11th International Junior Science Olympiad

Multiple Choice Questions

December, 4 2014

EXAMINATION RULES

1. No competitors are allowed to bring any tools except his/her personal medicine or any personal medical equipment

2. Each competitor has to sit according to his/her designated desk.

3. Before the examination starts, each competitor has to check the stationary and any tools (pen, ruler, calculator) provided by the organizer.

4. Each competitor has to check the Question and Answer Sheets. Raise your hand, if you find any missing sheets. Start after the bell rings.

5. During the examination, competitors are not allowed to leave the examination room, except in case of emergency. In such case, the examination supervisor will accompany them.

6. You are not to disturb other competitors. If you need any assistance you may raise your hand and wait for a supervisor to come and assist you.

7. There will be no questions or discussions about the examination questions. The competitors must stay at their desks until the time allocated for the examination is over, even if they have finished the examination earlier and do not wish to continue working.

8. At the end of the examination time there will be a signal (the ringing of a bell). You are not allowed to write anything on the Answer Sheet at this time. All competitors must leave the room quietly. The Question and Answer Sheets must be put neatly on your desk.

READ CAREFULLY THE FOLLOWING INSTRUCTIONS:

1. The time available is 3 hours.
2. The total number of questions is 30. Check that you have a complete set of the test questions and Answer Sheet.
3. Use only pen provided (not pencil).
4. Write down your name, code, country and signature in your Answer Sheet.
5. Read carefully each question and choose your correct answer by crossing one of the lowercase letters in your Answer Sheet. There is only one correct answer for each question.
6. Additional sheets are provided for rough work.

Example:

1	a	b	c	d

7. If you want to change your answer, you have to circle the first answer and then cross a new letter as your correct answer. You are allowed to make only one correction.

Example:

1	a	b	c	d

A is the first answer and D is the corrected answer

8. Competitors are not allowed to bring any tools from outside. After completing your answers, all the Question and Answer Sheets should be put neatly on your desk.
9. Point rules:

Correct answer: +1.0 point
Wrong answer: -0.25 point
No answer : 0.0 point

TEST COMPETITION

The Aconcagua Mountain has the highest elevation in the Southern Hemisphere. It belongs to the frontal range formation of the Andes and is located in the Central-West of Argentina, in Mendoza province. It is the highest mountain in America. It has two main peaks, one of 6 962 m a.s.l. (meters above sea level); and the other, the Southern peak, of 6 930 m a.s.l. Several glaciers are located on its hillsides, the most important ones being the Northeast or Polish Glacier, and the Eastern or English Glacier.

Reaching the Aconcagua peak is a challenge that calls for climbers worldwide.

Figure 1

1. The temperature of the human organism, considering deep tissues, is around 37ºC, which is subject to slight variations according to the time of day, physical activity, environmental temperature and certain metabolic processes.

Various neural feedback mechanisms can regulate body temperature. Some mechanisms are:

1. Tremblings caused by increased muscle tone.
2. Evaporation of water from the skin and mucous membranes.
3. Increased basal metabolism and thyroxine blood level.
4. Panting and peripheral vasodilation.
5. Peripheral vasoconstriction.

When a man faces a sharp drop in temperature as it happens at the Aconcagua summit, compensatory mechanisms that tend to maintain body temperature are:

a. 1, 2 and 4
b. 2, 3 and 4
c. 1, 3 and 5
d. 2, 3 and 5

2. *The skin is the interface between the internal and external environments, and the exchanges of energy that affect body temperature. These exchanges between the body and the environment are the result of mechanisms such as radiation, conduction, and convection.*

In a low temperature environment (as the one at the Aconcagua summit) the amount of energy lost through convection by a person without insulating clothing, will be proportional to:

a. The surface area of their body, and the difference in temperature between his body and the environment
b. The fourth power of their body temperature
c. Only the difference in temperature between their body and the environment
d. The person's height and the difference in temperature between their body and the environment

3. The atmospheric pressure at sea level is called normal pressure. The concentration of oxygen (O_2) under these conditions is 20.9 %v/v, so that the partial pressure of oxygen (pO_2) is 21.2 kPa. For the human body this concentration is sufficient to saturate the hemoglobin in blood. As one climbs the Aconcagua, the atmospheric pressure decreases, while the fraction of O_2 and all other gases remain constant.

Figure 2 represents the percentage change of atmospheric pressure as a function of altitude.

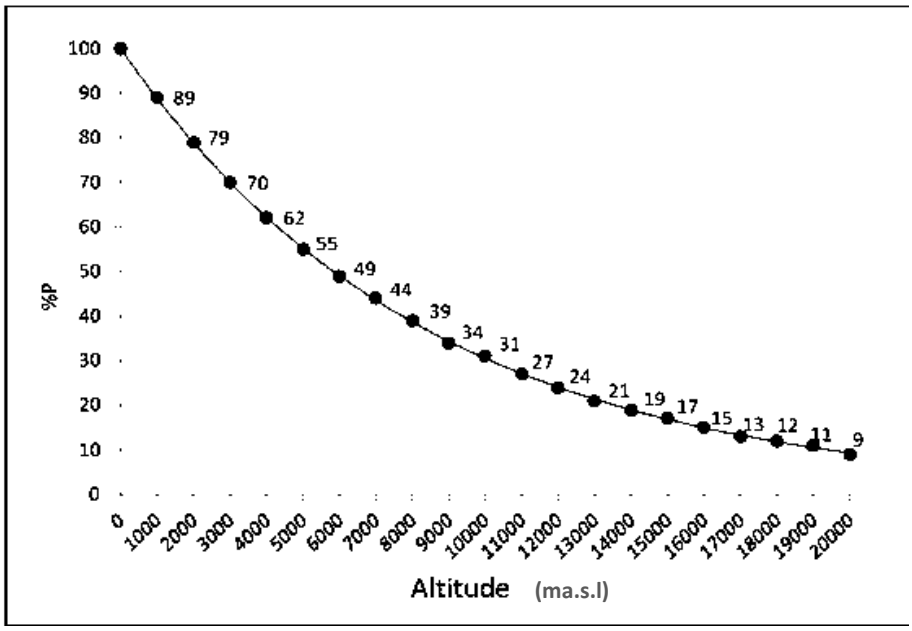


Figure 2

Knowing that Mount Aconcagua has a maximum altitude of 6 962 m a.s.l. (consider 7000 m), the pO_2 at the summit will be:

a. 44.00 kPa

b. 9.33 kPa

c. 21.00 kPa

d. 0.44 kPa

4. The curve shown in Figure 2 is the graph of:

a. A quadratic function

b. An exponential function

c. A linear function

d. A trigonometric function

5. The slope value of the tangent to the curve at the point with coordinates (3 000 m a.s.l.; 70 %) in Figure 2 is:

   a. Null

   b. Positive

   c. Negative

   d. Cannot be determined

6. *A climber is training to participate in an expedition to the summit of Aconcagua. For this reason, he goes to an area of high altitude to acclimatize and avoid acute mountain sickness, caused by air pressure reduction and consequently a low partial oxygen pressure (pO_2).*

   One of the acclimation mechanisms is:

   a. Pulmonary hyperventilation by venous chemoreceptors stimulation.
   b. Increase in number of erythrocytes.
   c. Decreased supply of oxygen to the muscles.
   d. Decreased oxygen diffusion by increased capillary surface.

7. Figure 3 shows the arterial oxygen saturation as a function of altitude when breathing air or pure oxygen. According to this, in the ascent to Mount Aconcagua, at 4 500 m a.s.l. , breathing air implies a saturation of hemoglobin with oxygen of approximately:

   a. 100%
   b. 92%
   c. 82%
   d. 72%

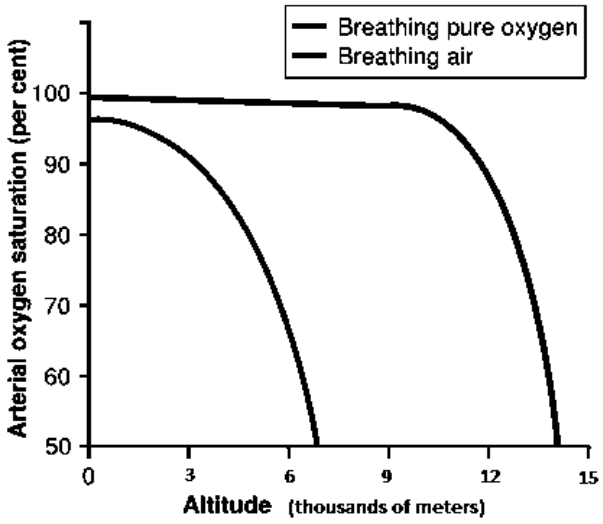


Figure 3

8. The differences in the chemical composition of the extracellular and intracellular fluids are vitally important for cells. The components of these liquids are almost equal; however, concentrations vary between both of them.
Mark the incorrect option taking into consideration the chemical composition of the intra- and extracellular fluids, transport mechanisms and the organs responsible for regulating the internal environment.

   a. Blood plasma and other extracellular fluids have similar ionic composition but vary in their protein content.
   b. Calcium ion (Ca^{2+}) is almost exclusively present in extracellular fluid. However, it is of vital importance within muscle cells.
   c. Extracellular fluid contains higher concentrations of chloride (Cl^-), potassium (K^+), hydrogen carbonate [$(HCO_3)^-$] ions than intracellular fluid.
   d. Metabolic products are eliminated mainly through the lungs and kidneys.

9. Assume the dissociation of a diatomic molecule, $Y_2(g) \rightleftharpoons 2\ Y(g)$ is an endothermic reaction. Figure 4.1 schematically shows the equilibrium state of dissociation and Figure 4.2 schematically shows the equilibrium state after a change.

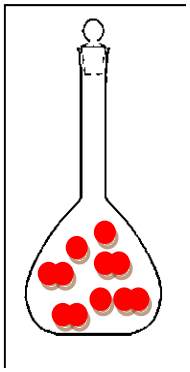


Figure 4.1

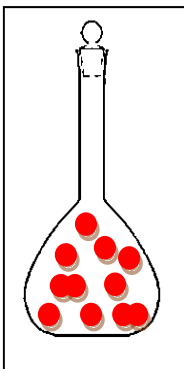


Figure 4.2

The introduced change that leads to equilibrium shown in Figure 4.2 is:

   a. The addition of Y atoms
   b. A decrease in temperature
   c. A decrease in volume
   d. An increase in temperature

Echo is an acoustic phenomenon produced when a sound wave is reflected and returns to its source. In the case of human hearing, in order for the echo to be perceived it is necessary for it to overcome acoustic persistence: required minimum time difference between the perception of two sounds by the ear, so that the human brain can distinguish them as different. In the case of dry sounds (like words) the acoustic persistence is 70.0 ms.

10. A climber, as shown in Figure 5, is standing at wall A and shouts toward wall B. The two walls can be considered perfectly vertical and flat. Considering that the speed of sound in air at that height is 344 ms^{-1}, the minimum distance from the wall that he must be at in order to hear the first echo is:

a. 24.1 m
b. 12.0 m
c. 241 m
d. 4.9 m

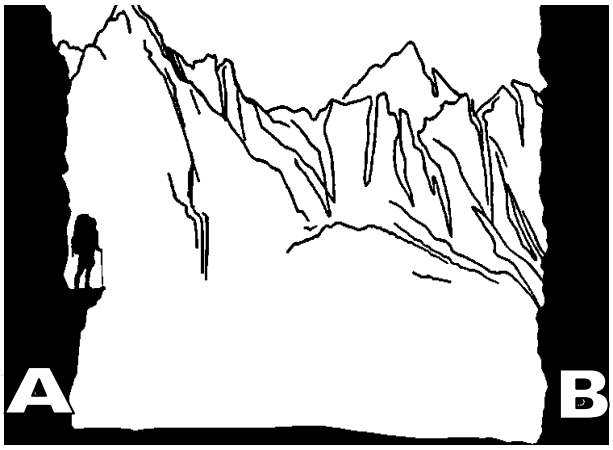


Figure 5

*11. The energy transported by a wave per unit time and per unit area through a surface perpendicular to the propagation direction is called intensity. If the waves propagate equally in all directions from a source of power P, the intensity **I** at a distance **r** is therefore* $\boldsymbol{I = \frac{P}{4\pi r^2}}$

Knowing that the distance between wall A and wall B is r, the intensity with which the climber hears the second echo, compared to the intensity with which he hears the first echo (I_0), will be (disregard energy loss in wall B reflection, and any interference effect):

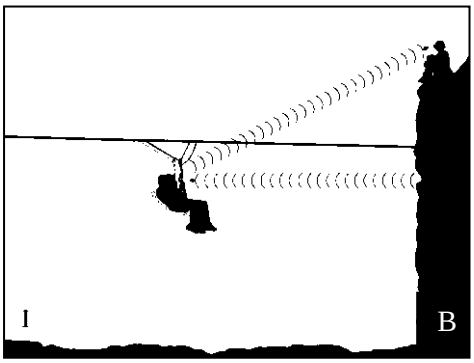
a. $I_0/4$
b. $I_0/2$
c. I_0
d. $2I_0$

The climber finds an old Tyrolean traverse (taut wire that connects the two sides of the mountain canyon) and decides to cross over. During his journey, on which he moves with constant velocity towards and perpendicular to wall B, the climber blows his whistle continuously. The pictures below are just a schematic representation of the situation.

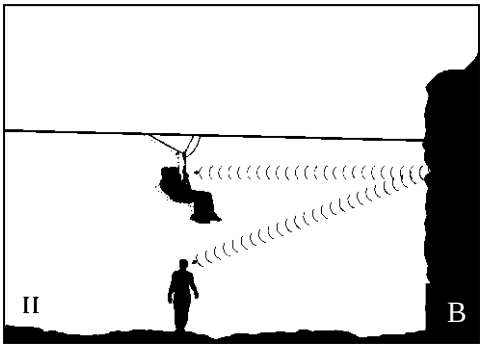
12. Taking into account the Doppler effect and neglecting the effect of wall A, select the correct option:

The frequency of the wave coming from wall B is perceived by the climber to be:

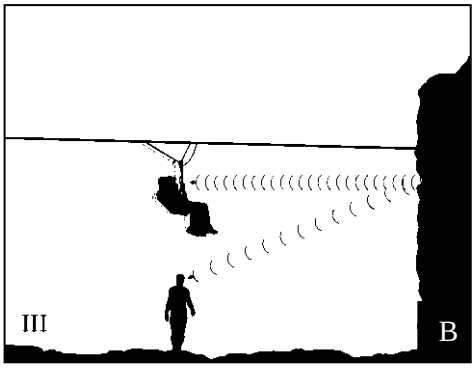
a. Equal to the frequency emitted by the whistle in motion that a person at rest sitting on the wall B, would receive



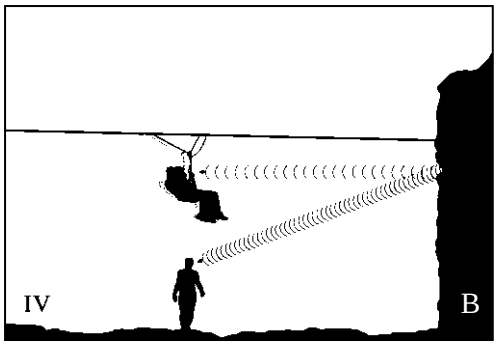
b. Equal to the frequency that a person at rest located at the same distance as the climber, would perceive coming from the wave reflected from wall B



c. Higher than the frequency that a person at rest located at the same distance as the climber, would perceive coming from the wave reflected from wall B



d. Lower than the frequency that a person at rest located at the same distance as the climber, would perceive coming from the wave reflected from wall B



13. Adult human hemoglobin (HbA) consists of four polypeptide chains, two α with 141 amino acids and two β with 146 amino acids.

The amino acid sequence of the polypeptide chain is known as:

a. The quaternary structure of hemoglobin
b. The tertiary structure of hemoglobin
c. The primary structure of hemoglobin
d. The secondary structure of hemoglobin

14. Hemoglobin may become abnormal by a mutation in the β chain of the normal protein thereby forming insoluble superpolymers that precipitate and generate sickle-shaped erythrocytes (Figure 7).

Figure 7

The synthesis of abnormal hemoglobin type "S" (Hb S) is governed by a recessive allele. Two parents are heterozygous for sickle-shaped erythrocytes. The percentage chance of genotypes in their offspring is:

a. 50% heterozygous and 50% recessive homozygous
b. 50% heterozygous and 50% dominant homozygous
c. 25% dominant homozygous, 25% recessive homozygous, 50 % heterozygous
d. 25% dominant homozygous, 50% recessive homozygous, 25% heterozyg

15. People who are homozygous for HbS suffer from falciform anemie disease /sickle cell anemia. What can be deduced from the altered hemoglobin and the abnormal shape of the erythrocyte?

a. Oxygen transport to tissues is canceled
b. Blood flow is reduced because of abnormal erythrocytes blocking blood vessels
c. Oxygen transport is not altered
d. Blood flow in vessels is decreased by precipitation of hemoglobin/HbS

16. Precipitation of red blood cells causes blockages in the blood vessels preventing normal tissue nutrition. The cross sectional area of an arteriole can be reduced up to 1/5 of the normal value.

When the cross sectional area of the arteriole is reduced, the velocity through this reduced section will be:

a. Higher than in the surrounding area where the cross sectional arteriole area is greater
b. Equal to the velocity in the surrounding area where the cross sectional arteriole area is greater
c. Lower than in the surrounding area where the cross sectional arteriole area is greater
d. Unchanged

17. Hemoglobin is composed of four pyrrole groups bound to Fe^{2+} (ferrous ion).

Fe (iron) is a transition metal, with atomic number 26 and mass number 56.

The ion of Fe (iron) above mentioned, will have:

a. 26 protons, 26 electrons and 30 neutrons
b. 26 protons, 24 electrons and 30 neutrons
c. 26 protons, 24 electrons and 24 neutrons
d. 26 protons, 26 electrons and 32 neutrons

18. Given the following standard reduction potentials for the redox pairs chemical species:

Chemical species	Standard Reduction Potential: Eº (V)
Fe^{2+} / Fe	-0.44
Cu^{2+} / Cu	+0.34
Zn^{2+} / Zn	-0.76
Ag^{+} / Ag	+0.80

It is possible to state that, in standard conditions, when arranging these species in pairs in an electrochemical cell:

a. Fe^{2+}(aq) is reduced when paired with all other elements (Cu(s), Zn(s) and Ag(s))
b. Fe^{2+}(aq) is an oxidizing agent when paired to Ag(s) and Cu(s), and Fe(s) a reducing agent when paired with Zn^{2+}(aq)
c. Fe(s) is oxidized when paired wih Ag^{1+}(aq), Cu^{2+}(aq), and Fe^{2+}(aq) is reduced when paired with Zn(s)
d. Fe(s) is oxidized only when paired with Zn^{2+}(aq)

19. In Mendoza flora, <u>Atriplex</u> <u>lampa</u> are an angiosperm with imperfect (unisexual) flowers.

These flowers are on separate plants, so in this case we are referring to a plant type:

a. Monoecious with flowers with stamens and carpels together
b. Monoecious with staminate flowers and carpellate flowers
c. Dioecious with staminate flowers and carpellate flowers
d. Dioecious with flowers with stamens and carpels together

20. *Osmosis modifies cells shape. If <u>Atriplex</u> <u>lampa</u> leaves are placed in a media with high salt concentration with respect to the plant, the solution is of the type:*

a. Hypotonic, causing movement of water to the outside of cells, the leaf wilts
b. Hypertonic, causing movement of water to the outside of the cell, the leaf wilts
c. Hypertonic, causing movement of water into the cell increasing turgor pressure
d. Hypotonic, causing movement of water into the cell, increasing turgor pressure

21. Regarding the formation of images by thin optical lenses, it is correct to ascertain that:

a. If the lens is divergent, as an object approaches the focal point from far away, the formed image is real, inverted, and more distant
b. If the lens is convergent, as an object approaches the focal point from far away, the formed image is real, upright, and more distant
c. If the lens is divergent, as an object approaches the focal point from the center of the lens, the formed image is real, upright, and smaller than the object
d. If the lens is convergent, as an object approaches the focal point from the center of the lens, the formed image is virtual, upright, and bigger than the object

22. *Different structures in animals allow maintenance of salt and water balance and excretion of metabolic waste. The functions of a species' excretory organs, and the composition of its excretions, both depend on the environment they inhabit.*

In the following table, column A presents different taxa belonging to the Animal Kingdom. Column B shows some excretory structures.

Select the right option which indicates the correlation between the number in column A and the letter in column B:

Column A		Column B	
1	Mammalia (Vertebrata)	A	No excretory organs
2	Gastropoda (Mollusca)	B	Kidneys
3	Hexapoda (Arthropoda)	C	Malpighian tubules
4	Asteroidea (Echinodermata)	D	Nephridia

a. 1-A; 2-B; 3-C; 4-D
b. 1-B; 2-D; 3-C; 4-A
c. 1-B; 2-A; 3-D; 4-C
d. 1-C; 2-B; 3-D; 4-A

23. The chemical element **A** has atomic number 11 and the chemical element **B**, 17. The electronic configuration of these elements in the ground state is :

a. A: $1s^2\ 2s^2\ 3s^3\ 3p^4$ B: $1s^2\ 2s^2\ 3s^2\ 2p^6\ 3p^6$
b. A: $1s^2\ 2s^2\ 2p^6\ 3s^1$ B: $1s^2\ 2s^2\ 3s^2\ 3p^5$
c. A: $1s^2\ 2s^2\ 3s^3\ 3p^4$ B: $1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^5$
d. A: $1s^2\ 2s^2\ 2p^6\ 3s^1$ B: $1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^5$

24. If the pressure of an ideal gas is halved and the absolute temperature of the gas is doubled, the volume of the gas:

a. Will increase by four times its original value
b. Will decrease to one fourth of its original value
c. Will stay the same as its original value
d. Will increase by two times its original value

25. 1.0 mole of CO_2 is placed in a sealed container with 1.0 mole of water at 25ºC. Then, half of the water from the container is siphoned out while the temperature is kept constant. The concentration of CO_2 gas dissolved in the remaining water inside the container:

a. will decrease because the amount of water in the container evaporates easily
b. will decrease because the volume of CO_2 gas will increase and so will its pressure
c. will decrease because the smaller amount of water decreases the solubility CO_2
d. will decrease because its pressure decreases and so does its solubility

26. A yellow-brown crystalline substance is heated strongly in a test tube at 280ºC. A clear liquid condenses around the mouth of the tube and the crystals gradually lose their yellow color and become dark green powder. Every gram of yellow-brown crystalline substance produces 0.39 g of clear liquid and 0.61 g of dark green powder. The same weight relationships are observed for samples of the crystals taken from many different sources.
These observations are consistent with the hypothesis that the yellow-brown crystalline substance is:

a. A Solution
b. A heterogeneous mixture
c. A hydrated salt
d. An element

27. Water is a compound with anomalous physical properties, giving it particular characteristics that allow the existence of life. These properties can be explained by stating that:

a. Water molecules can interact amongst themselves through hydrogen bonds
b. Water molecules make ionic bonds between oxygen-hydrogen giving water solubility to other compounds and forming solutions
c. Water molecules show only London forces
d. Water molecules show very weak interactions amongst themselves, and this is the reason of their easy ionization

28. Consider an experiment in which a particle moves in two dimensions on a table. The particle goes from point P1 to point P2 with constant speed along four different paths (Figure 8), but the time it takes to go from P1 to P2 is the same for all of them. Suppose now that the physicist carrying out the experiment measures the kinetic energy of the particle at point X in each trajectory. Select the path for which the kinetic energy at point X has the minimum value:

a. A
b. B
c. C
d. D

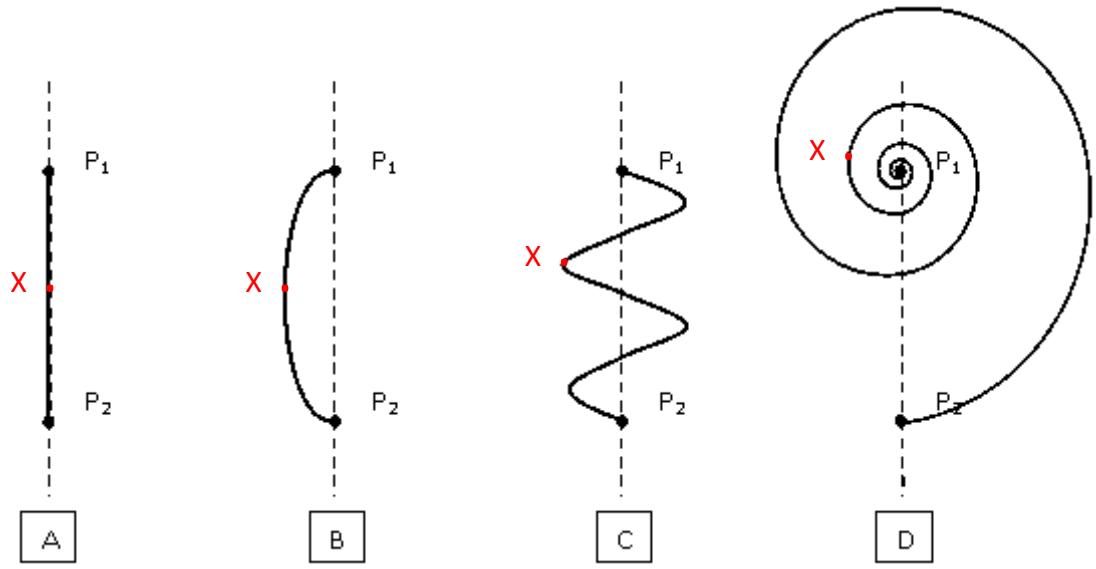


Figure 8

29. *Ozone in the stratosphere is formed under the effect of solar radiation on oxygen molecules. The total volume of ozone in the atmosphere is equivalent to a layer that under conditions of 25 ° C temperature and 1 bar pressure, would cover the Earth with a thickness of 3 mm. Its presence in the stratosphere is vital to maintaining life on Earth.*

Ozone is an:

a. Oxygen isotope
b. Oxygen ion
c. Oxygen allotrope
d. Oxygen isomer

30. Consider the circuit shown in Figure 9. If the resistance of each edge of the cube is R, the resistance between points a and h is:

a. 12R
b. (5/6)R
c. R
d. (3/2)R

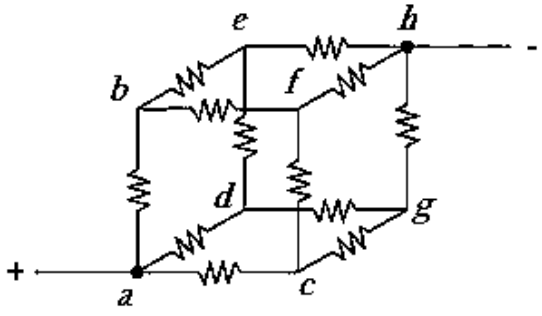


Figure 9

11th International Junior Science Olympiad

Multiple Choice Questions

December, 4 2014

NOMBRE	
APELLIDO	
CÓDIGO:	
PAÍS	
FIRMA:	

Nº	RESPUESTA			
1	a	b	c	d
2	a	b	c	d
3	a	b	c	d
4	a	b	c	d
5	a	b	c	d
6	a	b	c	d
7	a	b	c	d
8	a	b	c	d
9	a	b	c	d
10	a	b	c	d
11	a	b	c	d
12	a	b	c	d
13	a	b	c	d
14	a	b	c	d
15	a	b	c	d

Nº	RESPUESTA			
16	a	b	c	d
17	a	b	c	d
18	a	b	c	d
19	a	b	c	d
20	a	b	c	d
21	a	b	c	d
22	a	b	c	d
23	a	b	c	d
24	a	b	c	d
25	a	b	c	d
26	a	b	c	d
27	a	b	c	d
28	a	b	c	d
29	a	b	c	d
30	a	b	c	d

11th International Junior Science Olympiad

Theory Questions

December 6, 2014

EXAMINATION RULES

1. You have to sit at your designated desk.
2. Before the examination starts, you must check the stationery and any tools (pen, ruler, calculator) provided by the organizers.
3. You are not allowed to bring any tools except personal medicine or approved personal medical equipment.
4. You have to check the Question and Answer Sheets provided. Raise your hand, if you find any missing sheets. Start tasks after the start whistle is blown.
5. During the examination, you are not allowed to leave the examination room except in an emergency. If such is the case, you will be accompanied by a supervisor/volunteer/invigilator.
6. You are not to disturb other competitors. If you need any assistance, you may raise your hand and wait for a supervisor to come and assist you.
7. There will be no discussion about the examination tasks or problems. You must stay at your desk until the examination is over, even if you have finished it.
8. At the end of examination time you will hear a whistle blow. You are not to write anything on the Answer Sheets after the stop whistle. You must leave the room quietly when asked to do so. The question and Answer Sheets must be left neatly on your desk.

READ THE FOLLOWING INSTRUCTIONS CAREFULLY

1. The time available is 3 hours and 30 minutes.

2. Check that you have a complete set of test questions and Answer Sheets. The total number of questions is 3 (26 pages).

3. Write down your ID code on each page of your answer sheet.

4. Write your final answer in the smaller box provided. Write the steps clearly in the larger box.

To calculate $4^{-2.5}$, do the following

1) Press 4
2) Press y^x (just above number 8) key
3) Press 2.5
4) Press +/- key (next to key 0)

PROBLEM 1

The rapid increase in world population (currently of over 6700 million people) and the methods we have employed to extract, transport, transform, and use our resources are making our planet increasingly smaller and less sustainable for its inhabitants. During the last few years, global environmental changes have gained much importance due to a series of impacts that have been registered. Although the varied chemical, physical, and biological components interacting in our world have always been subject to change, the consequences of human activity have played, in the last century, a fundamental role in determining the nature of these changes.

A team of environmentalists of a non-governmental organization (NGO), and associated with the Ministry of Environment, have set among its goals, to divulge and assess the ecosystemic importance of the genus *Larrea*, (an Argentine plant species) which is widely distributed throughout the country. The NGO identifies its uses as:

1. Implementation in vegetative coverings of lands disturbed by human activity.
2. Social and cultural importance as a native species.
3. Applications in cosmetics and medicine, among others.
4. Use as ground hardener in areas prone to collapses.

The *Larrea* genus, of the Zygophyllaceae family (common local name: "jarilla"), inhabits arid areas. There are four species in Argentina: *Larrea cuneifolia, L. divaricata*, *L. nitida* y *L. ameghinoi*. Its leaves are coated by a "resin" composed in its greatest proportion by $C_{18}H_{22}O_4$, nordihydroguaiaretic acid (NDGA). This resin contributes to the prevention of water loss, and presents a level of toxicity for some herbivores.

1.1. In order to justify the use of *Larrea* as a native species for the revegetation of disturbed areas, a group of researchers worked in an area affected by mining activity. They proceeded as follows:
Two sample sites were chosen within the affected region. One of them was revegetated with *Larrea*, while the other one was left to natural succession. Ten years later, the two sites were sampled.

Since *Larrea* species modify the conditions under their coverage, favoring the colonization of other species; the goal of this test was to determine if the diversity of species observed in the community reflected this favored tendency for colonization.

Species diversity is an emergent property of biological communities, and can be measured by:

1. Species richness (S): number of different species in the community.
2. Species evenness or equitability: proportion of all individuals belonging to each species (relative abundance).

The Pielou index (J) is one of the indexes used to measure evenness and it is given by this equation:

$$J = \frac{H'}{H'_{max}}$$

Being:

H' the Shannon- Wiener index (values are shown in table 1 and 2 on the next page)

H'_{max} = ln(S)

S: species richness

Remember that:

1. Shannon- Wiener index is one of the indexes to measure biodiversity.
2. Species abundance: total number of individuals of a given species.

The data obtained from each sample is shown in the following tables and graphs:

Table 1: Species, Abundance and Forms of Life in the Community revegetated with *Larrea* (Sample site 1)

Species	Abundance	Form of Life
Acantholippia seriphioides	300	shrub
Condalia microphylla	250	shrub
Larrea cuneifolia	400	shrub
Larrea divaricata	250	shrub
Lycium tenuispinosum	250	shrub
Montea aphylla	150	shrub
Senna aphylla	220	shrub
Pyrrhocactus pachacoensis	700	cactus
Aristida mendocina	220	grass
Cottea pappophoroides	210	grass
Erodium cicatarium	750	herbaceous
Fabiana peki	450	herbaceous
Helenium donanium	650	herbaceous
Hoffmannseggia eremophila	500	herbaceous
Prosopis flexuosa	150	tree
Shannon-Wiener Index (H´)	**2.57**	

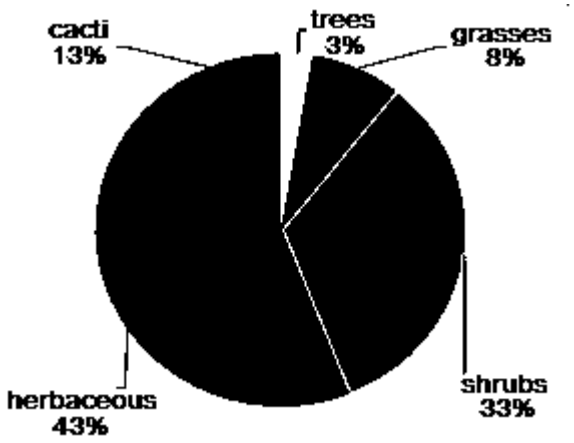


Figure 2: Forms of life percentages.

Table 2: Species, Abundance and Forms of Life in the Community left to natural succession (Sample site2)

Species	Abundance	Forms of Life
Spartium junceum	80	shrub
Larrea cuneifolia	200	shrub
Lycium chilense	70	shrub
Spartium junceum	50	shrub
Aristida mendocina	1000	grass
Cottea pappophoroides	250	grass
Stipa sp.	300	grass
Arjona longifolia	70	herbaceous
Buddleja mendozencis	25	herbaceous
Hysterionica jasionoide	30	herbaceous
Junellia aspera	40	herbaceous
Lecanophora heterophylla	55	herbaceous
Oenotera odorata	20	herbaceous
Salsola kali	25	herbaceous
Cercidium praecox	50	tree
Shannon-Wiener Index (H´)	**1.95**	

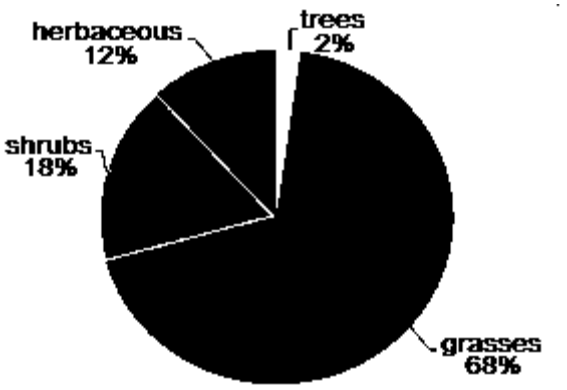


Figure 3: Forms of life percentages.

1.1.1. Fill in Table 3 in the Answer sheet with the information related to each community.

Community	**Species Richness (S)**	**Total number of individuals (N)**	**Name of dominant species**	**Abundance of dominant species**	**J**
Sample 1: Community revegetated with *Larrea*					
Sample 2: Community left to natural succession					

1.1.2. Indicate which community has a greater biological diversity.

1.1.3. Mark true (T) or false (F) appropriately for each explanation shown on the Answer Sheet, in order to justify the answer given in 1.1.2.

A. After ten years, the community left to natural succession and the community revegetated with *Larrea exhibited* a wide large difference in species richness, to *Larrea spp.*'s facilitating effect.	
B. The presence of *Larrea* in sample 1 increased the abundance of shrubs and herbaceous forms. This could indicate that *Larrea* favors the development of other species, augmenting the diversity of this community.	
C. The favoring effects are indicating that the revegetated community (sample site1) presents less diversity of forms of life than the community left to natural succession (sample site 2).	

1.2. Once the appropriate species for revegetation has been determined, the NGO is requested to revegetate an arid area located on the foothills of Mendoza. Figure 4 shows the region to be revegetated. Calculate the areas A, B, C, D, and calculate total surface area of the region. Express the final result in square meters.

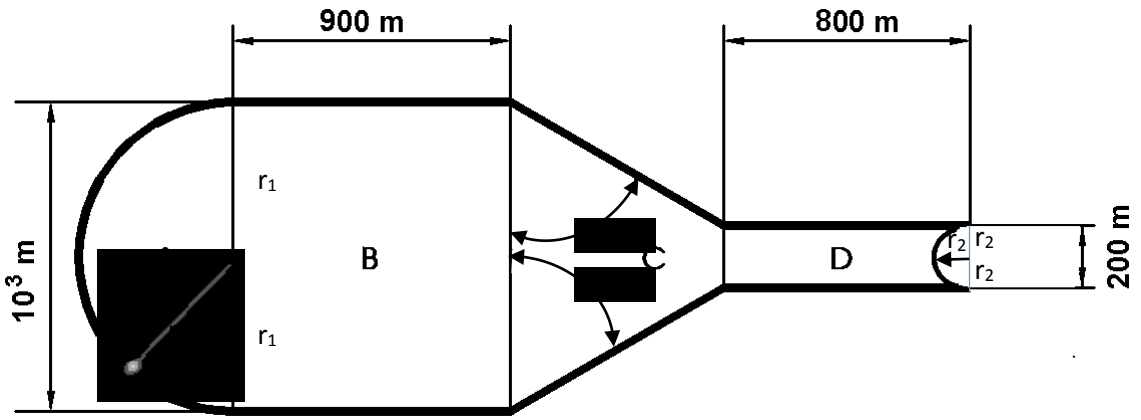


Figure 4: Schematic representation of the region to be revegetated.

1.3. Another difficulty generated by the loss of natural flora, mainly in terrains with a slope, is the detachment and consequent falling of rocks. A side-view of a foothill in Mendoza is shown on Figure 5.

Figure 5: Side-view of the terrain.

1.3.1. While carrying out their revegetation duties, a group of workers located at point I notice a rock sliding (without rotating) down the hillside, with a velocity of 10.0 m s^{-1}. From point I to point II there is a distance of 50.0 m, and the kinetic coefficient of friction between the rock and the ground in that stretch is 0.46. Neglecting air resistance, and assuming, the surfaces is

flat, find the velocity of the rock when it passes point II. (Acceleration due to gravity: 9.81 m s^{-2})

1.3.2. The presence of vegetation helps to prevent rock detachment, and to stop the rocks from sliding if they are detached. Suppose the 200.0 m stretch from point II to point III has been revegetated, and that the rock from exercise 1.3.1 reaches point III with zero velocity**.** Find the kinetic friction coefficient of this stretch, assuming it is constant throughout it.

1.4. In order to improve the condition of the soil, the researchers performed chemical analyses on the disturbed soil and determined the optimum values that *Larrea* spp. need for their development.
The results are shown on Table 4.

Variables	Composition of disturbed soil	Optimum soil composition for *Larrea*
Organic Matter (% w/w)	0.90	3.33
Total Nitrogen (mg Kg^{-1})	1 033.00	1 353.00
Extractable Phosphorus (mg Kg^{-1})	1.00	8.12
Extractable Potassium (mg Kg^{-1})	1 199.00	1 444.00
Humidity (% w/w)	10.52	12.97
pH	7.54	7.10

Table 4: Composition of the disturbed soil versus optimum soil composition for the development of the *Larrea* species.

Larrea is tolerant to disturbed soils in the desert, but has a low tolerance to soils with high amounts of phosphorus. The disturbed soil must be prepared with fertilizer at a depth of 0.3 m in order for the soil to reach its optimum level of nutrients. The composition of nutrients by mass in the proposed fertilizer is 30% N, 15% P_2O_5 and 15% K_2O.

(Soil density 1.63 x 10^3 kg m^{-3}; relative atomic mass P: 31; O: 16; K: 39; N: 14)

1.4.1. Determine which nutrient you would choose to use as a reference to determine the

amount of fertilizer to be used.

1.4.2. Compute how many kilograms of fertilizer must be added per square meters of terrain. Round results using two decimal after the unit.

1.4.3. Determine the concentration of hydroxide ions $(OH)^{1-}$ responsible for the pH value measured in the disturbed soil shown in Table 4.

1.4.4. Indicate which of the pH indicators shown in Table 5 you would choose to determine qualitatively the disturbed soil pH, and what color the chosen indicator would take. Write the indicator and the color using the code in parentheses)

Indicator	pH interval	Color
Methyl Orange (MO)	3.1 – 4.4	Red (R)-Yellow (Y)
Bromothymol Blue (BB)	6.2 – 7.5	Yellow (Y)-Blue (B)
Methyl Red (MR)	4.2 – 6.2	Red (R)-Yellow (Y)
Metacresol Purple (MP)	7.8 – 9.2	Yellow (Y)-Purple (P)
Phenolphthalein (PP)	8.3 – 10.0	Colorless (C)-Violet (V)

Table 5: Relationship between indicator, pH interval and color.

1.4.5. The pH of the soil can be high in regions of elevated concentrations of soluble Na_2CO_3 salt (sodium carbonate) and poor precipitation. This is due to the hydrolysis reaction between the carbonate ion and H_2O (water).
Write the balanced chemical equation for the total ionization reaction of Na_2CO_3 in water.

1.4.6. Write the balanced ionic hydrolysis equation that justifies the pH elevation in the soil due to the carbonate ion.

1.5. *Larrea* had numerous utilities for native people, including the use of *Larrea cuneifolia* as a compass according to the position of the sun. This plant positions itself so that it captures the morning and afternoon mild sunlight, but avoids the strong sunlight at noon, thus reducing transpiration. Figure 6 shows four illustrations of a *Larrea* leaf, each with a different orientation.

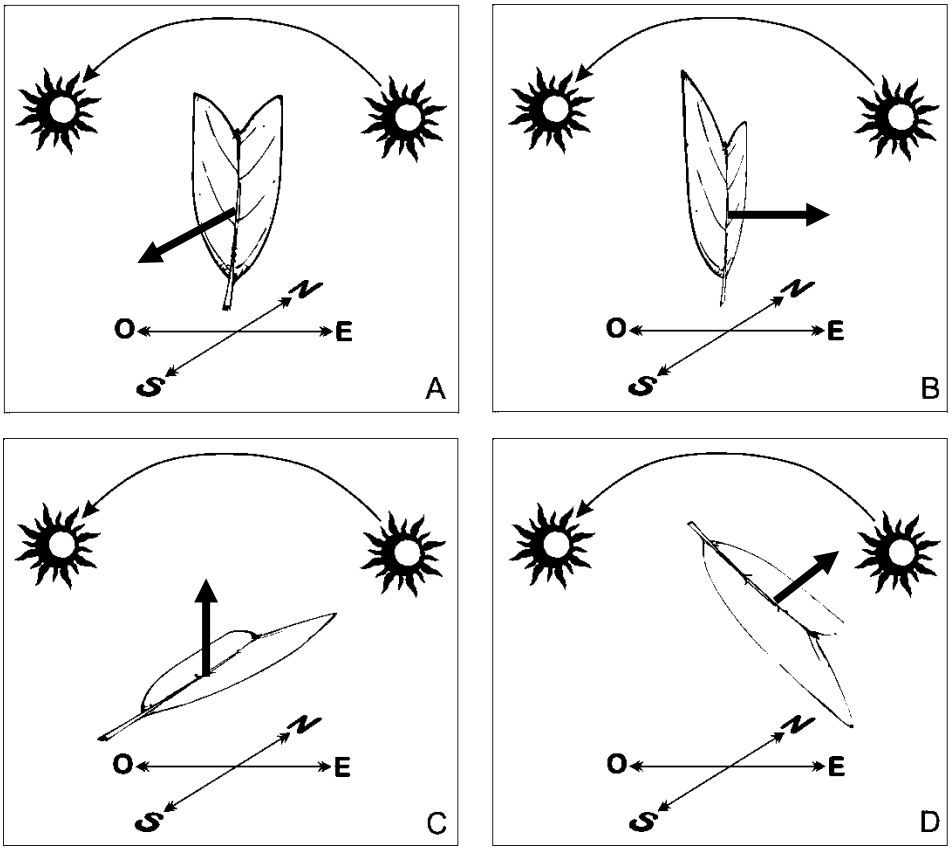


Figure 6: *Larrea cuneifolia* leaves with different orientations. The arrows show the normal direction of the upper surface of the leaf.

1.5.1. Indicate which one illustration best expresses the description given in 1.5.

1.5.2. Select from the following options the statement that provides the reason to the answer to 1.5.1:

a) The wavelength of the light emitted by the Sun is different at different times during the day

b) The frequency of the light emitted by the Sun is different at different times during the day

c) The surface of the leaf exposed perpendicularly to the Sun's radiation at different times during the day

d) The environment temperature is different at different times during the day

1.6. The nordihydroguaiaretic acid (NDGA) ($C_{18}H_{22}O_4$), present in the resin of *Larrea* leaves, has many uses in cosmetics and medicinal industries due to its properties as an antioxidant, anticarcinogen and antiviral.

A method to identify this acid is based on its reaction with ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}.4H_2O$) to produce an orange colored complex. The absorbance of an incident ray of light of certain wavelength is measured after it traverses a sample of the colored solution placed in a transparent cuvette as shown in Figure 7. The absorbance will depend directly on the concentration of the colored complex present in the solution, which absorbs radiation at that particular wavelength.

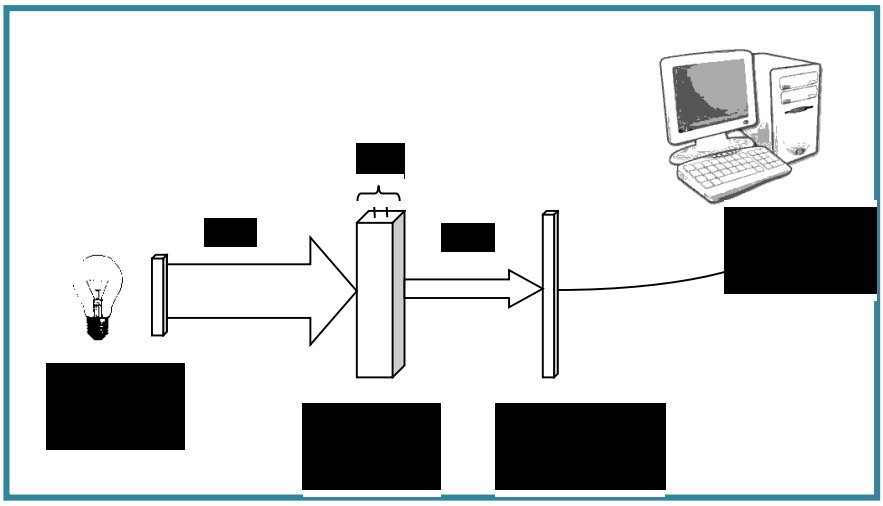


Figure 7: Schematic setup for the measurement of absorbance

In the process of extraction of NDGA from the leaves of *Larrea sp*., other substances are extracted together with it. These substances interfere with the quantitative determination of NDGA. Since they have some functional groups in common with NDGA, they react with the ammonium molybdate producing the colored complex.

Alcohol extracts were obtained from the leaves of *L. divaricata* and *L. cuneifolia*. Absorbance measurements were performed on them at different wavelengths, using the ammonium molybdate method. The results are shown on Table 6.

Wavelength (nm)	400	450	500	550	600	650
Average absorbance of other substances in the extracts	0.82	0.33	0.15	0.07	0.05	0
NDGA absorbance in *L. divaricata*	1	0.84	0.53	0.22	0.09	0.03
NDGA absorbance in *L. cuneifolia*	0.99	0.81	0.46	0.20	0.06	0.04

Table 6: Absorbance of NDGA and other substances in *L. divaricata* and *L. cuneifolia,* and the corresponding wavelengths.

1.6.1. Draw a Cartesian graph of comparative lines for absorbance as a function of wavelength for the three samples, based on Table 6. Use different colors for each type of absorbance line.

1.6.2. Using the information given in Table 6, indicate the wavelength that is needed to best distinguish NDGA from other substances.

A relation between the absorbance and the concentration of the concerned substance, which is given by the following expression:

$$\mathcal{A} = C\varepsilon L$$

where $\mathcal{A}$ the absorbance of the sample (dimensionless); C the concentration of the substance (M); L the optical travel length (see Figure 7); ε the molar absorptivity coefficient, which depends on the substance being analyzed, the wavelength concerned and experimental conditions (M^{-1} cm^{-1}).

A chemist obtained two extracts, one from the leaves of *L. divaricata* and another from *L. cuneifolia*, but forgot to label them. He decided to place them in two vessels labeled A and B, and measured the absorbance of each sample at 500 nm using a cuvette of 1 cm optical travel length.
With ε = 8 920 M^{-1} cm^{-1} and the molar mass of NDGA being 302 g mol^{-1}, the absorbance for extract A was 0.47, while the absorbance for sample B was 0.52.

1.6.3. Calculate the NDGA concentration (mg L^{-1}) in each extract. Consider the extract was purified from other substances.

1.6.4. Using the information in Table 6 and the values for each absorbance, indicate the species corresponding to each extract.

1.6.5. A 500 ml aqueous solution at 0.2% w/v of NDGA is to be prepared from *L. divaricata* leaves. It is known that dry *L. divaricata* leaves contain 7% w/w of NDGA. Determine the mass of fresh *L. divaricata* leaves that you need considering that fresh leaves have a moisture content of 8% w/w (water density at 20°C is 1 g cm^{-3}).

PROBLEM 2

Climate change is understood as a long-term change in the average weather conditions produced by nature and/or human activity. It is currently an environmental issue of major worldwide concern.

Some of the topics related to climate change that are causing concern in the scientific community include:

a. *Acid rain affecting the fresh water reservoirs of the planet, and generating conflicts in the distribution of water to human population.*
b. *Melting glaciers as a direct consequence of a global rise in temperature.*

The increased concentration of certain gases in the atmosphere, such as the so-called Greenhouse Gases (GG) among which we find carbon dioxide (CO_2), methane (CH_4), and nitrogen dioxide (NO_2), is a result of human activity associated with the use of fossil fuels, intensive agriculture, and soil alteration. The accumulation of these gases has produced a rise in the average temperature of the earth's surface known as "Global Warming". Another consequence of the greater concentrations of GG together with sulfur dioxide (SO_2), is the formation of a type of precipitation known as acid rain, which is also naturally produced by volcanic eruptions and thermal springs. The harmful effects of low pH levels in aquatic ecosystems are immediate and it also has other consequences such as the deterioration of construction materials, sculptures and stone monuments. Besides, acid rain dissolves metals such as lead (Pb), and copper (Cu) present in water pipes. Nevertheless, sulfur in acid rain may have benefits. Sulfur dioxide is converted into sulfate aerosols that enhance the reflection of solar radiation and also limits global warming by counteracting the natural production of methane gases by microbes in wetland areas; and thereby tending to cool Earth's surface.

2.1. Normal precipitation has an average pH of approximately 5.65 due to the presence of CO_2 which, together with atmospheric water, forms carbonic acid (H_2CO_3). If the pH value is lower than 5, it is considered acid rain, sometimes reaching a pH as low as 3. The toxic constituents of acid rain are SO_2 and nitrogen monoxide (NO), originated from the combustion of carbon or hydrocarbons. Through a series of chemical reactions, SO_2 and NO turns into sulfur trioxide (SO_3) and nitrogen dioxide (NO_2), respectively combining afterwards with atmospheric water and producing the corresponding acids responsible for a low pH.

2.1.1. Write the chemical reactions needed to obtain the acids derived from SO_3 and CO_2 when combined with water.

2.1.2. The unbalanced formation reaction of nitric acid in acid rain is:

$$NO_2\,(g) + H_2O\,(l) \rightarrow HNO_3\,(aq) + HNO_2\,(aq)$$

Write the half-reactions and the balanced equation.

2.2. Unwanted chemical reactions such as the dissolution of the aluminum hydroxide ($Al(OH)_3$) present in soils, occurring at pH levels lower than 4, are facilitated with severe consequences for fish, amphibians, and insects due to aluminum's toxicity. The water acidity causes serious complications for invertebrates with an exoskeleton composed of calcium carbonate ($CaCO_3$) in the form of calcite, since it favors the dissolution of this mineral.

The inhabitants of a city located in the Andes detected the disturbance of a lake ecosystem and its near surroundings up to a few kilometers away. The alterations were clearly manifested in the deterioration of the native flora and fauna, where a great number of fish and amphibians were found dead on the lakeside. With the object of determining the cause of the problem, and thus provide a solution, researchers decided to analyze a sample of water. It was found that fish mortality was due to aluminum poisoning.

2.2.1. Determine the solubility in moles per liter of aluminum hydroxide ($Al(OH)_3$) in the lake water which has a pH of 5.2, Knowing that the Kps (solubility product) value is $5x10^{-33}$.

2.3. The water piping network in the above mentioned city is represented in Figure 8, where V_1-V_{11} are the distribution points, and V_1 the main receptor connected to the water plant.
In the past, water pipes were made of lead. They have been degraded over time and caused cases of intoxication and saturnism (lead poisoning) (in people) due to the presence of this heavy metal in water.
The old water pipes must be replaced by others made of polyvinyl chloride (PVC). In order to avoid the cost of unnecessary connections, the current number of connections must be reduced.

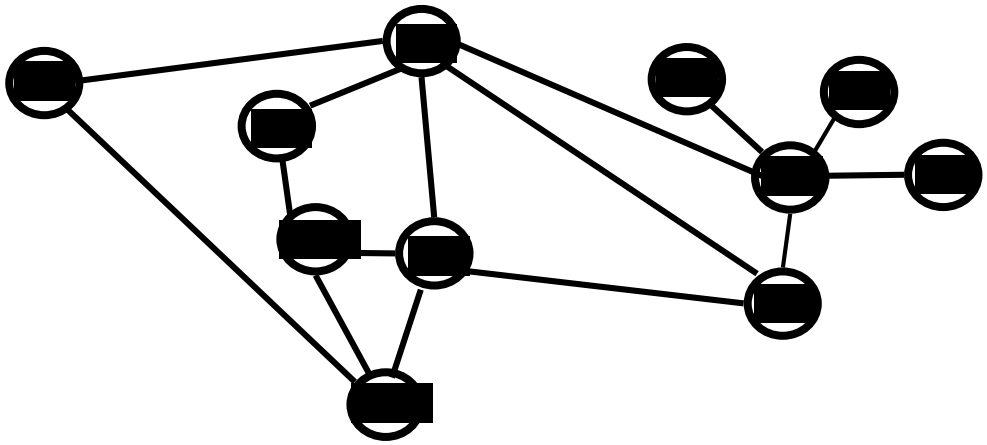


Figure 8: Water piping network

2.3.1. Determine the maximum number of pipes that can be removed without interrupting the water supply in every distribution point.

2.4. Lead accumulates in the body and normally affects the liver, the kidneys, and the nervous system. Its excessive ingestion affects the neurological system, resulting in mental retardation, low average growth heights and disruptions in daily activity. Even low quantities of absorbed lead can result in damages to the central nervous system of small children and fetuses.

The toxic levels of lead in blood for adults range from 0.038 mg/100 ml upward, while in children it starts at 0.005 mg/100 ml. The levels of lead concentration obtained from blood tests of 5 adults (sample 1) and 5 children (sample 2) of the city under discussion are shown on the following table7.

Sample	Blood lead concentration (mg/100 ml)				
1. Adults	0.057	0.020	0.087	0.060	0.065
2. Children	0.004	0.010	0.009	0.008	0.007

Table 7: Blood lead concentration (mg/100 ml) in adults and children.

2.4.1. Compute the average value of lead in blood for each sample. Indicate which of them, if any, is above the toxicity levels, writing an A for adults, a C for children and a B for both

2.5. Life on Earth is made possible by the energy it receives from the Sun, and by the presence of the Greenhouse Gases, which absorb the infrared radiation emitted by the surface of the Earth. Part of this absorbed radiation is reemitted to the upper parts of the atmosphere, while another part is reemitted to the surface of the Earth increasing its average temperature.

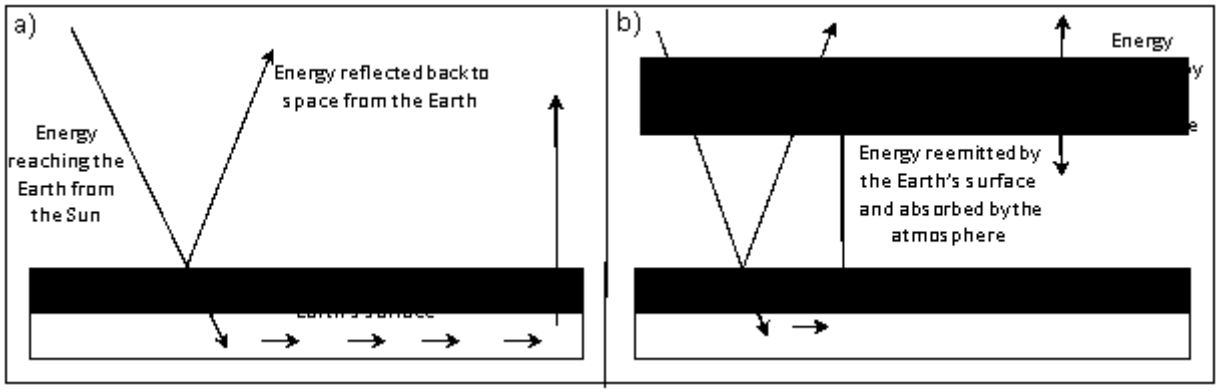


Figure 9. a) without atmosphere. b) with atmosphere

The surface of the Sun radiates energy as a black body at a temperature of 5500°C. A black body is an object that absorbs all the energy of the incident radiation. Part of this energy reaches the top of the Earth's atmosphere with an average incoming intensity of approximately 341W m^{-2} over the whole surface of the atmosphere. Thirty percent (30%) of this intensity is reflected back to space and the rest is absorbed by the surface of the Earth, which reemits it at larger wavelengths (infrared radiation).

2.5.1. Calculate Earth's equilibrium temperature without the absorption of the atmosphere (Figure 9a). Express your results in K and °C.

The intensity I (W m^{-2}) radiated by a black body follows Stefan-Boltzmann's Law:

$$I = \sigma T^4, \quad \text{where} \sigma = 5{,}67 \times 10^{-8} \text{W m}^{-2} \text{K}^{-4}$$

Assume that the Earth radiates energy as a black body.

2.5.2. The atmosphere is transparent to most of the high frequency radiation coming from the Sun, but not to low frequency radiation (infrared part of the electromagnetic spectrum), such as that reemitted by the Earth's surface. This is mainly due to the presence of greenhouse gases in the atmosphere.

Find the equilibrium temperature of the Earth with the atmosphere (Figure 9b), assuming that the atmosphere absorbs all the radiation emitted by the surface, and reemits it back to both the Earth and Space in equal proportions. Start out with intensity balance on the Earth surface and on the atmosphere.

Hint:

Assume that the atmosphere only absorbs energy from the Earth surface, and that both emit energy as a black body.

2.6. One of the theories concerning climate change affirms that the planet is warming due to the Greenhouse Effect, and this in turn is causing the melting of glaciers.

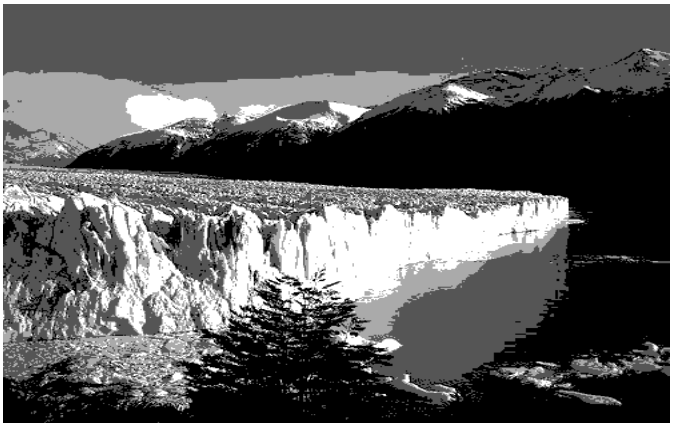


Figure 10: Glacier Perito Moreno, Santa Cruz, Argentina

2.6.1. In this context, calculate the minimum amount of energy needed to completely melt a block of 10 000 kg of ice, initially at -10°C.

Thermodynamic constants:

Specific heat capacity of Ice: $c = 2.093\ J\ g^{-1}\ K^{-1}$

Latent heat of ice fusion: $l = 333.7\ J\ g^{-1}$

PROBLEM 3

Sports physiology studies the responses and adaptations of the human body to physical effort. These responses and adaptations vary depending on multiple factors such as the intensity, duration or frequency of physical activity performed; or others, such as diet, environmental conditions or genetic inheritance of each individual.

A 13 year old teenager enjoys physical activity. For several months, she has been training for an athletic competition. Today she got up early and had breakfast, which consisted of a cup of milk, a slice of toast and a banana.

3.1. Carbohydrate digestion results in the catabolism of the large molecules into simpler molecules. The enzymes contained in the body catalyze this transformation.

3.1.1. Based on the information shown on Table A and Table B, mark with a cross (X) on Table A the secretory structure which produces the enzyme involved in digesting the starch that is present in the slice of toast. Then, use the corresponding number given to the enzyme shown on Table B, to match the Structure where this enzyme is produced.
Hint: the same enzyme may be produced by more than one structure.

Table A		
STRUCTURE	SECRETORY STRUCTURE INVOLVED IN THE DIGESTION OF STARCH	ENZYME
Liver		
Stomach		
Salivary Glands		
Large intestine		
Pancreas		
Esophagus		
Small Intestine		

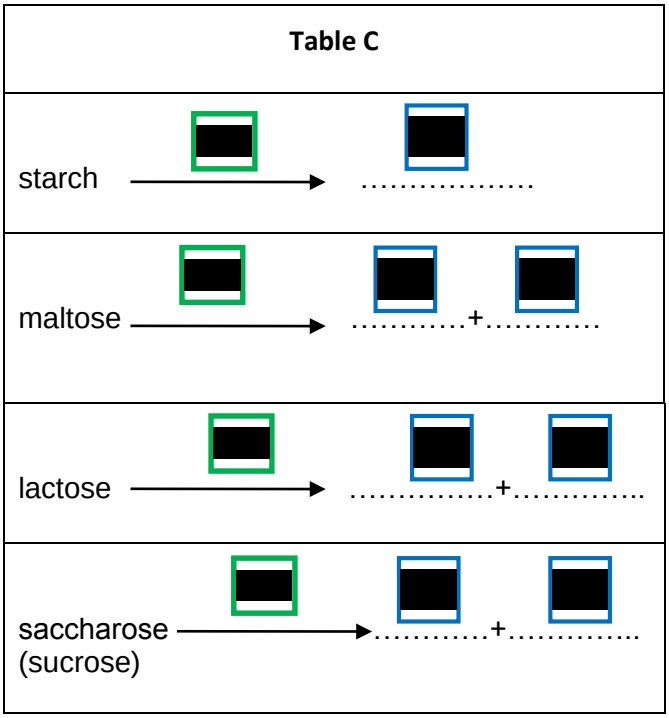
Table B	
1	Phospholipase
2	Maltase
3	Amylase
4	Lipase
5	Glucosidase
6	Sucrase

3.1.2. At breakfast, she also incorporated carbohydrates from milk and fruit that in the digestive process will be split into simpler sugars due to the activity of specific enzymes.

Fill in Table C (indicated on the Answer Sheet) which shows enzymatic reactions. Write the corresponding letter of the enzyme in the green box and the corresponding number for the products in the blue boxes (each number may be used more than once).

ENZYMES		PRODUCTS	
A	Creatin kinase	1	Maltose
B	Amylase	2	Glucose
C	Lactase	3	Fructose
D	Glucosidase	4	Lactose
E	Sucrase	5	Galactose
F	Maltase	6	Saccharose (sucrose)

Table C



After breakfast, the athlete goes to the sports field for her daily workout routine. If we study in detail the movement of the athlete and the processes involved in the musculature, we can say:

3.2. *". Musculature together with the skeletal system it forms the musculoskeletal system, which is responsible for movement of the human body. The mechanism of muscular contraction can be explained by an ordered sequence of processes".*

3.2.1 Table D shows muscular contraction processes. Complete Table E (indicated in Answer Sheet), indicating the letter of the process in the order in which they occur.

Table D: Processes of muscle contraction
A. ATP is hydrolyzed to ADP + Pi (inorganic phosphorus) and the myosin head is separated from the active site.
B. Acetylcholine acts on a local area of the sarcolemma to open multiple membrane protein channels. This allows the entry of large amounts of sodium ions into the sarcolemma, which initiates an action potential in the muscle fiber.

C. The action potential depolarizes the sarcolemma. The release of Ca^{++} ions from the sarcoplasmic reticulum occurs.
D. Ca^{++} ions are pumped back into the sarcoplasmic reticulum, where they remain until the arrival of a new action potential to the muscle.
E. An action potential reaches the neuromuscular junction (synapse) of a motor neuron and a muscle, acetylcholine is released from the axon terminal.
F. Ca^{++} ions initiate attractive forces between the actin and myosin. Filaments of myosin and actin are arranged next to each other within the sarcomere so that that they can interact in an organized fashion resulting in muscle contraction. During contraction, myosin heads bind actin and pull the filaments in towards the center.

Tabla E	
ORDER	CORRESPONDING LETTER
1	
2	
3	
4	
5	
6	

3.2.2 Figure 11 represents muscle contraction. Write the letter corresponding to each process in the space provided.

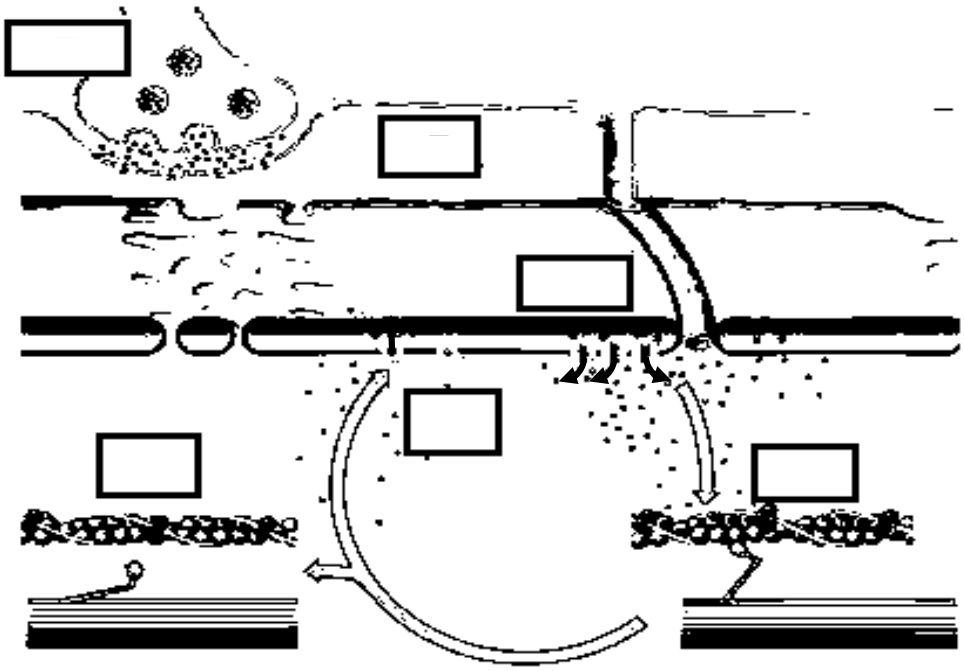


Figure 11: Muscle contraction representation.

The contraction of skeletal muscle, involves multiple simultaneous shortening of sarcomeres. This means that the sliding of actin and myosin filaments occurs, making them work as an engine. For this process to occur, mechanical forces and energy are required. The forces are generated by the filament cross-bridges and the energy comes from the hydrolysis of ATP to ADP + Pi.

3.3 As part of her daily training, the athlete also performs a weight lifting routine in order to strengthen her muscles.

3.3.1. The typical maximum force of an "engine" of myosin-actin as those found in muscles, is 5.0×10^{-12} N. Calculate the minimum amount of "engines" a muscle should utilize in lifting a mass of 50 kg. (Acceleration of gravity: 9.81 m s^{-2})

3.3.2. These engines consume ATP. The typical force exerted by a myosin engine is 5×10^{-12} N, and its typical speed is about 11×10^{-9} m s^{-1}. Calculate the power developed by the myosin engine measured in J s^{-1}.

3.4. Different foods provide the organism with different amounts of energy to carry out its functions. The combustion equation of glucose ($C_6H_{12}O_6$) is one of those most frequently used to describe this process.

3.4.1. If the food ingested at breakfast by the athlete is equivalent to 90 g of glucose, calculate the mass of carbon dioxide (CO_2) produced as a result of complete combustion. (Relative atomic mass C= 12; O=16; H=1).

3.4.2. Calculate the number of oxygen atoms in 90 g of glucose molecules.

3.5. Then the athlete decides to do a short speed test in a straight line first and then in a circular movement.

3.5.1. During her straight line path, her velocity follows the graph as shown in the figure below. Find the instantaneous acceleration at points A, B and C.

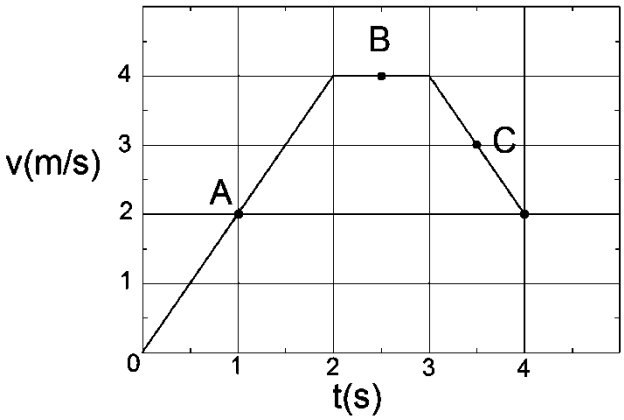
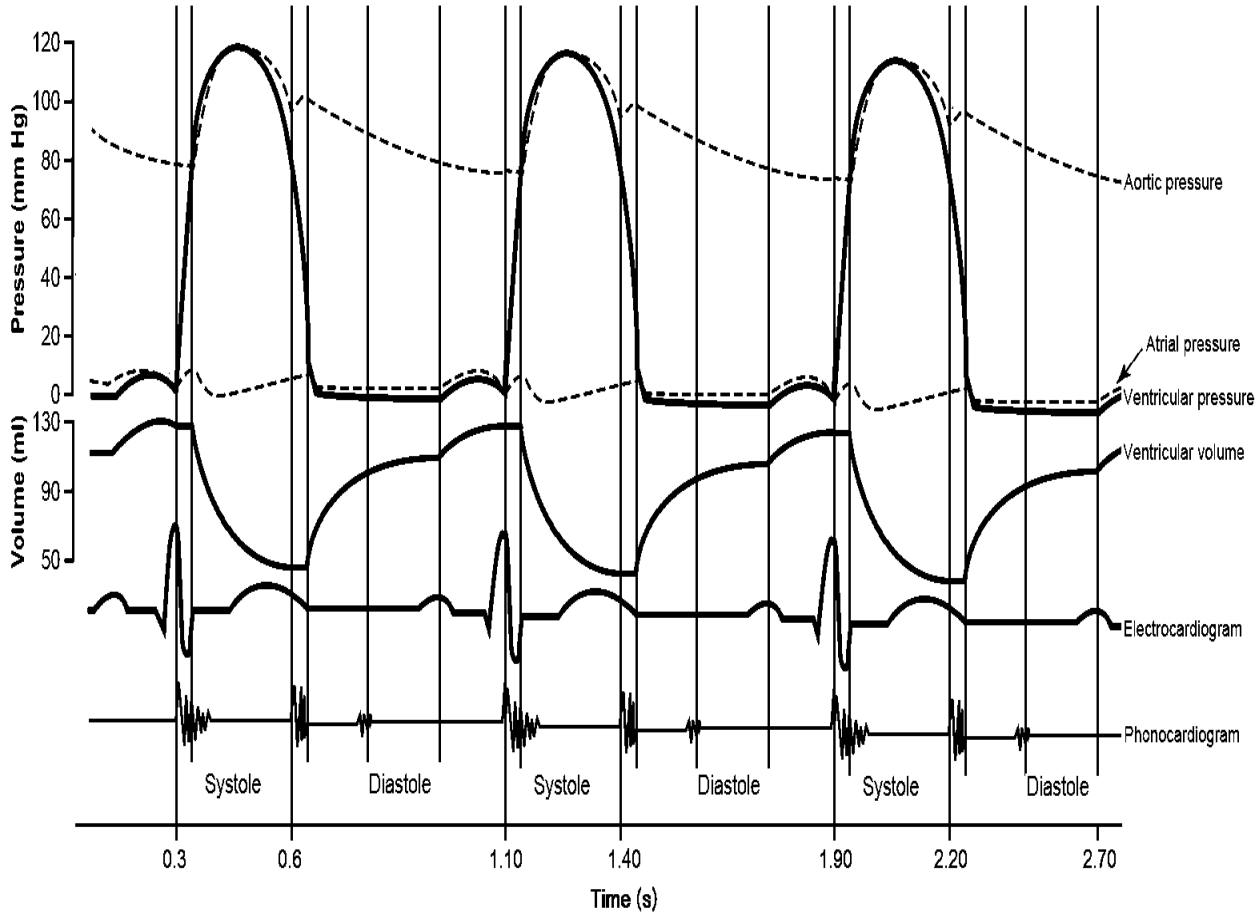


Figure 12: Velocity as a function of time

3.5.2. Calculate the distance she runs in the first two seconds the race.

3.5.3. The circular path starts at t= 4 s, with the speed shown in Figure 12. The limiting force friction between the footwear and the ground of the athlete does not allow her to have more than 3.0 m s^{-2} centripetal acceleration. Calculate the minimum radius of her circular path. Consider a constant speed along the whole path.

3.6. Before starting with her physical activity, the athlete underwent a medical checkup. The pressure and volume for different parts of the heart in relation with time, as well as an electrocardiogram and phonocardiogram are illustrated in Figure 13.



[redacted]

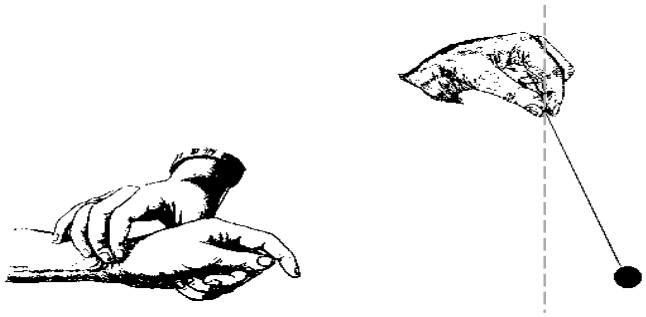
3.6.1. Using the information shown in the graph, compute the time period of one cardiac cycle (in seconds).

3.6.2. Calculate the corresponding heart rate (cardiac cycles per minute).

3.6.3. Indicate in which time intervals ventricular volume decreases considering that intervals in which the volume remains constant last 0.05 seconds.

3.6. 4. Indicate the maximum value observed for ventricular pressure.

3.7. After physical activity the athlete felt excessively agitated, so she decided to ask someone to take her pulse. At that time no one had a watch, but a Physics student said that he could do it by improvising a simple pendulum and making it oscillate slightly around its equilibrium position (Figure 14). Every 15 complete cycles of the pendulum, the student measured 20 beats and found that the heart frequency of the athlete was twice the one recorded in the previous exercises (frequency calculated in point 3.6.2).



3.7.1. Calculate the length of the pendulum used by the student, the period of simple pendulum is given by $T = 2\pi\sqrt{\frac{L}{g}}$

Being:
L the length of the pendulum
g= 9.81 m

11th International Junior Science Olympiad

Mendoza, Argentina

Experimental Test

December 8th, 2014

Time : 4 hrs
Marks : 40

Experimental test

EXAMINATION RULES

1. Participants must not enter prohibited items (tools, papers, books, medicine, etc.). Required medicine or personal medical equipment must be previously notified to the monitor.
2. Only examination sheets and the material provided by the monitor can be used throughout the examination.
3. The use of personal safety items: gloves, goggles, and lab coat; are mandatory throughout the whole Experimental Test.
4. Participants must work safely. The equipment and the working station should be kept clean and tidy.
5. Each participant must stay at their designated location and is not allowed to leave the classroom before the Experimental Test time elapsed.
6. It is forbidden to eat or drink throughout the examination. If necessary, the participant can call the monitor and make a request for permission to take a bite out of the examination location.
7. If a participant needs to go to the bathroom, they must be authorized by a monitor.
8. Participants of different teams cannot communicate with each other. Working discussions must be done sparingly and quietly. If the participant needs assistance, they can require it from the monitor.
9. No questions regarding the examination will be answered.
10. A beeping signal will announce the expiration of examination time. From that moment it is forbidden to write anything on the Answer Sheet.
11. After completing the examination, the working material should be organized like at the beginning of the test.
12. The yellow Answer Sheet will be collected by the monitor at the assigned place for each group after the finalization signal.
13. Participants must wait until the monitor collects the Answer Sheet before leaving the room.

Time : 4 hrs
Marks : 40

Experimental test

INSTRUCTIONS

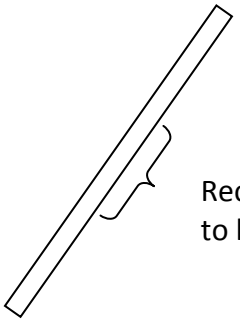
The Experimental Test consists of two parts, which are independent from each other. Therefore, they can be made consecutively or simultaneously. The team should decide how to best optimize working time.

1. Each participant must make sure to have all the working material provided by the monitor (pencil, eraser, ruler and calculator).
2. Each participant must check the papers given, which consists of two sets: experimental instructions and questionnaire, and the yellow Answer Sheet (for submit) and the white Answer Sheet (for draft). **Only the yellow Answer Sheet will be marked.**
3. Each participant must check the personal protective equipment provided (gloves, goggles and lab coat).
4. Each group must make sure to have all the necessary material and reagents for each experiment according to the detailed lists.
5. Each team must indicate on the first page of the Answer Sheet: name of each member of the group, seat numbers, country and signatures. In addition, the team must write the group code, and the participant codes on the top of each page.
6. Results must be written in ink on the yellow Answer Sheet in the assigned space for this purpose. Those results written out of the assigned location, will not be considered. **The results in the white Answer Sheet will not be considered.**
7. The time available to perform the experimental work and record the results on the Answer Sheet is 4 hours. Thirty minutes before and five minutes before expiration time, there will be an announcement indicating the end of the examination approaching. Upon exam time finalization, the group must stop working immediately and wait until the Answer Sheet is collected by a Monitor.

LABORATORY EQUIPMENT SUGESTIONS

Recommendation for glass rod use:

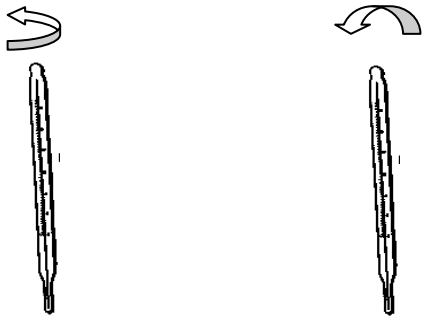
In order to prevent the glass rod from breaking, it is recommended to hold it from its middle section as indicated in the following drawing.



Recommended place
to hold the glass rod

Experimental test

Recommendation for thermometer use:
For temperature measurements hold the thermometer with the scale pointing towards you.
A thin line of mercury should be seen.
If the mercury line is not evident, move the thermometer slightly (either rotating it or tilting it) until you see the end of the mercury line.



General consideration
If there is any doubt about the use of laboratory equipment, raise your hand to be assisted by the monitor.

Calculator instructions

How to calculate $e^{-2.5}$:

1. Press 2.5
2. Press +/- key
3. Press 2ndf key
4. Press e^x key

How to calculate ln(4):

1. Press 4
2. Press ln key

SECTION 1: ALCOHOLIC FERMENTATION OF GLUCOSE [25.0 Marks]

INTRODUCTION:

Alcoholic Fermentation refers to the decomposition of organic matter with fast and vigorous gas release. It is a biological process in which sugars such as glucose, fructose and sucrose are transformed by microorganisms to obtain energy. The metabolic process can be performed by yeasts, such as Saccharomyces cerevisiae, under anaerobic conditions (absence of air, in particular oxygen). Yeasts are eukaryotic microorganisms classified in the Fungi kingdom. They are unicellular, and have no flagellum. They are round or ovoid cells with a 5 to 10 µm diameter. In particular, alcoholic fermentation (called as such due to the metabolic product ethanol), is the basis for various biotechnological applications including manufacture of alcoholic beverages such as wine, beer and cider drinks; as well as manufacture of bread products. Today it is also used for the large-scale synthesis of ethanol, which is then used as biofuel.

*The yeast Saccharomyces cerevisiae (**Figure 1**) will be used in this experiment. The metabolic process is represented by **equation 1.** In this process the cells get energy and generate ethanol (CH_3-CH_2-OH) and carbon dioxide (CO_2) as waste products. By measuring the volume of released gas during the metabolic process, glucose consumption and ethanol production can be estimated. The identity of the produced gas can also be confirmed.*

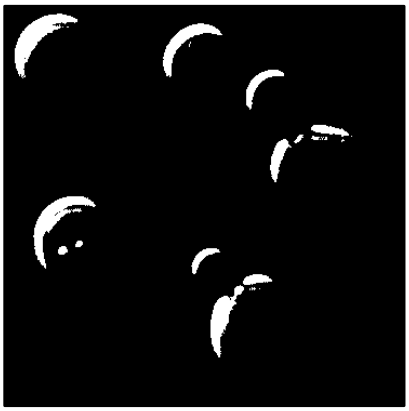


Figure 1: *Saccharomices cerevisiae*
(Image achieved by differential interference contrast microscopy)

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \xrightarrow{\text{yeast}} \underset{\text{Energy}}{238.5\ \text{kJ}} + \underset{\text{Ethanol}}{\mathbf{2}CH_3CH_2OH} + \underset{\text{Carbondioxide}}{\mathbf{2}CO_2(g)}$$

Equation 1

Experimental test

EXPERIMENTATION

Reagents and materials

ID #	Safety material	Quantity
A	Latex gloves	8
B	Goggles	3
C	Absorbent paper roll	1
D	Wastebucket	2

ID #	Material for experimental assay	Quantity
1	Rectangular container	1
2	1000 ml graduated cylinder	1
3	250 ml beaker	2
4	Universal support	1
5	Three-finger clamp	1
6	15 x 15 cm rubber pad	1
7	1000 ml beaker	1
8	Glass rod	1
9	Thermometer, -10 °C to 150 °C	1
10	1000 ml conical/Buchner flask (Kitasato) with rubber stopper and latex tube (reaction flask)	1
11	Stopwatch	1

ID #	Reagent for experimental assay	Quantity
I	Water, 10 L	1
II	Bromothymol blue solution, 25 ml	1
III	Hot water thermos flask, app. 75 °C, 1 L	1
IV	Glucose sample, 4 g	4
V	Yeast sample, 50 g	2
VI	Saturated $Ca(OH)_2$ solution, 25 ml	1

PROCEDURE:

A. ***Instructions for assembling the gas measurement equipment, according to*** **Figure 2*.***

This equipment will be used to monitor one of the produced metabolites (gas) by fermentation.

A.1. Pour water into the rectangular container (ID# 1) up to half full. Use water provided in the 10 L container (ID# I) (Figure 2a).

A.2. Fill the graduated cylinder (ID# 2) with the water poured in step A.1 by using a 250 ml beaker (ID# 3) (Figure 2 b).

A.3. Position the universal support (ID# 4) with the three-finger clamp (ID# 5) close to the rectangular container (Figure 2c).

A.4. Cover the mouth of the graduated cylinder with the rubber pad (ID# 6) and hold it with the palm of your hand (Figure 2d).

A.5. Turn the graduated cylinder upside down while keeping it closed with the rubber pad.

A.6. Immerse the graduated cylinder into the rectangular container and take out the rubber pad as shown in Figure 2e.

Important: The graduated cylinder must remain as full of water as possible. Some air could remain trapped but it should be kept at a minimum after taking out the rubber pad.

A.7. Fix the graduated cylinder to the universal support leaving approximately a 2 cm separation between the bottom of the rectangular container and the graduated cylinder, as shown in Figure 2e.

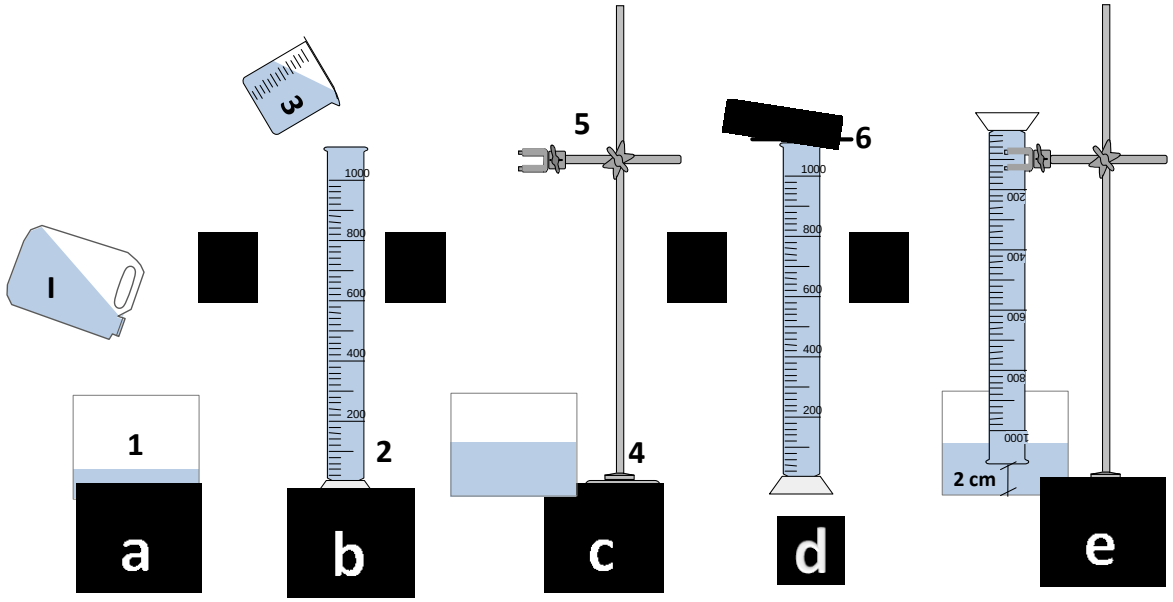


Figure 2

B. ***Instructions for assembling the fermentation reaction flask according to*** **Figure 3.**

B.1. Prepare approximately 750 ml of water within the range of 35-40 °C in the 1000 ml beaker (ID# 7). To this end, use the hot water (ca. 75 °C) provided in the 1 L thermos flask (ID# III) and the water provided in the 10 L container (ID# I). Measure the temperature with the thermometer (ID# 9) and record your reading on the Answer Sheet.

B.2. Label the 250 ml beakers (ID# 3) as "**A**" and "**B"** respectively.

B.3. Prepare **Suspension A** and **Solution B** using the warm water obtained in step B.1, as follows:

> **Suspension A:** Suspend in the 250 ml beaker labelled "A" (ID# 3) all 50 g of yeast supplied to you (ID# V) with 150 ml of warm water (35-40°C). Mix with the glass rod (ID# 8). Bring to a final volume of 250 ml with warm water (Figure 3a).
>
> **Solution B:** Dissolve in the 250 ml beaker labelled "B" (ID# 3) 4 g of glucose (ID# IV) with 150 ml of warm water (35-40°C). Mix with the glass stick (ID# 8). Bring to a final volume of 250 ml with warm water (Figure 3a).

B.4. Pour into the reaction flask (ID# 10) Suspension **A**. In order to ensure a quantitative transference of the yeast, rinse beaker A with a sample of Solution B. Do not dispose of the rinsing residues; pour all of them into the reaction flask. Gently swirl the solution in a circular

motion to homogenize. Place the reaction flask on the rubber pad (ID# 6), which is the same previously used (Figure 3b).

Important: The whole Suspension A and Solution B must be poured into the reaction flask. Do not use additional water for rinsing the beakers.

B.5. Measure the temperature of the resulting suspension and record it on **Table 1.1.** on the Answer Sheet (Figure 3c).

B.6. Cover the reaction flask with the rubber stopper firmly (Figure 3d).

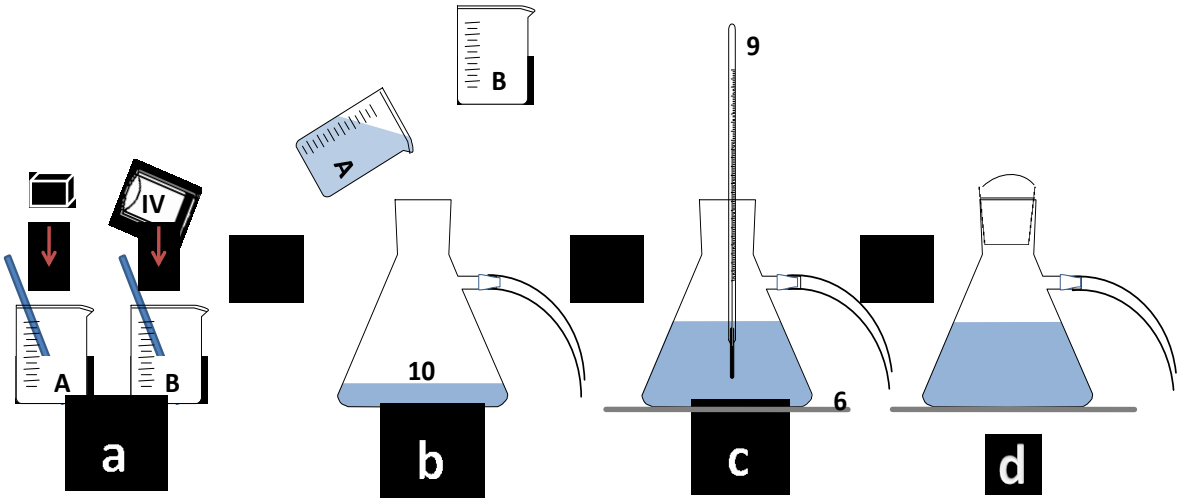


Figure 3

C. ***Instructions for assembling the fermentation reaction flask and the gas measurement equipment according to*** **Figure 4.**

C.1. Take the free end of the latex tube and insert it into the inverted graduated cylinder, as shown in (Figure 4). The free end of the latex tube should be at the top of the graduated cylinder after assembling.

C.2. Gently swirl the reaction flask in a circular motion in order to release air possibly trapped in the latex tube. Record the water level in the cylinder on the Answer Sheet. The resulting water level must be considered as the zero-point of the scale, which will be used for measuring the volume of released CO_2 (g).

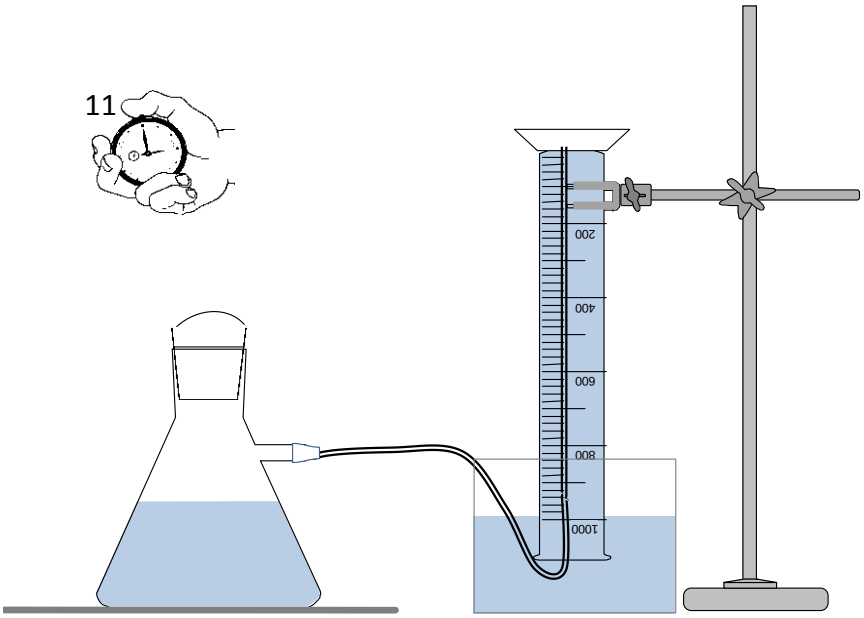


Figure 4

D. ***Instructions for recording the experimental data of the fermentation.***

D.1. As soon as the fermentation reaction flask and gas measurement equipment are assembled (Figure 4) start the stopwatch (ID# 11).

D.2. The reaction flask must be gently swirled in a circular motion, every 1 min for 5 seconds for the duration of the whole experiment.

Important: be careful when swirling the reaction flask because the latex tube should not slip out of the graduated cylinder.

As fermentation occurs in the reaction flask, the gaseous product is released; and most of it is transferred through the latex tube to the water column of the graduated cylinder. The transferred gaseous product will be trapped on the upper part of the graduated cylinder, where the total volume generated can be measured. The gaseous product accumulation will be evidenced by the decrease in the water column of the graduated cylinder. The remaining fraction of the gaseous product dissolves into the reaction flask solution.

D.3. The volume of accumulated gas must be measured every 2 minutes and recorded on **Table 1.1**. For 40 min, record data on the Answer Sheet.

Important: to carry out the reactivation process, do not stop the stopwatch.

Table 1.1: Recording fermentation data [8.5 Marks=7.0 (Record data) + 1.5 (Flow calculation)]

Initial temperature of warm water for preparing suspension A and solution B:		
Initial Temperature of suspension A+B (inside reaction flask):		
Time [min]	**Accumulated gas volume $V_a(t)$; [ml]**	**Flow F(t), [ml/min]**
0		
2		
4		
6		
8		
10		
12		
14		
16		
18		
20		
22		
24		
26		
28		
30		
32		
34		
36		
38		
40		

Experimental test

Instructions for recording experimental data of fermentation reactivation.

E.1. After 40 min of fermentation occuring, firmly hold the reaction flask and close off the latex tube by bending it tightly, so that gas does not pass through it.

E.2. Remove the rubber stopper from the reaction flask.

Important: be careful in order to avoid the latex tube from slipping out of the graduated cylinder.

E.3. Add the second sample of solid glucose (ID# IV) directly in the reaction flask and cover the reaction flask with the rubber stopper, making sure it is tightly closed.

E.4. Release the latex tube ensuring gas movement through it.

E.5. Gently swirl the reaction flask in a circular motion for 30 seconds in order to dissolve the new sample of glucose.

Important: if the graduated cylinder empties out completely it can be filled up again by following the procedure described in Section A. Or else do not disturb the setup of the graduated cylinder.

E.6. The reaction flask must be gently swirled in a circular motion, every 1 min for 5 seconds to record data.

E.7. The volume of the accumulated gaseous product must be measured every 2 minutes for 10 min and recorded on **Table 1.2** on the Answer Sheet.

TABLE 1.2.: Data of fermentation reactivation [2.5 Marks=2.0 (Record data) +0.5 (Flow calculation)]

Time [min]	**Accumulated gas volume $V_a(t)$; [ml]**	**Flow F(t), [ml/min]**
42		
44		
46		
48		
50		

E. *Identification of the gaseous product:*

F.1. Reaction with $Ca(OH)_2$

F.1.1. After 10 min of time elapsing of the reactivation stage, take out the latex tube from the graduated cylinder and immerse it into the saturated $Ca(OH)_2$ solution (ID # VI) as shown in **Figure 5**.

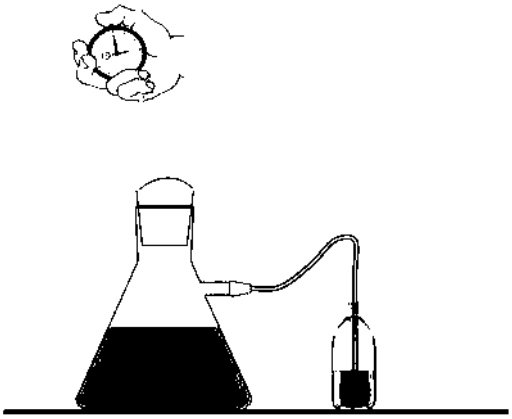


Figure 5

F.1.2. Swirl the reaction flask gently in a circular motion for 60 seconds for the gaseous product to bubble in $Ca(OH)_2$ solution.

F.1.3. Observe whether there is any change in the identification solution ($Ca(OH)_2$).

Choose the correct answer by ticking the appropriate box on the Answer Sheet. [0.5 Marks]

- White precipitate is observed
- Black precipitate is observed
- Precipitate is not observed

DO NOT FILL IN THIS TABLE

F.1.4. Formulate the stoichiometric equation of the reaction on the Answer Sheet. [0.5 Marks]

F.2. Reaction with bromothymol blue indicator.

F.2.1. Take out the latex tube from the $Ca(OH)_2$ solution and immerse it in bromothymol blue solution (ID# II) as shown in **Figure 6**.

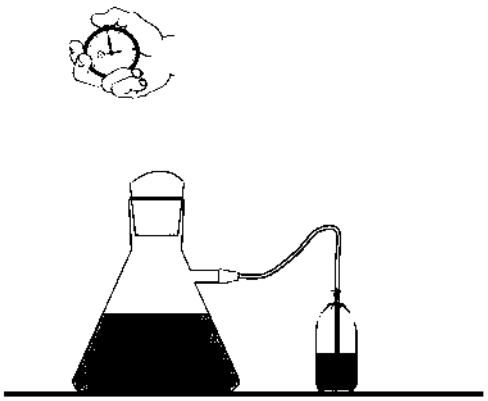


Figure 6

F.2.2. Gently swirl the reaction flask in a circular motion for 60 seconds for the gaseous product to bubble into the bromothymol blue solution.

F.2.2.1. Observe whether there is any change in the bromothymol blue solution.

Choose the correct answer by ticking the appropriate box on the Answer Sheet. [0.2 Marks]

- Yellow color is observed
- Blue color is observed
- Red color is observed
- White color is observed

DO NOT FILL IN THIS TABLE

F.2.2.2. What can you deduce from the observation of the bromothymol blue solution? (Mark the correct answer). [0.2 Marks]

- An increase in pH
- A decrease in pH
- No change in pH

DO NOT FILL IN THIS TABLE

F.2.2.3. Formulate the dissociation equation which explains the pH change in the indicator solution. [0.6 Marks]

G. *Data processing and analysis:*

Calculate and complete data in **Table 1.1** and **Table 1.2 (on the Answer Sheet)**.

G.1. Calculate the gaseous product flow (F) for each time interval (Δt=2 min) according to **Equation 2**:

$$F = \frac{V_a(t) - V_a(t - \Delta t)}{\Delta t}$$

Equation 2

Being:

- F: gaseous product flow [ml/min]
- $V_a(t)$: Accumulated volume at time t [ml].

G.2. Plot the data of **Table 1.1** and **Table 1.2** together on the supplied grid **on the Answer Sheet** by using Cartesian coordinate system as follows: [2.5 Marks]

G.2.1**. GRAPH A**: Accumulated gas volume ($V_a(t)$; [ml]) vs. Fermentation time [min].

G.2.2**. GRAPH B**: Gaseous product flow (F(t); [ml/min]) vs. Fermentation time [min]. [1.8 Marks=1.5 Marks (plotting data) + 0.3 identify fermentation stages]

G.3. Draw a cross (X) on the curve of **GRAPH A** at t = 40 min of fermentation.

The initial sample of glucose was partially metabolized after 40 min of fermentation. The glucose that is not metabolized remains in the reaction flask solution and is called ¨residual glucose¨. The metabolized glucose was transformed into gaseous product CO_2 and ethanol. Most of gaseous product CO_2 was transferred to the graduated cylinder through the latex tube. The remaining fraction of the gaseous product CO_2 was dissolved in the reaction flask solution. **Figure 7** *summarizes the process.*

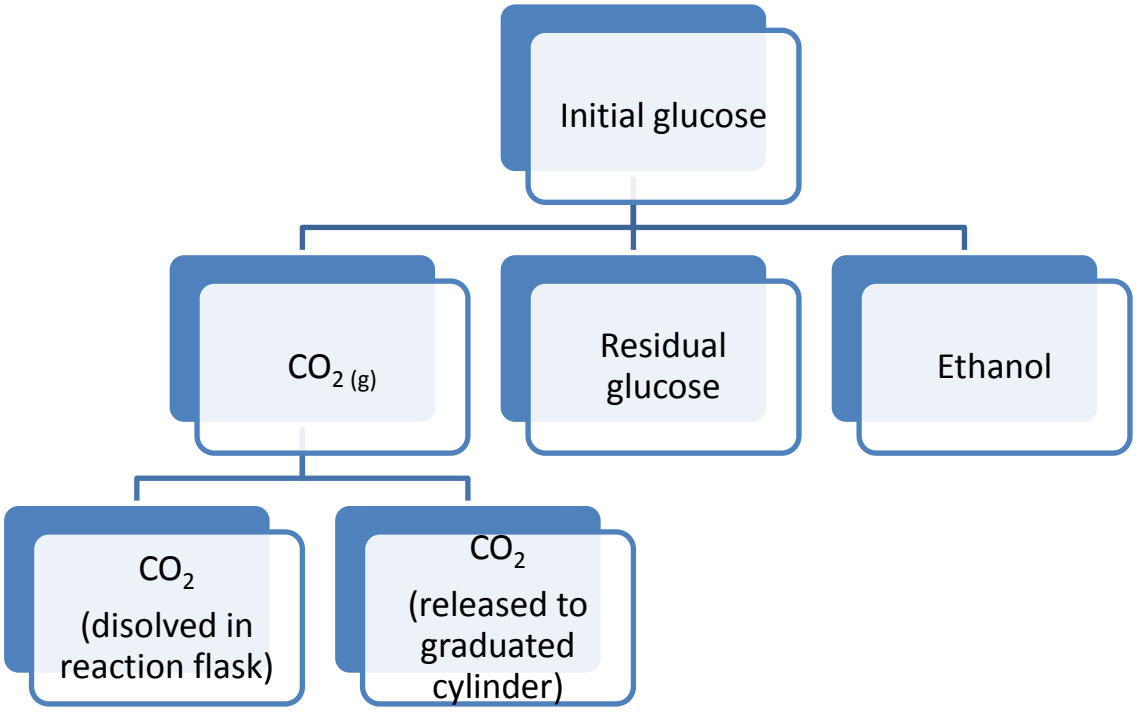


Figure 7

Perform the following calculations on the Answer Sheet.

G.3.1. Calculate the amount (in grams) of metabolized glucose that produced the CO_2 released into the graduated cylinder. To do this, consider the volume of CO_2 accumulated at t=40 min. Use stoichiometric calculations, **Equation 1** and the following data: [1.0 Marks]

Element	Relative Atomic Mass
C	12.010
H	1.008
O	16.000

CO_2 Molar volume at reaction conditions	22.4 L/mol

G.3.2. The amount of **residual glucose** can be estimated according to the following procedure:

G.3.2.1. Identify the time intervals of the fermentation stages (a, b and c) **up to t=40 min** on **GRAPH B**.

a. **An increase in the rate of gas production.**
b. **A decrease** in the rate of gas production.
c. **Constant** rate of gas production.

For such purpose, draw marks or bars in ink on **GRAPH B on the Answer Sheet** to indicate the different fermentation stages and write the corresponding letter.

G.3.2.2. Calculate the natural logarithm (ln) of measured gaseous product flow (F) during the fermentation stage ' A decrease in the rate of gas production' (Stage b, **GRAPH B**), and complete **Table 2.1**. with the calculated values on the Answer Sheet.

Table 2.1.: Calculate the natural logarithm (ln) of data of the fermentation stage corresponding to "A Decrease in the rate of gas production" [0.5 Marks]

Time [min]	F [ml/min]	ln(F)

G.3.2.3. Plot the data of **Table 2.1** on the supplied grid by using a Cartesian coordinate system as follows: ln F vs. time [min] (**GRAPH C**). [1.0 Marks]

G.3.2.4. Draw a straight line that best fits the data points of **GRAPH C**. [1.0 Marks]

G.3.2.5. Calculate the slope (A) and obtain the y-intercept (B) of the fitted line on the **GRAPH C**. Record the calculated values on **Table 2.2** on the Answer Sheet. [0.7 Marks]

Table 2.2: Linear equation of calibration curve

Linear equation	
Slope(A)	DO NOT FILL IN THIS TABLE
y-intercept(B)	

G.3.2.6. There is a hypothetical CO_2 volume (V_h) associated to the residual glucose. This is the volume of CO_2 that could be released into the graduated cylinder if the residual glucose were metabolized. This CO_2 volume is calculated through Equation 3. Calculate and record the value of V_h on the Answer Sheet. [0.2 Marks]

$$\mathbf{V_h = -\frac{e^{[A \times t_h + B]}}{A}}$$

Equation 3

Being:

- $t_h = 40$ min
- V_h: Hypothetical CO_2 volume [ml]
- A: slope of the fitting line obtained in Section G.3.2.5
- B: intercept of the fitting line obtained in Section G.3.2.5

G.3.2.7. Calculate stoichiometrically the mass of glucose that corresponds to the volume V_h of CO_2. That amount of glucose is the residual glucose in the reaction flask. [0.4 Marks]

G.3.2.8. Calculate the **total mass of glucose** that was fermented during the 40 min fermentation. Calculate and record the value on the Answer Sheet. [0.2 Marks]

G.3.2.9. Calculate the **total mass of CO_2 produced** by the glucose consumed calculated in section G.3.2.8. Record the value on the Answer Sheet. [0.4 Marks]

G.3.2.10. Calculate the **mass of CO_2 released** to the graduated cylinder during the 40 min fermentation. Use the V_a (40 min) for calculation. Record the value on the Answer Sheet. [0.4 Marks]

G.3.2.11. Calculate the **mass of CO_2 dissolved** in the reaction flask. Record the value on the Answer Sheet. Assume that no CO_2 is dissolved in the water outside the reaction flask during the whole process. [0.2 Marks]

G.3.2.12. Calculate the **solubility of CO_2** (in g/L) in the reaction flask. Record the value on the Answer Sheet. [0.3 marks]

G.3.3. Calculate the **amount of ethanol** (**in moles**) produced at t=40 min by using stoichiometric calculations and **Equation 1**. Resolve the calculation and record the answers on the Answer Sheet. [0.4 Marks]

G.3.4. Calculate the resulting **concentration of ethanol** (in g/100 ml) in the reaction flaskat t=40 min by using the amount of ethanol previously calculated in G.3.3. Resolve the calculation and record the answers on the Answer Sheet. [0.5 Marks]

G.3.5. Gas production rate slows down at stage ¨b¨ (**GRAPH B**) for some of the reasons detailed below. Consider alcohol concentration calculated previously (G.3.4), and toxic concentration threshold for yeast, which is 14 g/100 ml. [0.5 Marks]

Choose the primary reason by ticking the appropriate box on the Answer Sheet.

a. Yeast death

b. Inhibition of yeast due to the concentration of alcohol.

c. Shortage of fermentable substrate

DO NOT FILL IN THIS TABLE

Time : 4 hrs
Marks : 40

Experimental test

SECTION **2: REFRACTOMETRIC DETERMINATION OF SUCROSE CONCENTRATION** [15.0 Marks]

INTRODUCTION:

Refractometry is a physical testing method of measuring the refractive index of substances. Since the refractive index is a constant for a particular liquid at a given temperature, it can be used to help identify substances, check for purity, and measure concentrations. In fact, the refractive index for many binary mixtures changes linearly with concentration over a wide range of concentrations.

Food and beverage industries use the refractive index to find the concentration of sugar in different samples. The instrument used for this purpose is called ***refractometer.*** *The refractive index is a measure of the speed of light in a medium and therefore, when light passes from one medium to another with different refractive indexes, the path of light is deviated from the original path. This phenomenon is the fundament of the refractometer.*

The refractive index of juices or drinks is influenced by the concentration of soluble solids, which are mainly constituted by sucrose. In addition to sucrose, other soluble solids such as organic acids and salts, can affect the measurement. Consequently, it is considered that the measurement in these samples corresponds to sucrose content, with an accuracy of about 0.5%. The total sucrose concentration is expressed in Brix degrees (° Bx), whose units are grams of sucrose per 100 g of solution [% w/w]. For example: A honey of 25 ° Bx contains 25 g of sucrose per 100 g of honey.

In this experiment the sucrose concentration unit will be g/100 ml.

EXPERIMENTATION

Reagents and materials

ID #	Material for experimental assay	Quantity
12	Home-made refractometer	1
13	Laser source	1
14	Funnel	1
15	50 ml volumetric flask	1
16	125 ml plastic bottle	5
17	25 ml pipette	1
18	Pipette filler	1
19	Marker pen	1

Experimental test

ID #	Reagent for experimental assay	Quantity
VII	Initial solution, 62.5 g/100 ml sucrose, 100 ml	1
VIII	Water squeeze bottle	1
IX	Plastic Pasteur pipette	1
X	Sucrose solution, unknown concentration, 50 ml	1
XI	Honey solution, 50 ml	1
XII	Beverage sample, 50 ml	1

PROCEDURE:

CAUTION:
Do not point the laser beam to the eyes. Turn it on only for the calibration and measuring processes.
Laser specifications: <1 mW, red.

A. ***Instructions for assembling the refractometer according to*** **Figure 8*.***

This equipment will be used to construct a calibration curve and to determine the sucrose concentration of the Samples. The home-made refractometer is almost completely assembled (ID# 13). Only the laser beam alignment is required. For such purpose:

A.1. Turn on the laser source (ID# 13) and move it side to side on the main wooden base until the beam falls on the center of the millimetric scale, as show in **Figure 8**.

A.2. Mark the correct position of the laser source on the main wooden base using the marker provided.

A.3. Take the laser source and peel off the adhesive tape cover on the bottom. Place the laser source in the previously marked position on the main wooden base (A.2) and exert gentle pressure to ensure proper adhesion. Ensure that you have placed the laser source in the correct position before fixing it on the wooden base.

Experimental test

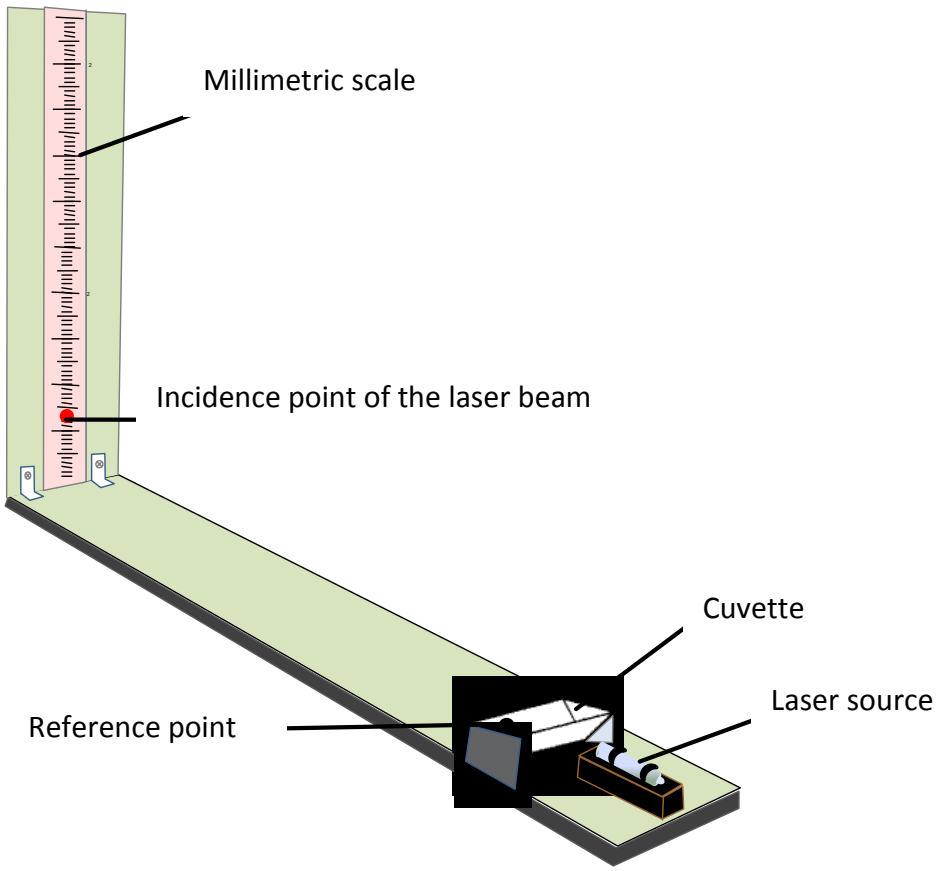


Figure 8

B. ***General instructions for the preparation of the calibration curve:***

B.1. Preparation of calibration solutions

B.1.1. From the initial solution of the sucrose (ID# VII), 5 other solutions of sucrose need to be prepared at 5, 10, 15, 20 and 25 g/100 ml concentration. To do this , some volumes of the initial solution are diluted to the final volume of 50 ml. Calculate the volumes of the stock solution needed to prepare 5, 10, 15, 20 and 25 g/100 ml sucrose solutions (considering that the sucrose initial solution (ID# VII) has an initial concentration of 62.5 g/100 ml and the final volumes of each stock solution will be 50 ml) Record the calculated values on **Table 3.1** on the Answer Sheet. [1.2 Marks]

Table 3.1: Summary of calibration solutions data

Solution identification	Initial Concentration [g/100 ml]	Initial Volume [ml]	Final Concentration [g/100 ml]	Final Volume [ml]
A	62.5		5	50
B	62.5	DO NOT	10	50
C	62.5	FILL IN	15	50
D	62.5	THIS TABLE	20	50
E	62.5		25	50

B.1.2. Prepare sucrose dilutions calculated in the previous section (B.1.1) as follows.

B.1.2.1. Label the plastic bottles (ID# 16) with identification letters A, B, C, D and E, which correspond to different concentration levels of the standard solutions [A=5 g/100 ml, B=10 g/100 ml, C=15 g/100 ml, D=20g/100 ml, E=25g/100 ml]. Different solutions are to be prepared using the pipette (ID# 17), the pipette filler (ID# 18), and the 50 ml volumetric flask (ID# 15). Prepare the solutions in order of increasing concentration.
B.1.2.2. Take the calculated volume of 62.5 g/100 ml sucrose solution (ID# VII) using the pipette and pipette filler.
B.1.2.3. Transfer the taken volume to the 50 ml volumetric flask.
B.1.2.4. Fill the volumetric flask with the water squeeze bottle (ID# VIII) up to a volume close to the calibration mark. Gently shake to homogenize.
B.1.2.5. Complete the volume up to the calibration mark using the plastic Pasteur pipette (ID# IX).
B.1.2.6. Transfer the solutions to the corresponding labelled plastic bottle (ID# 16).

B.2. Data acquisition for the calibration curve using the home-made refractometer (ID# 12).
Special considerations for measurement:

- The cuvette must be fully filled for measurement.
- The cuvette must be placed at the same position for all the measurements. For such purpose, a reference point is drawn on one of the cuvette´s side (**Figure 8**).
- Dry the outside of the cuvette with absorbent paper (ID# C) before each measurement.

General procedure for measurements:

B.2.1. Remove the cuvette from the refractometer, empty it, dry the inside of it, put it back in the original position, fill it with the respective solution, and dry it on the outside..
B.2.2. Turn on the laser source (ID# 13). The laser beam stays on while holding down the switch
B.2.3. Use the marker to draw the point of incidence of the light beam on the millimetric scale.

General procedure to build the concentration scale
The scale should be built from low to high sucrose concentration after measuring the zero of the scale.

B.2.4. Determination of the zero point of the scale: follow the procedure described above (B.2.1-B.2.3) using water (ID# VIII) for determining the reference point (zero) of the scale.
B.2.5. Determination of scale points:
B.2.5.1. Repeat the procedure described above (B.2.1-B.2.3) with sucrose solution ¨**A**¨.
B.2.5.2. On the millimeter scale, measure the linear distance between marks of zero and concentration of sucrose solution ¨**A**¨.
B.2.5.3. Record the obtained data on **Table 3.2** on the Answer Sheet.
B.2.5.4. Repeat the procedure described above (B.2.5.1-B.2.5.3) with the remaining sucrose solutions (**B, C, D** and **E**).

Table 3.2: Calibration curve data [4.0 Marks]

Solution Name	Sucrose Concentration [g/100 ml]	Laser beam deviation from the zero point [mm]
Water	**0**	0
A	**5**	
B	**10**	DO NOT
C	**15**	FILL IN
D	**20**	THIS TABLE
E	**25**	

B.3. Graphic of the calibration curve (**GRAPH D**): [3.0 Marks]

B.3.1. Plot the data recorded on **Table 3.2** (Laser beam deviation from the zero point[mm] vs. Sucrose concentration [g/100 ml]) by using a Cartesian coordinate system.
B.3.2. Draw a straight line that best fits the data points of **GRAPH D**.

B.3.3. Calculate the slope (A) and the y-intercept (B) of the fitted line of **GRAPH D**. Record the calculated values on **Table 3.3** on the Answer Sheet. [0.9 Marks]

Table 3.3: Linear equation of calibration curve

Linear Equation	
Slope	DO NOT FILL IN THIS TABLE
y-intercept	

C. *Instructions for sample analysis (ID# X,ID# XIand ID#XII):*

C.1. Data acquisition of samples with unknown sucrose concentration by using a home-made refractometer (ID# 12).

Procedure for measurements:

C.1.1. Rinse the cuvette with water (ID# VIII). Dry the inside of it with the paper towel provided.
C.1.2. Fill up the cuvette with the sample of unknown sucrose concentration (ID# X).
C.1.3. Dry the outside of the cuvette and place it at the original position for measurement.
C.1.4. Turn on the laser beam.
C.1.5. With a marker, draw the point of incidence of the light beam on the millimeter scale.
C.1.6. Measure the distance between the marks of zero and the sample on the millimeter scale.
C.1.7. Record the obtained data on **Table 3.4** on the Answer Sheet.
C.1.8. Repeat the described procedure (C.1.1-C.1.7) with the remaining samples of unknown sucrose concentration (ID# XI and ID# 0XII).

Table 3.4: Data of analyzed samples [2.1 Marks]

Samples	Millimeters deviation [mm]
ID # X	DO NOT FILL IN THIS TABLE
ID # XI	
ID # XII	

C.2. Determine the sucrose concentration of the samples: [2.3 Marks]

C.2.1. Graphic determination:
C.2.1.1. SampleUse GRAPH D to determine the concentrations of samples X, XI, XII.

C.2.1.2. Record the data obtained in **Table 3.5** on the Answer Sheet.

C.2.2. Analytical determination:
C.2.2.1. Calculate sucrose concentration for the samples using the linear equation recorded in **Table 3.3** and data recorded in **Table 3.4**. Record the calculations on the Answer Sheet.
C.2.2.2. Record the results on **Table 3.5** on the Answer Sheet.

C.2.3. Calculate the percentage concentration difference [D(%)] between graphic and analytical determination by using **Equation 4**. Record the data obtained on **Table 3.5** on the Answer Sheet.

$$D(\%) = \frac{C_G - C_A}{C_A} \cdot 100$$

Equation 4

Being:

C_G: Sucrose concentration graphically obtained
C_A: Sucrose concentration analytically obtained

Table 3.5. Graphical and analytical determination of sucrose concentration in analyzed samples

Sample	Sucrose concentration graphically obtained [g/100 ml]	Sucrose concentration analytically obtained [g/100 ml]	Percentage ConcentrationDifference D[%]
ID # X			
ID # XI	DO NOT FILL IN THIS TABLE		
ID # XII			

D. Additional calculations and data analysis of samples XI and XII:

D.1. Calculate the original sucrose concentration (in °Brix) of the honey using the corresponding sucrose concentration analytically obtained for the honey sample (ID# XI), and considering that for its analysis the honey sample (ID# XI) was prepared as follows: 16 g of honey were diluted in water reaching a final volume of 100 ml. Record the data obtained on **Table 3.6** on the Answer Sheet. [0.3 Marks]

Table 3.6.: Sucrose concentration in original honey sample

Samples	Sucrose original concentration [°Brix]
ID # XI	DO NOT FILL IN THIS TABLE

Honey must have more than 65 °Brix for safe conservation.

D.2. Indicate whether the original honey sample meets the sucrose concentration requirement for a secure conservation. Mark with a cross the correct answer on **Table 3.7** on the Answer Sheet.

Table 3.7.: Safe preservation of honey [0.2 marks]

SAFE	NOT SAFE
DO NOT FILL IN THIS TABLE	

D.3. Calculate the theoretical deviation of the laser beam [mm] for a sucrose solution of 8.5g/100 ml concentration using the two procedures: the graphic representation and the analytical calculation (B.3). Record the calculations on the Answer Sheet. Record the results on **Table 3.8** on the Answer Sheet. [0.5 Marks]

Table 3.8.: Theoretical deviation of the laser beam [mm] for a solution 8.5g/100 ml sucrose concentration

Calculation	Theoretical deviation of the laser beam [mm]
Analytical	DO NOT FILL IN THIS
Graphical	TABLE

The label on the package of the analyzed beverage states that it contains a sucrose concentration of 15 ± 2 g *per serving of 200 ml.*

D.4. Considering the sucrose concentration found in the analyzed beverage, indicate whether it matches the sucrose concentration stated on the package label. Record the calculations on the Answer Sheet. Mark with a cross (X) the correct answer on **Table 3.9** on the Answer Sheet. [0.5 marks]

Table 3.9.: Analysed beverage specification according to the statement on the package label

Yes	DO NOT FILL IN THIS TABLE
No	