Song and Dance through the eyes of science

EOES2023, 01.05.2023

Introduction to the task:

The Latvian Song and Dance Festival is one of the largest amateur choral and dancing events in the world and an important event in Latvian culture and social life. The All-Latvian Song Festival has been held since 1873, normally every five years, with the Latvian Dance Festival component added in 1948. Approximately 40,000 performers altogether participate in the event. As one of the Baltic song festivals, it is also a part of the UNESCO Masterpieces of the Oral and Intangible Heritage of Humanity list since 2008. Since 1960, a distinct Latvian School Youth Song and Dance Festival has been held in an alternate five-year cycle, on a matching scale.

In July 2023, 27th XXVII Latvian Song and XVII Dance Festival will take place.

In this task you will explore how the human body is able to dance and sing. For some experiments you will have to share resources- either samples or human resources.

Here are approximate times you will need to spend on each problem.
Problem 1 - Biochemical properties of the muscle - 1.5 hours
Problem 2 - Quantification of myoglobin - 1.5 hours
Problem 3 - Burning the calories - 1.5 hours
Problem 4 - Phonetics - 30 minutes
Problem 5 - Vowel simulation - 3 hours

Chemistry of dance

Problem1 - Biochemical properties of the muscles

Materials and equipment

- Three samples of muscle tissue, in Petri dishes on ice, labelled with “A”, “B” and “C”. Supplied when asked by student
- Dropper bottle with H_2O_2
- Mortar and pestle
- 6 empty centrifuge tubes, 2 ml
- Marker for marking the tubes, shared with problem 2
- 20 ml of Grinding buffer in the ice container in a 50ml centrifuge tube marked with “Grinding Buffer” and with black tape.
- Plastic spatula made of pipette for transferring ground muscle
- NAD solution in the ice container marked as “NAD” and with red line.
- 6 glass test tubes in rack
- 3 gradulated Plasticc pipettes marked for NAD, grinding buffer and reaction buffer with corresponding colours
- 3 micropipettes (P10, P100, P1000), shared with problem 2
- Micropipette tips, shared with problem 2
- Distilled water in wash bottle
- Waste container
- Paper tissues
- Enzyme reaction buffe, in a 50ml centrifuge tube marked with “REACTION BUFFER” and with yellow tape. It will be given to you by a lab assistant together with samples A1, B1 and C1 before proceeding with 1.4.3. Tubes A1, B1, C1 are also used in Problem2.

Introduction

For movement of various body parts we need muscles. This problem deals with muscle energetics and biochemistry.

Human muscles are divided into striated muscles, smooth muscles and cardiac muscles. The striated muscles can be divided into two types - red muscles and white muscles. As can be seen from the name, the colour of the muscle fibres differs. This is due to differences in muscle metabolism: red muscles are coloured by the protein myoglobin, which binds oxygen, and these muscles mainly produce energy aerobically (using oxygen). Red muscles are supplied with nutrients by the blood and are able to work for long periods. White muscles, on the other hand, produce energy anaerobically (without oxygen), so they can produce energy more quickly, but the end products of metabolism accumulate in the muscle, contributing to muscle fatigue. White muscles normally use reserve substances in the muscle itself for energy production. Many muscles will have mixed fibers - with red and white muscle properties.

In this work we will simulate red and white muscles with samples from chicken muscles. The samples are from chicken breast, thigh and gizzard.

Problem 1.1 . Sample description

1.1. *Observe the samples in front of you, match the letter with the correct term in the corresponding boxes in the answer sheet, so the sentence below would be correct. (2 p)*

While [1] is not a striated muscle, metabolically it is very close to [2] muscle fibers, that can be also deduced from its high myoglobin content.

Choices for [1]

a) Breast
b) Thigh
c) Gizzard

Choices for [2]

a) red
b) white

Problem 1.2. Metabolism of muscle

Look at the simplified schemes of metabolism for red and white muscle fibers:

1.2.1. *In the ANSWER SHEET write "W" for the scheme corresponding to metabolism of the white muscle fibres and "R" to the red. (2p)*

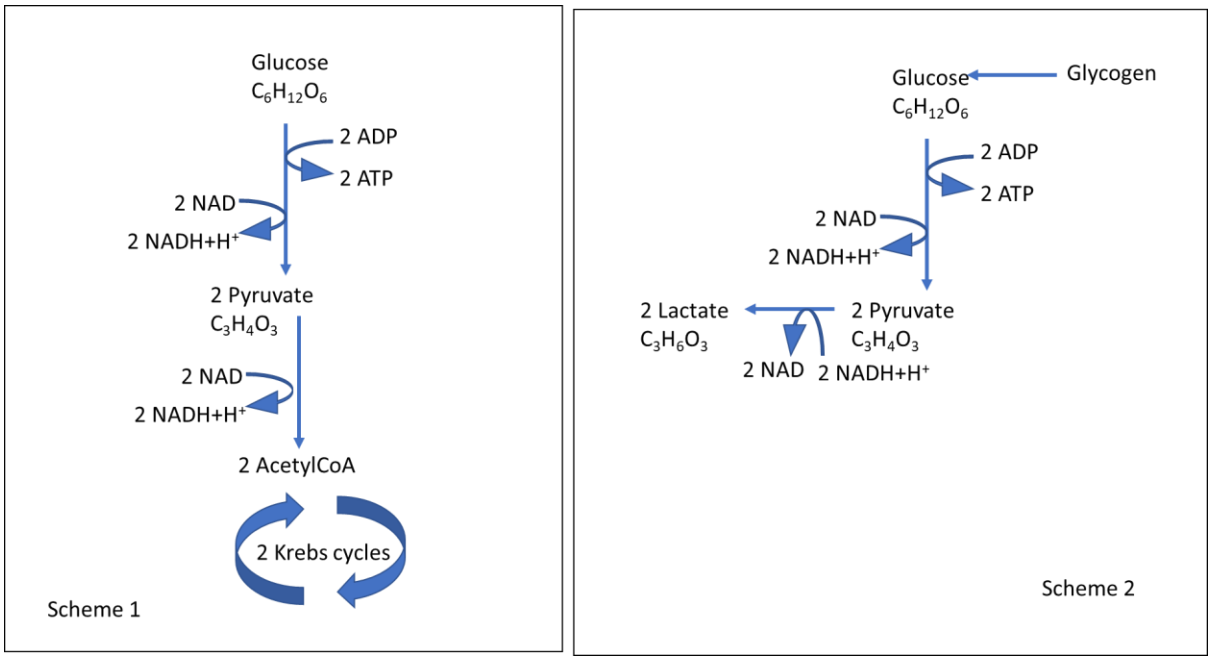


Figure 1.2.1. Simplified schemes of muscle metabolism

Human energetic metabolism is orientated towards production of energy - ATP. ATP can be produced in two ways - directly - by moving phosphate group on to ADP during biochemical reactions or indirectly. During indirect energy production at first reduced cofactors - $NADH+H^+$

and $FADH_2$ are produced. The energy of these cofactors is later converted to ATP in mitochondria, in the electron transport chain, where electrons travel through several protein complexes and are accepted by oxygen. During travel through these protein complexes protons are transported across mitochondrial membrane. The proton gradient ensures energy for ATP formation. Every cofactor ($NADH^+ + H^+ + FADH_2$) donates two electrons. See the scheme below.

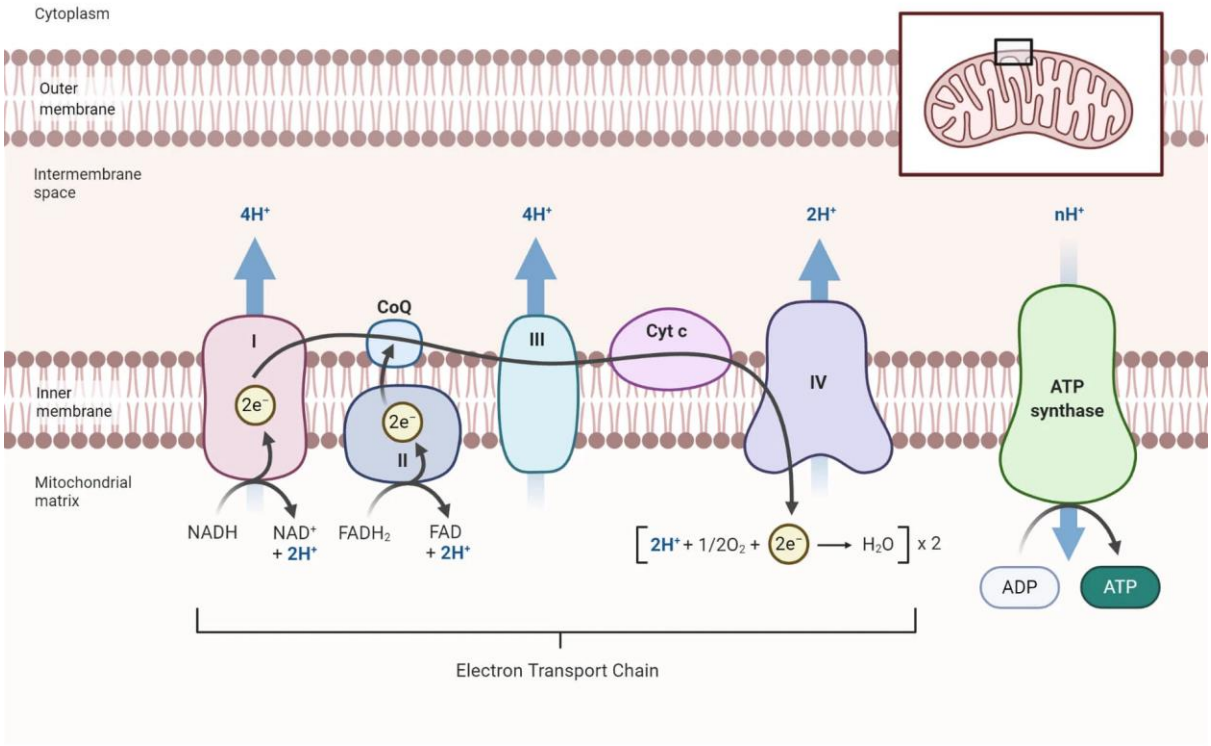


Figure 1.2.2. Electron transport scheme within mitochondria

***1.2.2**. Based on the information provided in the task, calculate how many ATP molecules can be produced from 1 glucose molecule in each type of muscle fibre and how many oxygen molecules would be used. Write the numbers in the answer sheet (4 p)*

In calculations use the following data: 1 Krebs cycle gives 1 ATP, 3 $NADH+H^+$ and 1 $FADH_2$. In the presence of oxygen, $NADH+H^+$ and $FADH_2$ are converted to ATP energy in the electron transport chain, 1 $NADH+H^+$ corresponds to 2.5 ATP and 1 $FADH_2$ to 1.5 ATP.

Metabolic reactions from scheme 1 produce _____ ATP molecules from 1 glucose molecule

Metabolic reactions from scheme 2 produce _____ ATP molecules from 1 glucose molecule

Number of oxygen molecules needed to produce your calculated ATP amount in scheme 1

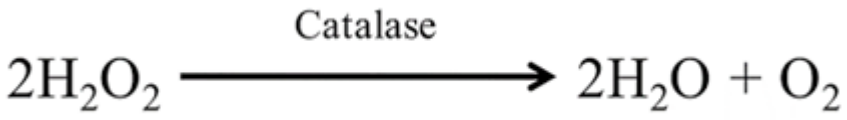
_____

Number of oxygen molecules needed to produce your calculated ATP amount in scheme 2

_____

Problem 1.3. Catalase reaction

While oxygen is a useful molecule as an electron acceptor in mitochondria, sometimes mistakes in electron transport within protein complexes happen and highly reactive oxygen species such as O_2 and H_2O_2 are formed. To eliminate these molecules our cells have antioxidant enzymes. One such enzyme is catalase. Catalase is one of the most active enzymes in the human body. One molecule of catalase can catalyse the decomposition of approximately 4 x 10^7 molecules H_2O_2 per second!



Take the three muscle samples that you have on your table and put a drop of H_2O_2 on each sample.

Observe the samples.

1.3.1. *In some samples you should see bubbles forming. Pick the right cause for bubble formation (1p)*

a) *A very active reaction heats up the muscle by with the air, which is trapped in muscle fibres, escapes.*
b) *With influx of O_2, mitochondria start to produce additional CO_2*
c) *Oxygen produced by the action of a catalase leaves the reaction in a form of gas*
d) *Hydrogen peroxide is unstable in light*

1.3.2. *Arrange your samples according to their catalase activity, starting from the most active one.(3 p)*

1.3.3. *Which of the statements explain the observed differences in the catalase activity ? (1 p)*

a) *The more oxygen is used by muscle, the higher amount of H_2O_2 produced*
b) *The more oxygen is used by muscle, the lower amount of H_2O_2 produced*
c) *Muscles being closer to lungs will have higher catalase activity*
d) *The larger the muscle, the higher the catalase activity*
e) *Myoglobin is the source of H_2O_2*

Problem 1.4. Lactate dehydrogenase activity

Lactate dehydrogenase is an enzyme that catalyses the conversion of pyruvic acid to lactic acid or *vice versa*. The direction of reaction depends on the available concentration of molecules.

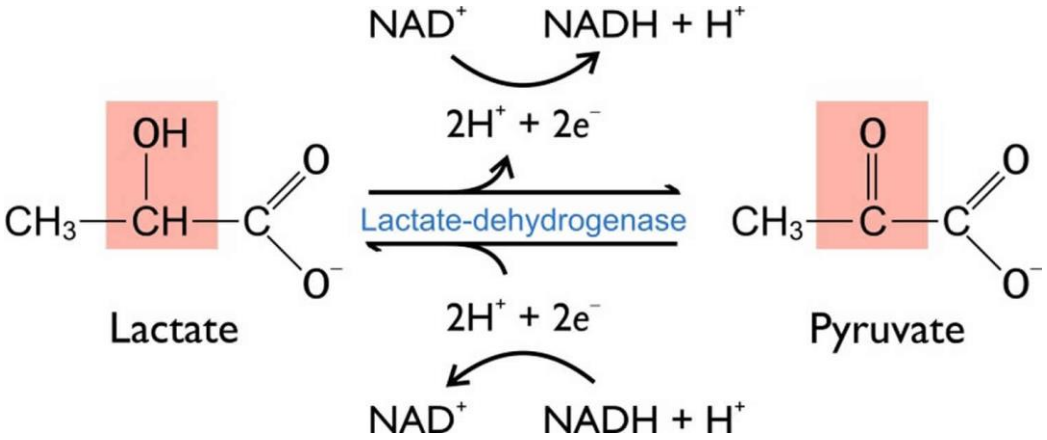


Figure 1.4.1. Reaction performed by enzyme lactate dehydrogenase

To visualise the activity of the enzyme in this lab work, we will use a dye that changes colour from yellow to purple when it is reduced.

1.4.1. *Complete the reaction scheme by writing the correct reactants in the answer sheet (2p)*

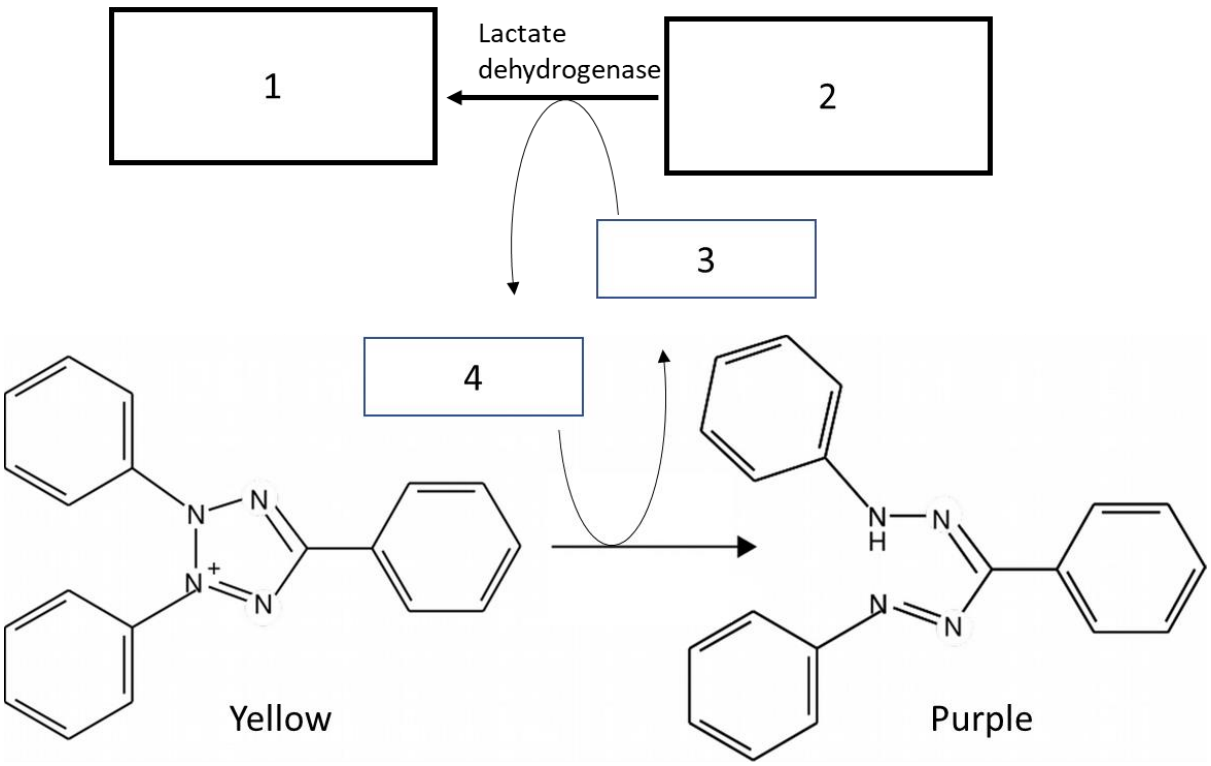


Figure 1.4.2. Reaction performed by enzyme lactate dehydrogenase

Assessment of lactate dehydrogenase activity

1. To obtain the enzyme, crush the muscle sample A in a mortar. It is ok to use the same sample you used for catalase activity assessment. Using the designated plastic pipette, add 4 ml of cold grinding buffer in the mortar to obtain a uniform mass.

1.4.2. When you have evenly mashed the tissue, *call the lab technician, who will record the result of your work together with your participant code.* You should do this with all three samples. (3p)

2. Transfer a part of your obtained paste in the centrifuge tube with a spatula (the tube should be filled up to 1.5 ml mark). Label it with the sample letter and your country abbreviation.
3. Clean the remaining tissue from the mortar and pestle with a paper tissue. Put the tube on ice
4. Repeat the same with samples B and C
5. Give all three filled and labelled centrifuge tubes to the lab assistant, they will take the tubes to the centrifuge and return them to you. Enzyme will be found in the supernatant.
6. Transfer as much supernatant as you can to clean a tube with the micropipette, try not to disturb the sediment. Label the new tubes with the same letters A, B, C. Note that these samples will also be used in Problem2

You will also receive 3 microcentrifuge tubes A1, B1 and C1 that are samples that were prepared by organisers from the same tissues, respectively as A, B and C, and have known enzymatic activity. All samples were prepared by completely homogenising equal amount of muscle. To test the activity of the enzyme you will have to record the colour of the tube contents every 30 seconds after the start of the reaction for 2.5 minutes (150 seconds) after the start of the reaction. Timer is projected on the wall.

To record colour use the following code

Yellow	*Orange*	*Red*	*Brown*	*Purple*
1	*2*	*3*	*4*	*5*

7. In the glass tube for every sample add reactants in following order:
   2 ml of reaction buffer
   10 microliters (µl) of muscle extract
   1 drop of NAD. HINT: Addition of NAD will start the reaction, therefore consider how to properly organise your pipetting

For addition of enzymes use micropipette, whereas for reaction buffer and NAD designated plastic pipettes should be used.

1.4.3. *Fill in the table with your recorded results in Answer Sheet. (6 p)*

After you have recorded the results, immediately call the lab assistant to verify your results. Points will be awarded for 1.4.3. only if the signature of the lab assistant is present

1.4.4. *Arrange samples A1 - C1 according to their lactate dehydrogenase activity, starting from the most active one in decreasing order.(3 p)*

1.4.5. *Compare the protein concentration in samples A and A1 based on your enzyme activity (1p)*

Problem 2 - Quantifying myoglobin in the sample

Note! You will have to use some solutions which you prepared during Problem 1.

Materials and equipment

- Wash bottle with distilled water shared with problem 1
- 1 ml cuvettes, 15 pieces each in a Petri dish
- 3 micropipettes (P10, P100, P1000), shared with problem 1
- Micropipette tips, shared with problem 1
- Spectrophotometer on a separate table, shared among the teams
- Chicken muscle extracts A, B, C prepared in Task1
- Samples A1, B1, C1, shared with problem 1
- 15 mL haemoglobin standard solution, with concentration 1.000 g/L
- 10 empty 15 mL tubes with caps in a rack
- Beaker for water
- Permanent marker, shared with Problem 1
- 10 mL measuring pipette
- 5 mL measuring pipette
- Pipette filler
- Millimeter paper
- Ruler
- Paper tissue, shared with problem 1

Introduction

Absorption of light by substances

Light is a form of energy. Therefore, all substances absorb light to a greater or lesser extent, using it as energy to excite atoms or molecules, including the breaking of bonds. Light absorption for dilute solutions in a certain range is directly proportional to the concentration of the substance - the more substance there is (the higher its concentration), the more light it absorbs, resulting in a higher absorbance A. This relationship is used for analytical purposes, most commonly for the determination of concentration of a substance.

Spectrophotometry

In a spectrophotometer a light source and a detector is used for measurements. Sample is inserted in a cuvette and placed between them. The light from the source passes through the sample and enters the detector. The apparatus then compares the intensity I_o of the light Io before it passes through the sample and the intensity I of the light on the detector. Knowing these two values, it is possible to determine the relative light absorbance.

Different substances absorb light more intensely in different wavelength (λ) regions. Therefore, for each analyte light is introduced at a wavelength which is as close as possible to the absorption maximum for that particular substance.

Calibration curve

The determination of the concentration of a substance by spectrophotometry is used for substances with known absorption peaks. Initially, several solutions are prepared using a substance with a known concentration. The solutions are prepared using the same solvents that are used in the solution with unknown concentration. The absorbance of the prepared solutions is then measured. The relation between the concentration of the substance and the absorbance of light will be linear within the measuring limits of apparatus.

The data obtained are plotted graphically as points with the concentration of the substance on the x-axis and the absorbance of light on the y-axis. Once all points are plotted, from point 0 (because no substance means no absorption) as close as possible to all experimentally obtained points, a straight line (approximation line, trendline) is drawn, because concentration of a substance and absorbance of light have linear relationship.

If any experimentally obtained point does not obviously follow the general relationship (is too far from the approximation line), it should be disregarded and discarded as a gross error at the time the line is obtained.

The resulting line is called the calibration line (curve). Using it, it is possible to determine the concentration of the same substance if the light absorbance of the solution is known. To find the concentration of a sample substance of unknown concentration, measure its absorbance and read the concentrations from the graph through the calibration line value.

Sample cuvettes for light absorption measurements

All cuvettes have two different sides - one pair of opposing sides is smooth and transparent, and the other matte and/or ribbed. The transparent part of the cuvette is designed to allow light to pass through. Thus attention should be paid that no fingerprints, drops of solution or scratches are found on transparent sides.

To avoid errors when handling cuvettes you should touch only matt/ribbed sides. Any drops of liquid remaining on the transparent part of the cuvette should be carefully blotted with a piece of paper.

Filling the cuvette

The filling of the cuvette with the sample solution is carried out using a pipette or micropipette so that no air bubbles form in the cuvette.

Inserting the cuvette into the spectrophotometer

The sample is always placed between the light source and the detector so that the light beam passes through the transparent sides of the cuvette from the light source to the detector. On all the cuvettes, there is a small arrow at the top of one of the transparent sides. Therefore, the cuvettes shall always be placed in the apparatus with the arrow pointing in the direction of the light (detector).

Setting the zero point

One of the advantages of the spectrophotometer for quantitative measurements is the possibility of independently setting a point at which the light absorbance A = 0 (concentration

of the substance to be detected in solution γ = 0 g/L). Thus, not only water but also other compounds, including those which have a colour to begin with, can be used as a solvent for the preparation of solutions of the substance to be detected.

How to use spectrophotometer of this lab

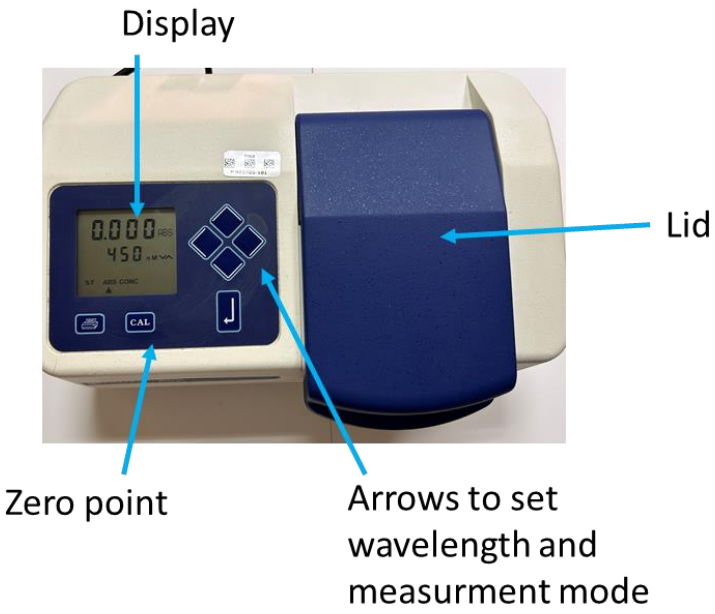


Figure 2.1. Spectrophotometer you will be using

1. Switch on the spectrophotometer, wait until it goes through startup procedure
2. Check if the Absorption is measured, indicated by “ABS” on the display. If not, use arrows pointing left and right to switch to the absorbance mode
3. Set the wavelength by up and down arrows
4. Insert the cuvette, filled with solvent for calibration. Be careful, observe the slit where light passes through to determine orientation of the cuvette. Close the lid.
5. Push CAL to calibrate, wait until the display shows 0.00(0). The last digit may change
6. Take out the first cuvette and insert the cuvette with the sample.

7. Close the lid, write down the measurement

When you are done with all measurements, set the wavelength to 600 nm

Description of myoglobin

Myoglobin is an iron-containing, oxygen-binding protein found in the heart and skeletal muscle tissue of vertebrates. Myoglobin is similar and distantly related to haemoglobin in blood, which is also an oxygen-binding protein. What makes the two molecules similar is the heme group, which is also responsible for the similar function. The heme group is also the reason why the two molecules have virtually identical light absorption peaks.
Myoglobin, with the oxygen it carries, can supply the muscle tissue and keep it functioning. The more myoglobin in the muscle tissue, the longer the aerobic load the body can withstand. For example, the muscles of divers have a particularly high concentration of myoglobin.

Myoglobin is able to attract and store oxygen because, like haemoglobin, it contains a heme group. The myoglobin molecule contains one such iron-containing heme group, which is able to bind oxygen.

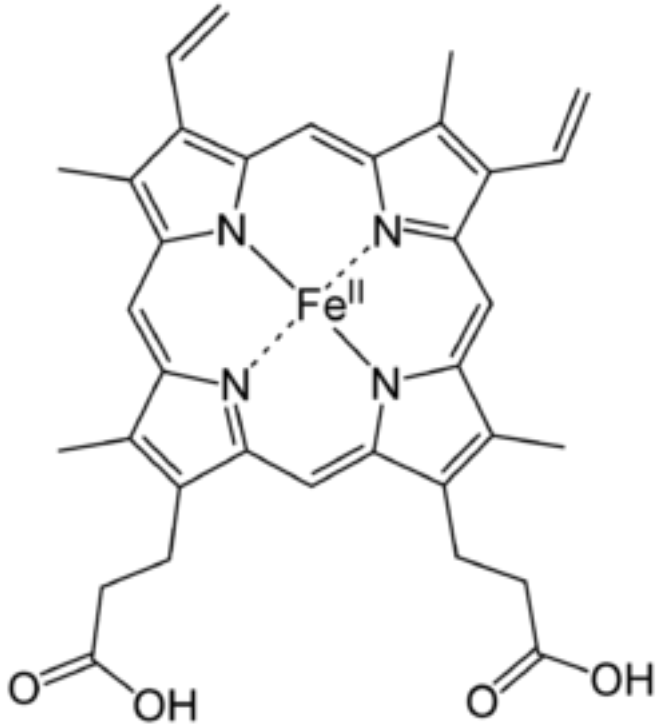


Figure 2.2. Heme group

Below you can see the UV-Vis spectrum for haemoglobin (remember that it is virtually identical to myoglobin).

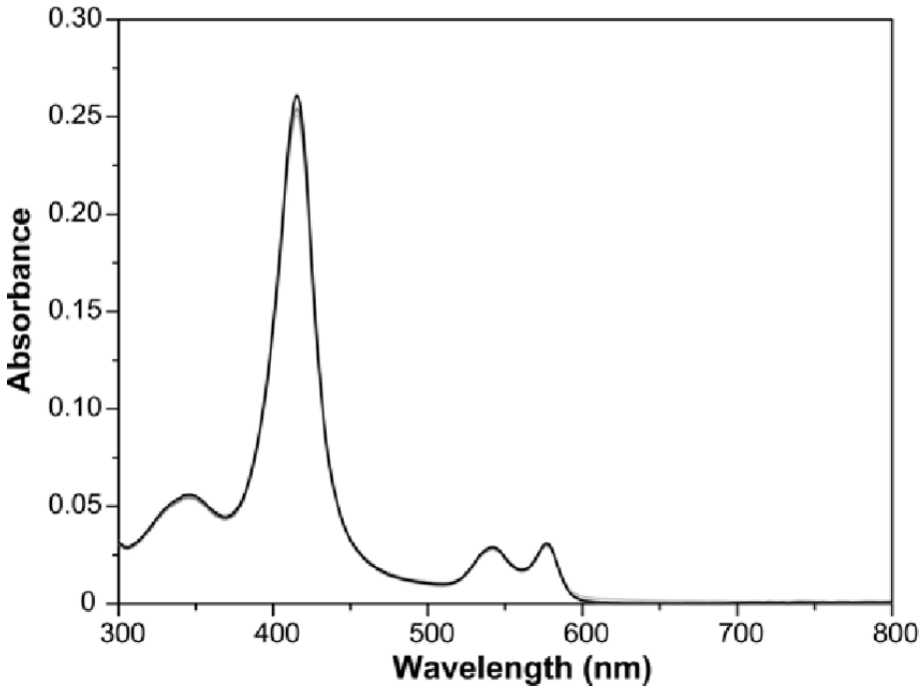


Figure 2.3. Absorption spectra of haemoglobin

2.1. Calibration curve

2.1.1. *Look at the graph 2.3 and choose the best wavelength to use for measuring haemoglobin concentrations. Indicate letter of the correct answer in the answer sheet (1 p)*

A: 350 nm
B: 420 nm
C: 520 nm
D: 380 nm

To prepare a calibration curve for measuring *myoglobin* concentration you will have to prepare six samples with known *haemoglobin* concentration.
To do that you should use the so-called serial dilution technique.

How to prepare serial dilutions

1. Prepare six 15 ml tubes. Number them 1 to 6.
2. Add 5.0 ml of water in each tube
3. Add 5.0 ml of haemoglobin standard solution in tube 1, mix thoroughly. Do not forget to close the cap.
4. Take 5.0 ml solution from tube 1 and transfer it to tube 2, mix thoroughly
5. Take 5.0 ml solution from tube 2 and transfer it to tube 3, mix thoroughly
6. Follow this sequentially through all the tubes

2.1.2. *Calculate and write concentrations of haemoglobin solutions you have prepared in the answer sheet, considering that the STANDARD haemoglobin solution's concentration is 1,0 g/L. Show calculation for tube 1 (3 p)*

Preparing calibration curve

1. On the spectrophotometer set the wavelength you have chosen in 2.1.1.
2. Using micropipette, fill 1 ml of water in the cuvette use this cuvette to set zero in the spectrophotometer
3. Take an empty cuvette and fill it with 1 ml of one of your prepared solutions, insert it in spectrophotometer and read the measurement
4. Repeat the process with all your haemoglobin solutions, including the standard solution that was prepared for you

2.1.3. *In the answer sheet, write absorbance measurements that you obtained (4.5 p)*

2.1.4. *On a provided millimetre paper plot the data that you will use for the calibration curve, Name it "Calibration curve". Do not forget to draw the calibration curve itself (6 p)*
Note that not only precision, but also neatness of the graph and labelling will be evaluated.

Measuring your samples

From Problem 1 you should have 6 centrifuge tubes with solutions A, B, C and A1, B1, C1
As there is a small amount available, you will dilute the samples in the cuvette.

First prepare a cuvette for each sample where you add 200 microliters of sample and fill it up until 1 ml with water.
You should also calculate dilution coefficient k that is $c_{(previous)}/c_{(new)}$

2.1.5 *In the answer sheet write how much water you should add and what will be the dilution coefficient in this cuvette (2 p)*

Measure all the samples with this dilution.

2.1.6. *In the answer sheet, write measurements that you obtained (3 p)*

If any of your measurements falls out of your calibration curve limits or spectrophotometer shows “1.” an indication that absorbance is too high. You can adjust your measurements by preparing more or less diluted samples.

2.1.7. *Write in the answer sheet, if additional measurements are needed and why (3 p)*

For the samples that need additional dilution, figure out needed amounts of sample and water and write them down in the answer sheet together with the dilution coefficient k. For samples that are out of measuring limits of spectrophotometer we suggest using k=20

2.1.8. *Fill out table in answer sheet for samples that needed additional dilution (2 p) Prepare those dilutions and write down measurements in the same table*

Using your calibration curve, measured absorbance of diluted samples and calculated k, calculate initial concentration of myoglobin in each sample.

2.1.9. *Write down calculations and answers in answer sheet (7 p).*

2.1.10. *In the answer sheet indicate which muscle (A1, B1, C1) will have largest oxygen reserves. (1 p)*

Problem 3 Burning the calories

Materials and equipment

- Potato crisp in a ziplock bag marked with “T3-1”
- Rice galette in a ziplock bag marked with “T3-2”
- Corn puff in a ziplock bag marked with “T3-3”
- Calorimetry device stand
- Aluminium can with a removable lid
- LabQuest 2 data logger device
- LabQuest temperature sensor
- Lab stand with a clamp
- Aluminium foil, 50 cm piece
- Scales
- Tweezers
- Metallic spoon
- 50 mL measuring cylinder
- Matches
- Container for waste
- Textile gloves
- Safety goggles

Note that in this experiment you will be dealing with **open flame** in a contained experimental setup. Be careful not to get anything other than the required samples in the flame.
If you have long hair, be sure to contain it in a way that it does not hang or slip into the flame.

Some parts of the calorimetry device setup can have sharp edges. Aluminium can opening can be sharp as well. Be careful during the whole experiment. If needed, use the thicker textile gloves provided.

Introduction

In problem 1 you analysed how metabolism of muscles works. You saw how glucose is used as fuel. Usually for energetic needs our cell will use carbohydrates and fats.

In this task you will explore the energetic value of three common snacks.
From packaging of all three food items you know following information

Per 100 g	Potato crisp	Rice galette	Corn puff
Energetic value	2070 kJ or 497 kcal	1619 kJ or 378 kcal	1480 kJ or 355 kcal
Fats (g)	27.0	2.8	0.9
Carbohydrates (g)	59.0	79.5	80.9
Proteins (g)	2.7	8.2	6.0
Salt (g)	1.7	0.1	0.0

To determine energetic value you will have to use a simplified calorimeter setup. All work must proceed in or under **fume hood**. Remember about safety measures - wear safety goggles.

Calorimeter device setup

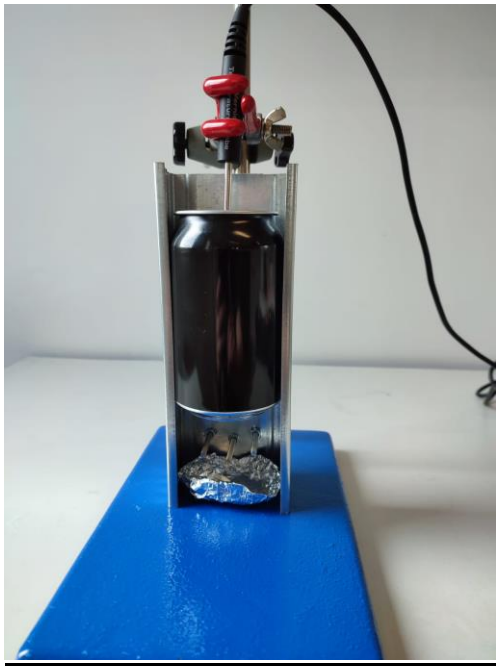


Figure 3.1. Assembled calorimeter

For a valid result you should do at least 4 measurements with each of the food items.

Using calorimeter

1. Using aluminium foil, make a weighing boat for the sample. It should be about 1 cm high and approximately the same size as the aluminium can bottom projection in diameter. The boat should easily fit under the bottom screws of the device setup.
2. Place the chosen food item (or a part of it) in the boat and weigh it. Record the measurement in the Answer sheet.
3. Measure 50.0 mL of distilled water and pour in the aluminium can. Place the can in the device.
4. Place the food object onto the screw rods under the can. Place the aluminium boat under the screws so that if anything falls or drips from the object it will get into the boat and be collected.

5. Fix the temperature sensor into a lab stand clamp. Place the sensor into the can so that it is immersed in water. Note that the sensor should not touch the bottom of the can or any of the walls. You can mark the height of the lab stand clamp on the stand pole for future reference.
6. Connect the sensor to a LabQuest 2 data logger device. Once connected, the device should start showing live temperature readings.
7. Wait for the temperature readings to stabilise. Write down the initial temperature in the Answer sheet.
8. Once the temperature is not changing, press the Play button ➤ on the device. It will start recording temperature measurements for 180 seconds.
9. Light the food object on fire using matches. It is suggested to place the match directly under the food object to minimise heating up the can with the match itself.
10. Note the temperature changes in the device. Once the food object has fully burned or is not burning any more observe the highest achieved temperature. You can also write down the highest reached temperature in your notes for double safety.
11. Once the device stops measuring, one can press the word "***Analyze***" -> "***Statistics***" -> "***Temperature***". The device shows the max and min temperature.
12. Record the highest reached temperature in the Answer sheet.
13. Using tweezers and/or the metallic microspoon place the burnt object in the aluminium boat. Weight the burnt object and record the measurement in the Answer sheet.
14. Repeat the experiment as many times as you deem necessary for a valid result but you should do it at least 4 times with each of the food objects.
15. Keep in mind to change water in the can after each try as the cooling of the water from previous experiments might interfere with your results.

Repeat the same experiment with the other two food objects.

3.1. *Register the data from your experiment in table in answer sheet (8 p)*

When we speak of the energetic value of food we usually use the term calories, which is now an obsolete non-SI unit of energy, equivalent to approximately 4.2 joules. This unit was widely used in chemistry and physics, being the amount of energy needed to raise the temperature of 1 gram of water by 1 °C

3.2. *Calculate the energetic value of all three food items from your data in kcal/100g, write your answers in the answer sheet. Show your calculation for potato crisp. (6 p)*

In problem 1 your team looked at how glucose is converted into ATP by muscles. Here you burn the food that also contains glucose.

3.3. *Mark to which form of energy most of the chemical energy contained in glucose is converted in each case. Write down corresponding letter in answer sheet (2 p)*

A: Chemical energy
B: Kinetic energy
C: Thermal energy

Which metabolic process of converting glucose into energy, aerobic or anaerobic, is more closely related to combustion if measured by the degree of decomposition of glucose's chemical structure?

3.4. *Mark if aerobic (A) or anaerobic (N) metabolism is closer to burning in the answer sheet (1 p)*

When estimating energetic values of food often gross and physiological calorific values are mentioned. Physiologic calorific value is the heat energy that is released when one gram of food is oxidised inside the body. Gross calorific value is obtained in the calorimeter from food directly.

3.5. *Evaluate following sentences and propose which is the correct one and write the letter of the correct one in the answer sheet (1 p)*

a) Physiologic calorific value usually will be lower as gross value as some energy is used for maintenance of the body
b) Physiologic calorific value usually will be lower as gross value as some heat is lost from human body
c) In your setup you estimate physiological calorific value
d) Physiologic calorific value usually will be higher as gross value as we are using nutrients more effectively

Take a look at the packaging information about composition of crisp, corn puff and galette provided previously.

3.6. *Estimate which nutrient has the highest caloric value per mass. Write corresponding letter in the answer sheet. (1 p)*

A: Fats, B: Carbohydrates, C: Proteins, D: Salt

Take a look at your data, probably you have obtained a smaller amount of energy than it was on the packaging information above.

3.7. *In answer sheet write letters corresponding to the processes that explain why your data would give you less calories than shown on packaging information (2 p)*

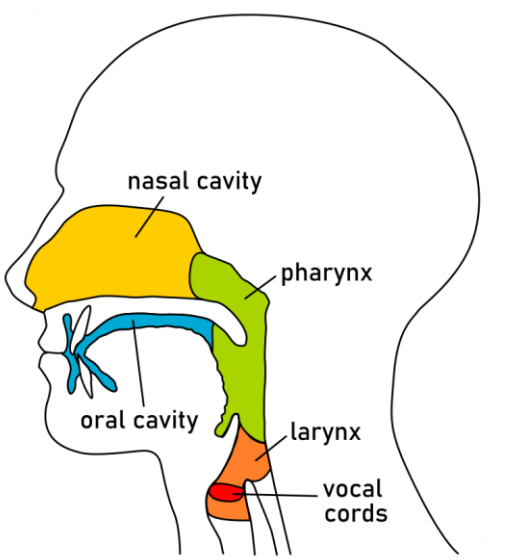
a) Heat loss by convection
b) Heat loss by conduction
c) Heat loss by thermal radiation
d) Heat loss by evaporative cooling
e) Endothermic chemical reactions between the thermometer and dissolved food particles in the water
f) Heat was lost for maintenance of system

Physics of song

People have long been fascinated by physics phenomena that can be directly observed by our own senses, thus the extensive research done in optics and acoustics (even though optics falls within the realm of electromagnetic wave analysis and acoustics could be well-tied with mechanical waves beyond what can be heard by the human ear like the seismic waves).

We are most familiar with simple, harmonic sound waves, that are described by:

- their pitch (determined by the temporal frequency of the wave)
- their loudness (determined by the wave amplitude)



Sometimes, when discussing more complex sounds (such as those produced by musical instruments), the timbre of a sound is noted (related to the overtones or additional sound waves produced that are some multiple of the initial pitch).
However, when talking, humans will often produce sounds at the same pitch and loudness yet distinguishable as different vowel sounds.
Speaking and singing involve a voice mechanism that is composed of three subsystems. Each subsystem is composed of different parts of the body and has specific roles in voice production (see the table below).

Subsystem	Voice organs	Role in sound production
Air pressure system	Diaphragm, chest muscles, abdominal muscles, lungs	Provides and regulates air pressure to cause vocal folds to vibrate
Vibratory system	Larynx and vocal cords (folds)	Vocal folds vibrate, changing air pressure to sound waves and producing “voiced sound” (described as the “buzzing sound”). Also varies the pitch of the sound
Resonating system	Pharynx, oral cavity, nasal passages	Changes the “buzzing sound” to recognizable speech patterns

Note: in the tasks we will use sound depictions from phonetics marked in square brackets. These sounds will be used: [ɑ], [i], [u], [f], [v]

Problem 4 – Phonetics (8 p)

Materials and equipment

- you

Phonetics is a branch of linguistics that studies how humans produce and perceive sounds. To produce any kind of sound, there must be a movement of air. To produce sounds that people can interpret as spoken words, the movement of air must pass through the vocal cords, up through the throat and, into the mouth or nose to then leave the body.

Vocal cords are folds of throat tissues located above the trachea in the larynx or voice box. Vocal cords can be open or closed. See the picture below - A open vocal cords, B closed vocal cords. Vibration of vocal cords produces sound.

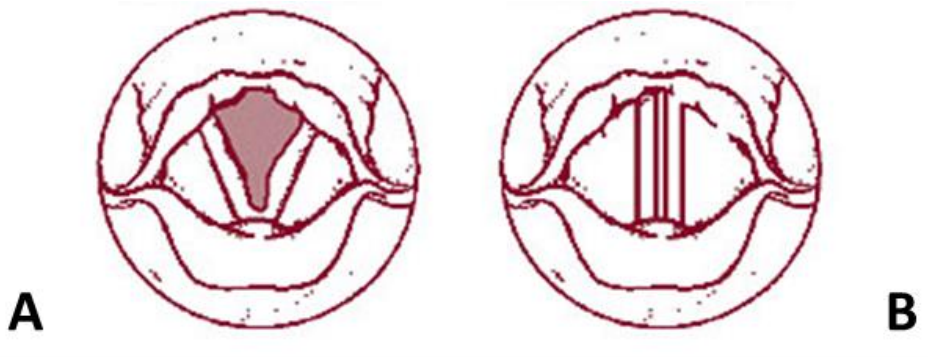


Figure 4.1. Vocal cord disposition

4.1. *Deduce if vocal cords are open (A) or closed (B), when breathing (1p)*

Size of the larynx changes while growing and is also influenced by sex hormones.

4.2. Observe the scheme of various muscles in the larynx that are involved in the vocal cord movement. *Deduce which muscles will open and close the vocal cords if contracted. Which muscle will change the length of the vocal cord? Fill in your answers in the answer sheet. (3p)*

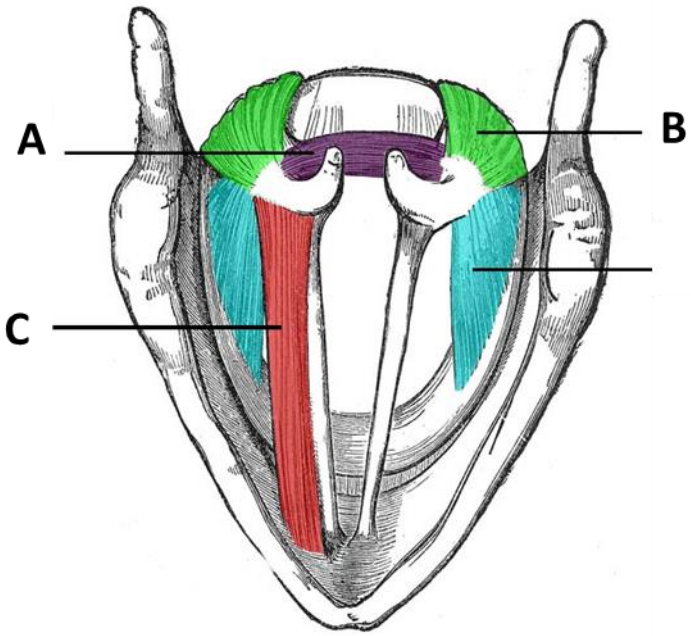


Figure 4.2. Muscles of a voice box

The technical names of speech sounds are based on the features of their production in the vocal tract. Sounds are grouped in consonants and vowels. Consonants form one group because they are produced when the airstream is impeded in some way as it moves through the vocal tract. The airstream of vowels is not impeded, but rather is shaped by the vocal tract, creating differences in sound qualities.

Consonants are grouped in voiced and unvoiced consonants. To discover the difference between them, touch your throat with your fingers and pronounce a continuous [f] sound. Now pronounce a continuous [v] sound.

***4.3**. Describe your observations and conclusions in the answer sheet. (4 p)*

Problem 5 Vowel simulations

Materials and equipment

- A personal computer with VA64.exe or VA32.exew software for audio recording, located outside the lab
- Duck-call parts (channel, stopper, tube, reed)
- Resonator parts: foam sleeve tube, 50 cm and plastic tube, 18 cm
- Scissors
- Ruler

Problem 5.1 – getting acquainted with frequency spectra

Fundamental frequency and harmonics

The fundamental frequency, also referred to as the first harmonic, is defined as the lowest frequency of a periodic waveform. The harmonics are multiples of the fundamental frequency. So, if the fundamental frequency is 100 Hz, the higher harmonics will be 200 Hz, 300 Hz, 400 Hz, 500 Hz, and so on. If the fundamental frequency were 220 Hz, the harmonics would be 440 Hz, 660 Hz, 880 Hz, and so on.

Below is a frequency spectrum of a guitar playing a single string.

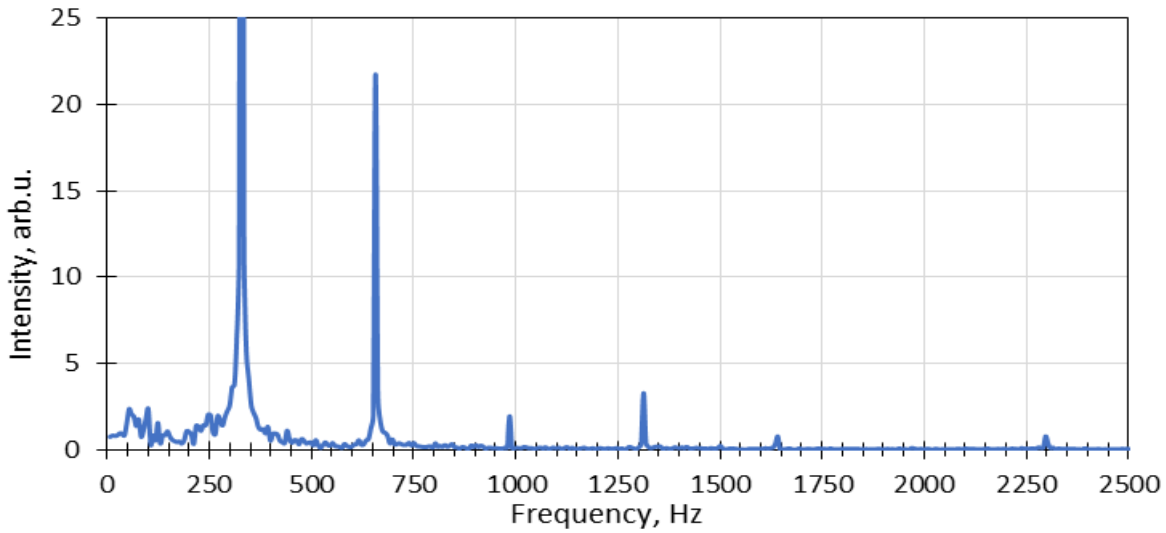


Figure 5.1.1

5.1.1. *Write in the answer sheet the fundamental frequency (the first harmonic) of this particular string on a guitar. (1 p)*

5.1.2 *Write in the answer sheet how many harmonics, including the fundamental one, can you see in the spectra and what their frequencies are. (3.5 p)*

5.1.3. *Write in the answer sheet are all the possible harmonics within the frequency range 0-2500 Hz present. If not - sketch in the graph where the missing harmonics would be (1.5 p).*

The impact of a resonator

You may have noticed that the intensity of some harmonics is much smaller than that of others. The relative intensities are determined by the shape of the guitar body which amplifies certain frequencies and diminishes some others. The resulting signal is dependent on the sound source and the impact of the resonator. The relationship between the sound source and the resonator is illustrated below using synthetic data (figure 5.1.2.). Mathematically the source intensity is multiplied by the resonator amplification to obtain the resulting spectrogram.

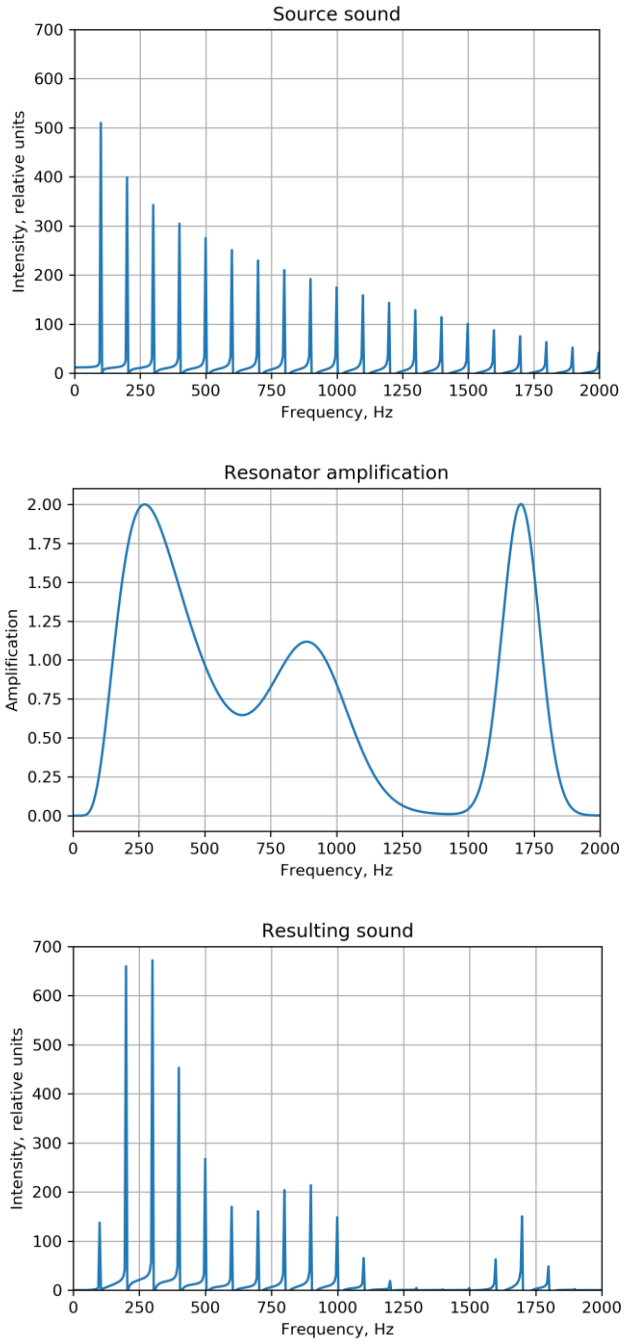
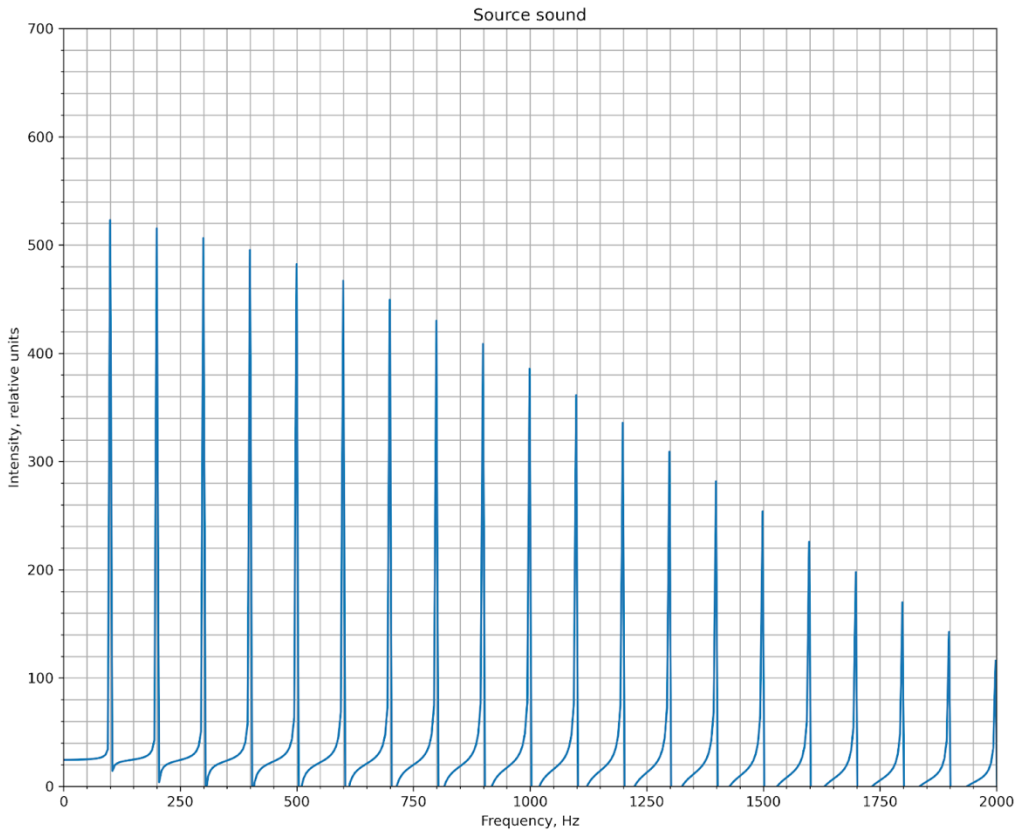
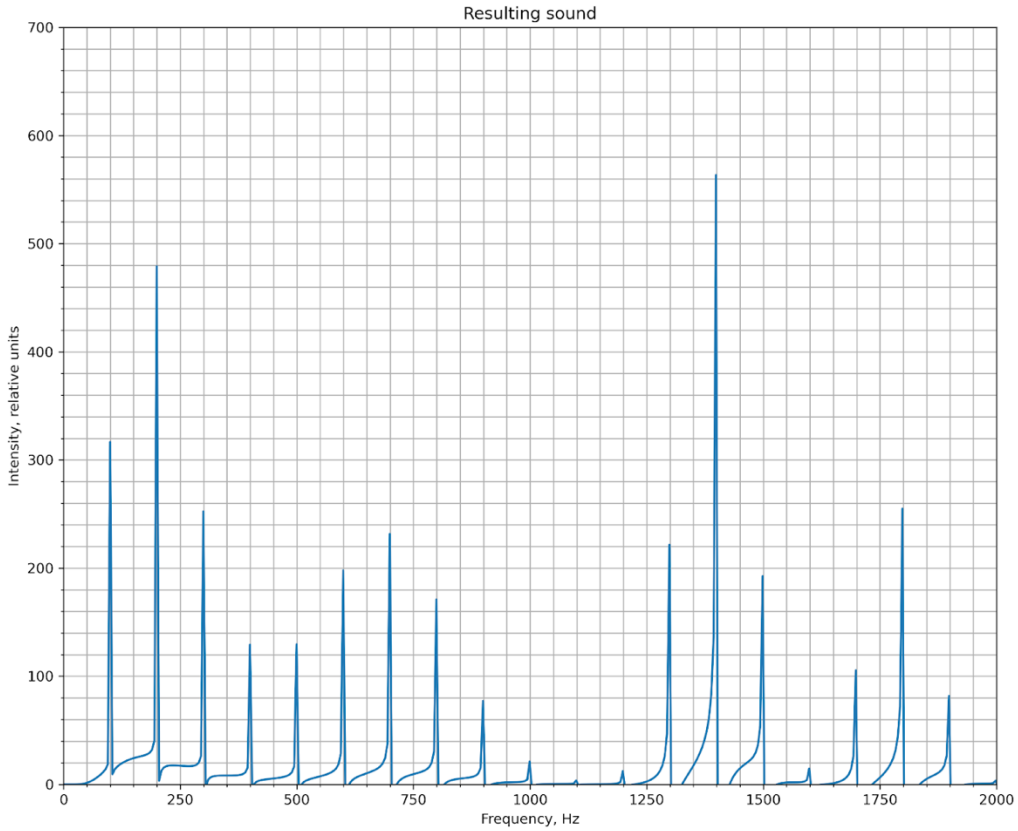


Figure 5.1.2. The resulting sound spectrogram (bottom) after the source sound (top) is amplified by the resonator (middle).

This particular resonator amplification response has got three maxima. **These maxima are called "formants" (and characterised by their frequency)** - formants describe how the resonator concentrates energy in certain frequencies while removing energy from others. For this filter, the first formant is f_1=270 Hz, the second formant is f_2=900 Hz, and the third formant is f_3=1700 Hz. Note that unlike the harmonics of the source spectrum, the higher formant frequencies are not necessarily multiples of the first one. This is important because humans distinguish different vowel sounds based on the formant frequencies produced by their resonating sub-system (the mouth and tongue shape).

5.1.4. *Determine and plot the resonator amplification as a function of frequency for the this source and resulting sound spectrum: (4 p)*





5.1.5. *What are the frequencies of the first two formants for this resonator? (1 p)*

Now that you are familiar with the basics of reading and interpreting frequency spectra, it is time to create a model of the human vocal system.

Problem 5.2 – assembling and calibrating the sound-source

Your first practical task is to build an analogue to the larynx - the sound source of human vocalisation. For this, we will use a duck call. It consists of four pieces: the channel, the stopper, the tube, and the reed (see figure 5.2.1.).

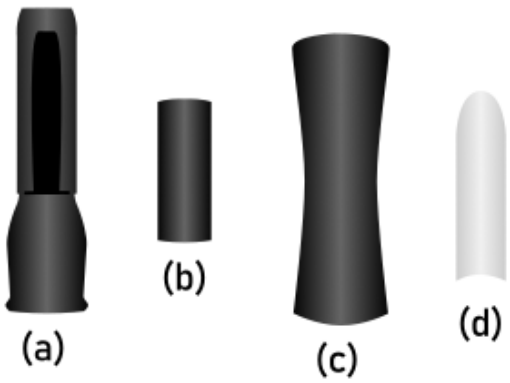


Figure 5.2.1 The pieces of the duck call a) the channel, b) the stopper, c) the tube, d) the reed.

Assembly process (see figure 5.2.2):

1. Take the channel and place the reed on top of it so that the reed is bending away from it. You can position the reed against the wall, or move it back - it will change the pitch of the duck call.
2. Cover the reed from the other side with the stopper.
3. Finally, insert the assembled structure inside the tube. It should go in quite deep, but not all the way through. Some force might be needed, but make sure not to overdo it, so that you can disassemble the duck call later.
4. Try out the duck call - blow some air from the tube side.

Figure 5.2.2. Duck call assembly steps 1.-3.

To obtain the sound spectra you will have to use a computer with a special program. To not disturb your teammates and other people taking data, ask the lab assistant to guide you to the nearest computer outside the lab. When you are finished, you can return to the lab. If needed, you can take a teammate with you to help with data acquisition.

Audio capture program configuration:

The program will be opened by a lab assistant. If you accidently close the program ask the lab asistent to open it again.

Measurement process:

1. Begin the audio capture process by clicking the “On” button on the top-left of the window.
2. Right click on the lower diagram and hover over “take spectrum screenshot”. Blow in the duck call as you would blow in the whistle to produce sound. The spectrum is shown on the bottom graph in the program. The audio signal is averaged over the last 5 seconds. Therefore, you will need to hold the sound you want to measure for that long. Make sure to blow with a constant force, otherwise, the pitch can change, and the spectrum will look “fuzzy”.
3. Once you are happy with the spectrum, click on “Take Spectrum screen shot”. This will create an image file in the folder Desktop/EOES/ScreenShot/. You can later rename the image in the folder.

5.2.1. *If you increase the length of the reed that is poking out of the stopper the frequency of the duck call increases, decreases or stays the same? Check the correct answer in Answer sheets. (1 p)*

5.2.2 *Adjust the reed position so that the duck call produces a sound with the fundamental frequency of 150 Hz. Measure the spectrum of the duck call sound, making sure that the frequency peaks are well-defined and not “fuzzy”. Rename the spectrum as “duck_call_YOURCOUNTRY.bmp”. (4 p)*

Problem 5.3 - making the resonators to model the human oral cavity

Introduction to the human oral cavity

The nose, pharynx, and mouth amplify and modify sound, allowing it to take on the distinctive qualities of voice - essentially they act as the resonator/filter that you saw in Part I when determining the formant frequencies.

Each vowel produced is obtained by changing the shape of the oral cavity and this shape in turn determines the formant frequencies. The shape of the cavity is, of course, very complicated, but it can be modelled by cylinders of different diameters to approximate this shape (see figure 5.3.2.). Below (Figure 5.3.1.) are MRI scans of a human pronouncing three vowels: /ɑ/ - like in “father”, /i/ - like in “meet”, and /u/ - like in “book”.

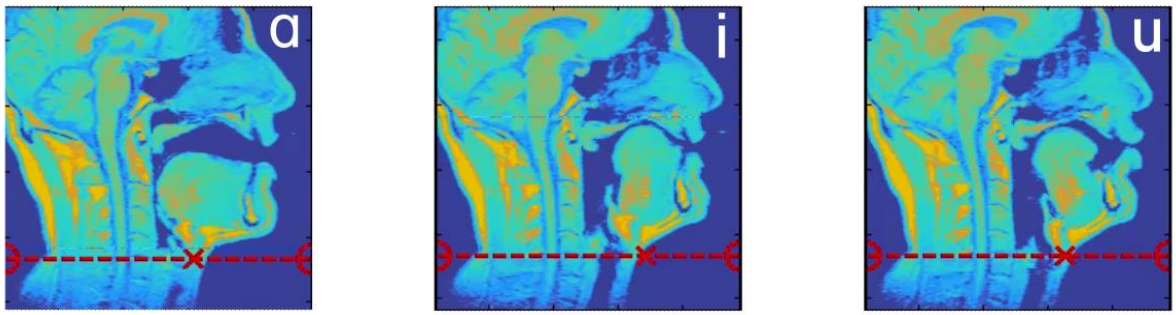


Figure 5.3.1 MRI scans of a person pronouncing the vowels /a/, /i/ and /u/. The vocal cords are on the red line.

5.3.1 *Look at the model blueprint for the three vowels below and match each of the models to the corresponding vowel based on what you can identify in the MRI scans. Record your choices on the answer sheet (3 p)*

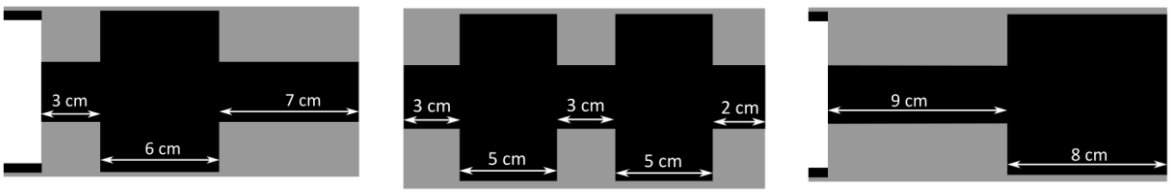


Figure 5.3.2. Blueprints of the model vowel resonators that you will have to make.

Vowel: _____ Vowel: _____ Vowel: _____

Assembling the three resonators

1. Use a model vowel resonator blueprints (see figure 5.3.2).
2. Cut from the foam sleeve tube cylinders of the correct size for the narrow sections in the blueprint.
3. Shove the foam cylinders inside the larger plastic tube according to the blueprint. Use a ruler to align them correctly. A sample resulting resonator is shown in figure 5.3.3.

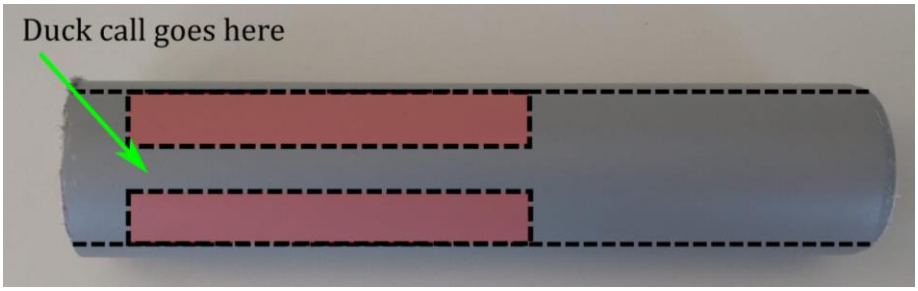


Figure 5.3.3. An assembled vowel resonator.

Problem 5.4 - measuring the frequency spectra of different vowel resonators

Having assembled the resonators corresponding to the ɑ, i, and u vowels, it is time to test them. Humans distinguish between vowels based on the **two lowest** formant frequencies. In this part, your task will be to characterise the frequencies amplified by the model resonators and determine their formants.

Measurement process:

1. If the audio capture program was closed, refer to section 5.2 “Audio capture program configuration”. Otherwise, the program is configured and you can proceed.
2. Insert the duck call **3 cm deep (!)** into the resonator from the “larynx” side (see figure 5.3.3.). Make sure not to dislodge the foam cylinders from their positions.
3. Blow into the duck call like in a whistle for approximately 5 seconds while the program averages the sound. Make sure to blow evenly like when the spectrum of just the duck call was captured, otherwise, the pitch can change, and the spectrum can look “fuzzy”. If done correctly, you should be able to roughly hear the expected vowel sound coming from the resonator.
4. Once you are happy with the spectrogram, right-click on it and select “Take Spectrum screenshot”. This will create an image file in the folder Desktop/EOES/ScreenShot. You will later rename the image in the folder.
5. Repeat steps 1.-4. for each resonator.

5.4.1. *Measure the spectra of the sound produced by the duck call and filtered through the ɑ, i, u vowel resonators. Make sure that the frequency peaks are well-defined and not “fuzzy”. Rename the spectra as “resonator_a_YOURCOUNTRY.bmp”, “resonator_i_YOURCOUNTRY.bmp”, “resonator_u_YOURCOUNTRY.bmp” for each of the corresponding ɑ, i, u vowel resonators. (6 p)*

5.4.2. *Compare the spectrum of the pure sound from the duck call with the spectra of the sound filtered through the ɑ, i, u vowel resonators. Make three sketches of the amplification v.s. frequency of the resonators - one for of each vowel ɑ, i, u. The scale of the amplification axis is arbitrary. For each sketch, below the first and second formant write down their approximate frequencies. (6 p)*

5.4.3. *Using your sketch of the amplification of each resonator, determine the first two formant frequencies for each ɑ, i, u vowel resonator. Mark them down as points in the vowel chart (Figure 5.4.1.) in the answer sheet. (3 p)*

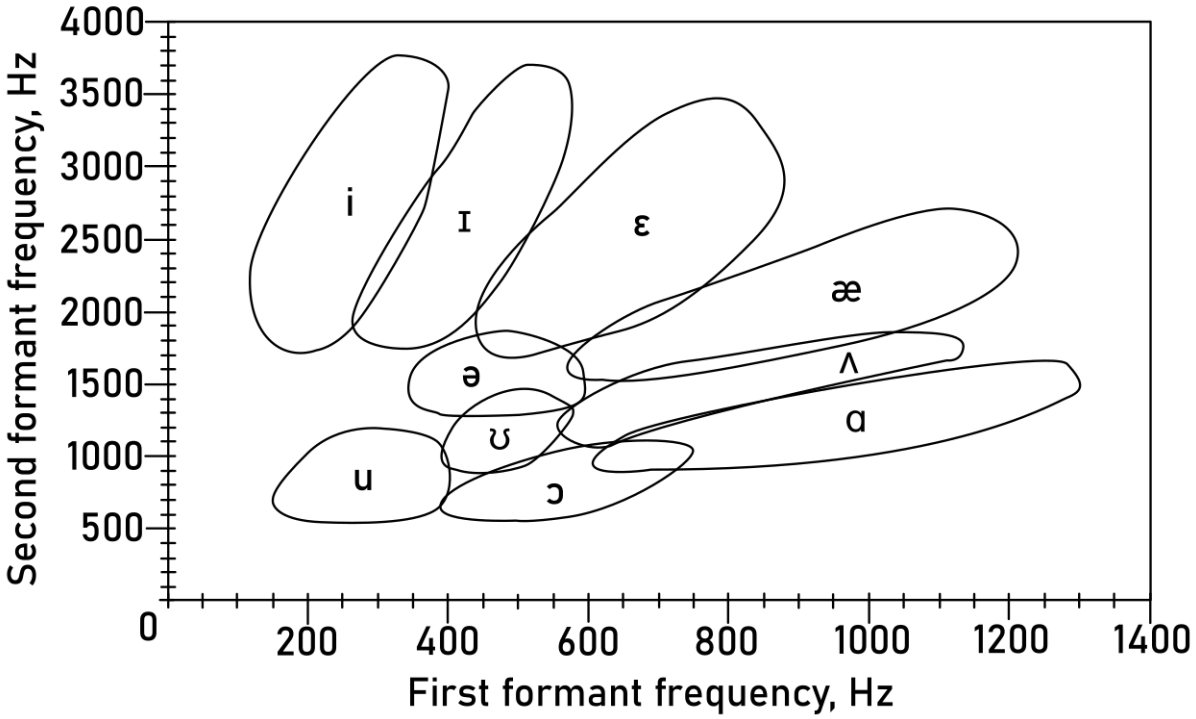
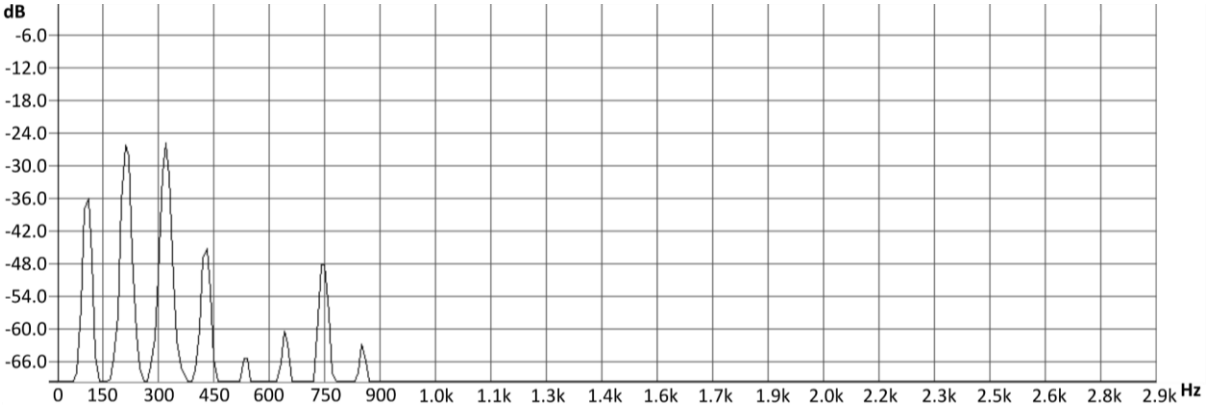


Figure 5.4.1. The vowel chart: shows which combinations of first and second formants are present in different spoken vowels.

Problem 5.5 Analysing sound spectra and determining speech patterns

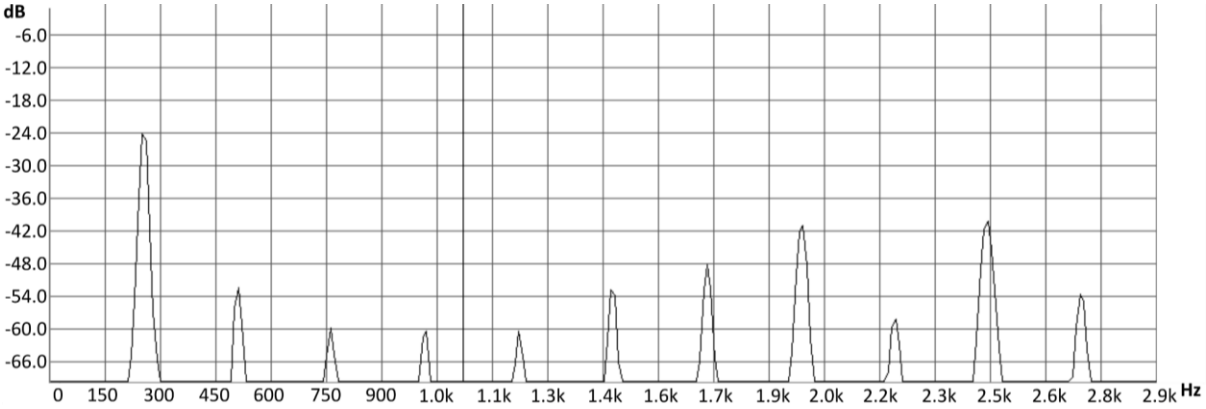
For the final test to show your now-acquired knowledge of the way humans create and distinguish sounds, let's look at the spectra of actual human speech. Below are the spectra of human-produced ɑ, i, u vowels. Assume that the spectrum of the vocal cords in the 0-3000 Hz range has approximately constant peak heights

5.5.1 *What is the vowel that produced this spectrum? (1 p)*

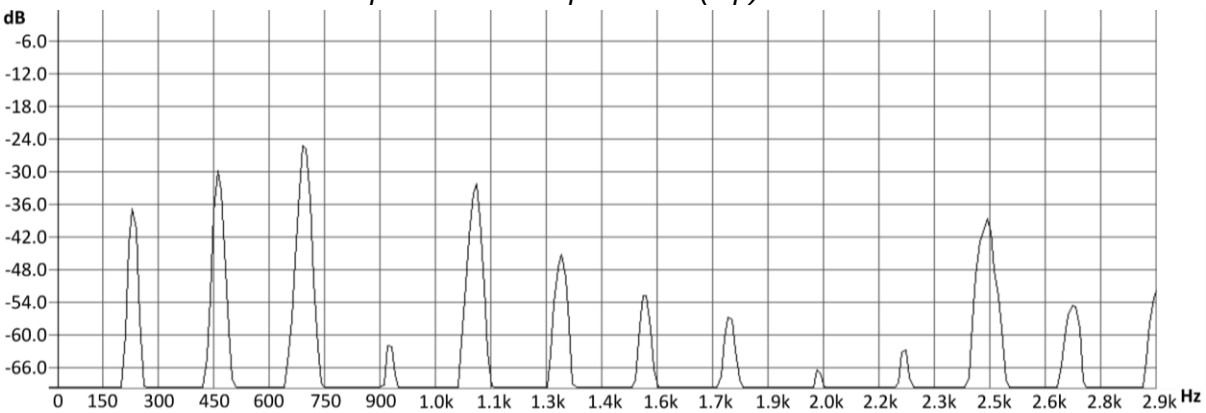


gram?

5.5.2 *What is the vowel that produced this spectrum? (1 p)*



5.5.3 *What is the vowel that produced this spectrum? (1 p)*



Problem 6

Have a little song and dance as this task is finished now! :)