SO_2}) = \frac{V_a \cdot \mathrm{K} \cdot 0{,}64 \cdot 1000}{V}$$

where
c_m is the concentration of sulfur dioxide, in mg/L
V_a is the volume of I_2, ($c(I_2) = 0{,}01\ mol.dm^{-3}$), in ml ($cm^3$)
V is the volume of sample, in ml (V=50 ml)
K is the factor for the iodine solution

Free sulfur dioxide, $w(SO_2)$ = ... mg/kg (ppm)

$c_m(SO_2)$ = ... mg/L

4. Calculate the content of total sulfur dioxide in mg/kg or mg/L using formula:

$$w(SO_2) = \frac{V_a \cdot K \cdot 0{,}64 \cdot 1000}{m}$$

Total sulfur dioxide, $w(SO_2)$ = ... mg/kg (ppm)

$$c_m(\mathrm{SO_2}) = \frac{V_a \cdot \mathrm{K} \cdot 0{,}64 \cdot 1000}{V}$$

$c_m(SO_2)$ = ... mg/L

5. Calculate the content of bound sulfur dioxide in mg/kg using formula:

   $w(SO_2, bound) = w(SO_2, total) - w(SO_2, free)$

   Bound sulfur dioxide, $w(SO_2)$ = ... mg/kg (ppm)

   $c_m(SO_2, bound) = c_m(SO_2, total) - c_m(SO_2, free)$

   $c_m(SO_2)$ = ... mg/L

6. Calculate the content of free sulfur in mg/kg:

   $$w(S) = \frac{V_a \cdot K \cdot 0{,}32 \cdot 1000}{m}$$

   Free sulfur, w(S) = ... mg/kg (ppm)

   Molar masses of individual forms:
   $M(SO_3^{2-})$= 80,063 $g.mol^{-1}$
   $M(SO_2)$= 64,064 $g.mol^{-1}$
   M(S)= 32,065 $g.mol^{-1}$

   $$w(S) = w(SO_2)\frac{M(S)}{M(SO_2)} = 0{,}501.w(SO_2)$$

7. Display the results in table below.

Table 5 Sulfur dioxide content in wine

Sample	Trial	Free SO_2		Bound SO_2		Total SO_2	
		mg/kg* (ppm)	mg/l	mg/kg* (ppm)	mg/l	mg/kg* (ppm)	mg/l
Sample 1	1						
	2						
	3						
	Average						
Sample 2	1						
	2						
	3						
	Average						

* The mass of sample is to be determined when results are given in mg/kg (ppm), otherwise results are given in mg/l.

According to COMMISSION REGULATION (EC) No 606/2009 (of 10 July 2009 laying down certain detailed rules for implementing Council Regulation (EC) No 479/2008 as regards the categories of grapevine products, oenological practices and the applicable restrictions), the total sulphur dioxide content of wines, other than sparkling wines and liqueur wines, on their release to the market for direct human consumption, may not exceed:
(a) 150 milligrams per litre for red wines;
(b) 200 milligrams per litre for white and rosé wines.

Source: https://eur-lex.europa.eu/legal-content/GA/TXT/?uri=CELEX:02009R0606-20150930

Conclusion

……

……

……

Questions

Food chemistry and technology

1. Why is sulfur dioxide added to wine? What are its functions?
2. What are the other applications of sulfur dioxide in food technology?
3. At pH 3,3 and 20°C, what percentages of total free SO_2 are present as H_2SO_3, HSO_3^-, and SO_3^{2-} in wine?
4. Which form of sulfur dioxide in wine exhibits antiseptic activity and how does pH affect this activity?
5. Why is sulfur dioxide not permitted for use in foods containing thiamine (vitamin B1)?
6. Evaluate the health implications of consuming foods preserved with sulfur dioxide, especially for individuals with sensitivities or allergies.
7. How has the use of sulfur dioxide in wine preservation evolved from Roman times to the present day?
8. What UNESCO world heritage wine regions would you like to visit and why?

Analytical chemistry

1. Describe why carbon dioxide is introduced into the sample during sulfur dioxide determination.
2. Describe the principle of determination of sulfur dioxide by iodometric titration.
3. Describe the principle of equivalent point indication using starch solution.
4. Explain why iodine is dissolved in potassium iodide solution to make an iodine solution.
5. Derive the formula to calculate free sulfur dioxide content $w(SO_2)$ in mg/kg wine. The final formula is given on page 5.
6. Derive the formula to calculate free sulfur content $w(S)$ in mg/kg wine.
   Algorithm of calculation: Mass fraction of sulfur → Mass of sulfur → Mass of sulfur dioxide → Moles of sulfur dioxide → Moles of iodine
7. Calculate how many mg/kg of free sulfur dioxide is equivalent to 1 ml of 0,01 M iodine in 1 g of sample.
   Result: …………. mg/kg of free sulfur dioxide is equivalent to 1 ml of 0,01 M iodine in 1 g of sample
   Algorithm of calculation: Moles of iodine → Moles of sulfur dioxide → Mass fraction of sulfur dioxide
8. Investigate modern analytical techniques used to measure sulfur dioxide levels in wine.

Applied informatics

1. To fumigate the wine potassium pyrosulfate is used. Find the IUPAC name, formula and CAS number of this chemical. To find the properties and structure of a pyrosulfate 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 potassium pyrosulfate.
   b. Find the solubility of potassium pyrosulfate in water at 25°C (20°C).
   c. Download the structure of potassium pyrosulfate in .mol format.
   d. Open the mol file in the ChemSketch program.

2. Draw the structure of amylose in the ChemSketch. Amylose is made up of alpha-D-glucose molecules bound with covalent bonds using an alpha (1,4) glycosidic bond.

   Tip: To insert pre-drawn templates of sugars use "Open Template Window" function ▦.
3. Which alpha,omega-dicarboxylic acid is one of the main organic acids identified in wine?
4. Find the dissociation constants (K_1 and K_2) of sulfurous acid in chemical tables (printed version) and online.
5. Find the diagram of ionisation forms of sulphur dioxide depending on pH value. 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. Based on the paper "Chemical and biochemical mechanisms of preservatives used in wine: A review"calculate the percentage of free sulfur dioxide in form of SO_2, SO_3^{2-} and HSO_3^- at pH = 3.
7. Investigate the regulatory limits for sulfur dioxide in foods using sources like the FDA or EFSA. How do these regulations ensure consumer safety?