Country: _____ Language: _____

FRUIT, JUICES and FOOD

TEST 2

Murcia, April 2nd, 2009

Country: _____ Language: _____

Use only the calculator provided.
The length of the test is 4 hours and 30 min.
Once you hear the order STOP *you should stop immediately*

Introduction

Mr. Hero has just been appointed as Managing Director of the company in which he has worked for many years. He remembered the time when he asked his father, manager at the main factory of the company for many years: "*Dad, will I be able to become Managing Director of the company one day*?" To which his father replied: "*Of course, you can, just like anyone else who wants to, but to be a good manager you will need to know about fruit trees, production processes – both technical aspects and laboratory analyses – you'll need to carry out marketing surveys, etc. All this and also you'll have to learn about human nature and know about the people who work in the factory. Only then will you be a good Director*".

Mr. Hero wanted very much to be Managing Director and help solving the problems that arose every day in the company and which could have negative economic consequences for the company which he was proud to work for. So far he had held other positions of responsibility in various factories of the company, normally associated with sales and purchasing raw materials.

The memory of the conversation with his father helped him in his next decision: he would have to bring himself up to date about the scientific procedures that enabled the company to transform the natural products he had so often bought into the canned products he had helped to sell. He communicated this decision to the board of directors and immediately set out for the place where he thought he would obtain the best and most complete training – the factory that the company had had since 1922 near Murcia, in the heart of the "Huerta de Murcia" known as the "Orchard of Europe" which provides the company with the excellent fruit it needs for its jams and canned products. Furthermore, the company had decided to concentrate its research into new products there and to open up new fields parallel to its core business – the production of baby foods, canned vegetables food and dietetic products.

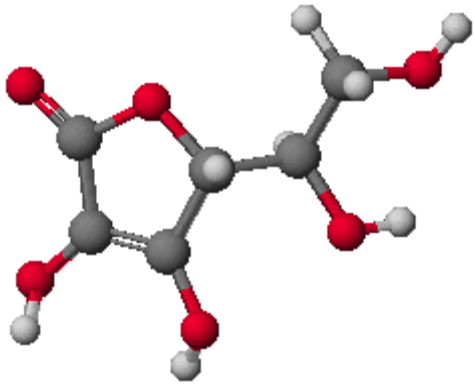
Surprised by his presence but aware of his motives, the technicians of the factory advised him to begin his training at the laboratories of the University of Murcia since they had for many years shared research projects with the schools of Chemistry and Biology. So, after a brief interview with the two Deans, it was agreed

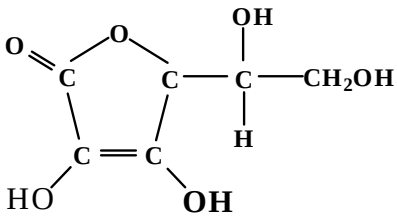
that he would begin his “scientific training” in the Department of Analytical Chemistry, where they would instruct him about the analytical techniques necessary to ensure that food products complied with the respective EU norms concerning food products.

By chance, participants in the EUSO programme, which facilitated exchanges between young scientists from all EU countries, had been working in the same department for several days and so he joined them in solving the tasks they had been set.

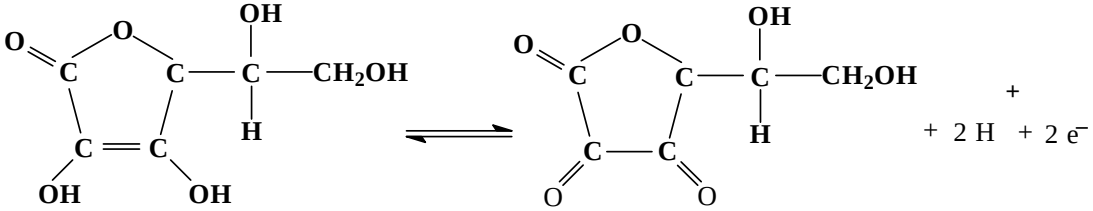
TASK A: VITAMIN C CONTENT OF A FRUIT JUICE

Ascorbic acid (L-ascorbic) or vitamin C is a γ-lactone synthesised by plants and almost all animals except primates and hamsters.





Its prolonged deficiency in the diet of humans can produce a disease known as scurvy, which is characterised by skin lesions, fragility of the blood vessels and poor wound healing. Furthermore, ascorbic acid is a powerful natural antioxidant present in fruit juices and widely used as a food additive. However, in manufactured products exposed to the oxygen of the air, vitamin C undergoes continuous oxidation since it is a reducing agent that reacts with mild oxidants to produce dehydroascorbic acid.



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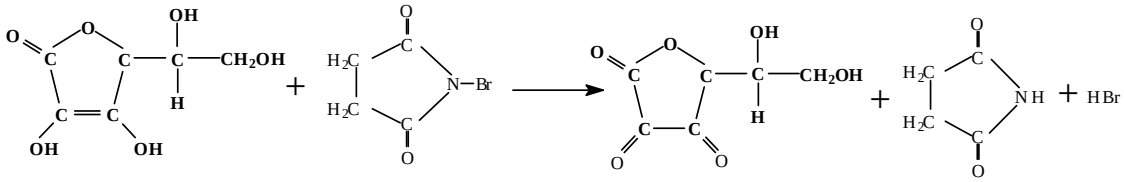
Schematically, the above equation can be written as

$$\mathbf{AA} \quad \Longleftrightarrow \quad \mathbf{DHAA} + 2\mathbf{H}^{+} + 2\mathbf{e}^{-}$$

(ascorbic acid) **(dehydroascorbic acid)**

Most chemical methods for determining ascorbic acid (**AA**) are based on its reducing character. One such method, which is both fast and reliable, is the tritation of the acid with an *N*-bromosuccinimide solution (**NBS**) that acts as oxidant. This converts the secondary alcohols into ketones (which produce dehydroascorbic acid, **DHAA**), which are then reduced to succinimide and hydrogen bromide. The reaction which is equimolecular and rapid, is represented by the equation



Since **NBS** is an oxidant, it releases iodine when it reacts with potassium iodide in acidic solution (acetic acid), but, in the presence of **AA**, it oxidises the ascorbic acid first. If both substances are found together in solution, iodine is only released when the **AA** has been completely oxidised. A slight excess of **NBS** after the oxidation of **AA** will mean that iodine will appear in the solution. This can be detected by previously adding a few drops of a starch solution, with which the iodine will form a complex of a characteristic blue, blue-violet colour.

Now, let's get down to business, put on the lab coat and, following the usual safety rules, carry out the following experiment.

EXPERIMENTAL PROCEDURE

To determine ascorbic acid (AA) or vitamin C in a juice sample you will need:

- a marker pen
- a magnetic stirrer
- three stirring bars
- a burette holder
- 250 mL plastic volumetric flask
- 25 mL burette
- micropipette and tips
- five 50 mL glass beakers
- 100 mL glass beaker
- 25 mL plastic measurement cylinder

- plastic funnel
- vial of solid ascorbic acid [labelled **Ascorbic acid**]
- N-bromosuccinimide solution [**NBS sol.**]
- 4 % potassium iodide solution, [**KI(aq), 4 %**]
- 10% acetic acid solution, [**Acetic acid, 10 %**]
- starch solution [**Starch**]
- fruit juice sample [**Juice sample**]

WARNING: When you have finished the titrations, deposit the wastes and residues in the appropriate containers next to the laboratory sinks.

A) Standardisation of the NBS solution

To determine the vitamin C in the problem sample (fruit juice), you must first standardise the NBS solution. For the sake of greater reliability, we shall do this by titrating several **AA** solutions of known concentrations. In this way, we can relate by means of a graph the amount of AA in each solution with the volume of the NBS solution used for its titration.

First, prepare an **AA** solution of known concentration.

1. Weigh into the glass beaker the amount of solid **AA** (molecular mass 176.13 g mol^{-1}) needed to obtain 250 mL of around $3 \cdot 10^{-3}$ M (3 mM) solution. Write the mass calculated and the mass weighed in the Answer Sheet (A.1). Add 50-60 mL of distilled water to the glass beaker, introduce a stirring bar and place the beaker on the magnetic stirrer, stirring gently. When all **AA** has dissolved, place the funnel in the mouth of the 250 mL volumetric flask and pour the solution into it; rinse the beaker with a small amount of distilled water three times and pour the water each time into the flask; dilute to the mark with distilled water in order to obtain the desired solution.
2. Label the 50 mL glass beakers, from 1 to 5, with the marker pen. Using the micropipette place exactly 1, 2, 3, 4 and 5 mL of the AA solution, respectively, in the numbered beakers. Write the amount of **AA** in each beaker in the Answer Sheet (A.2).

   Fill the burette with the **NBS** solution.

   To one of the glass beakers containing the **AA** solution, add

   2 mL of 4% KI solution,
   0.5 mL of 10% acetic acid solution,
   3 drops of starch solution and

approximately 10 mL distilled water (measured with the measuring cylinder).

Put a stirring bar into the beaker, place it on the magnetic stirrer and stir gently. Start to titrate by adding slowly the NBS solution until the drops falling produce a vanishing blue taint. Add two more drops of starch solution and add the NBS solution dropwise until a permanent blue colour remains in the solution.

Repeat the process with the rest of the beakers you have prepared.

Write in the Answer Sheet the volume of NBS solution needed to reach the end point in each case (A.2) and complete the table.

In a graph, plot the mass of AA contained in each beaker *vs.* the volume of NBS solution needed for the titration (A.3).

B) Determination of the AA **content in a fruit juice**

Weigh accurately, approximately 5 g of fruit juice into a clean and dry 50 mL or 100 mL beaker. Add 15-20 mL distilled water and the same amounts of KI, acetic acid and starch solutions as mentioned above. Now titrate this solution with NBS. Repeat the titration with a similar amount of the same fruit juice. Note the volumes of the two titrations in the Answer Sheet (A.4).

Using the graph, obtain the mass of AA corresponding to each sample of fruit juice titrated, according to the volume of NBS used. Write the values in the Answer Sheet (A.5).

Now calculate the percentage (mass/mass) of AA in the fruit juice and write the answer in the Answer Sheet (A.6).

Mr. Hero is amazed at the work and the results obtained. However, there is still one matter that bothers him. “If the EU recommends a daily dose of vitamin C of 60 mg, how many 200 mL cartons of the juice we have analysed would a person have to drink every day to satisfy the EU requirements?” he asks. Wishing to help him in as many ways as possible, his new companions suggest that the density of the fruit juice can be taken to be the same as that of water (A.7).

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TASK B

Mr. Hero is very interested in biotechnology, especially those aspects that are related with the products his company produces. He has read and heard that many revolutionary possibilities exist to use microorganisms to produce beverages – like the multifruit juices and/or milk-fruitshakes, which are expensive and problematic to produce. With the same eagerness as before he goes to the Department of Genetics and Microbiology of the Biology School where they can show how to recognise these microorganisms and possible ways of using them to obtain new products. As when analysing vitamin C, he found a group of EUSO students willing to demonstrate the techniques and procedures necessary to bring his scientific knowledge to the level his new post demanded and – who knows? - perhaps impress one or two people in the meetings he would have to attend.

The best we can do here is to provide him with some basic information.

> Microorganisms are living beings which, measuring less than 0.1 mm in diameter, can only be observed with the help of a microscope. There are microorganisms that belong to the three large domains of life: bacteria, archaea and eukaryota. In the first two domains, bacteria and archaea, the cells are prokaryotic, that is they do not have a differentiated nucleus, so that their DNA is in the cytoplasm. Among eukaryotic microorganisms are some fungi, which can be divided into moulds and yeasts. Moulds have a filamentous morphology and can form spores, which are grouped according to the genus. Yeasts are unicellular with an ovoid shape.
>
> Both eukaryotic and prokaryotic microorganisms are used in the biotechnological production of a large number of molecules: amino acids, vitamins, enzymes, etc. Some of the enzymes produced by microorganisms used in industry are the pectinases, which, as their name suggests, are capable of degrading pectin, which is an important component of the cell wall of vegetables. Pectinases are used in the food industry to hydrolyse pectins thus reducing the viscosity of juices and other foods.

With this basic information, Mr. Hero will be able to follow the two parts of the following task that the EUSO participants have been assigned.

In the first part, B1, the participants have to identify by direct observation using the microscope the microorganisms present in the microbial cultures corresponding to the samples provided. In the second part, B2, the presence of pectinase activity in the supernatant (extracellular medium) of these cultures will be evaluated. To do this, participants must measure the decrease of the viscosity of a juice sample resulting from the addition of different supernatants of microbial cultures. The viscosity of an untreated juice and one subjected to different treatments will be measured by means of

a viscosimeter, an instrument in which the time taken for a liquid sample to pass through a capillary will depend on its viscosity.

B1. Identification of pectinase-producing microorganisms of interest for elaborating fruit juices.

Attention: In order to save time, test B.1 must be done during the 40 min incubation time of test B2. Thus, start with test B2.

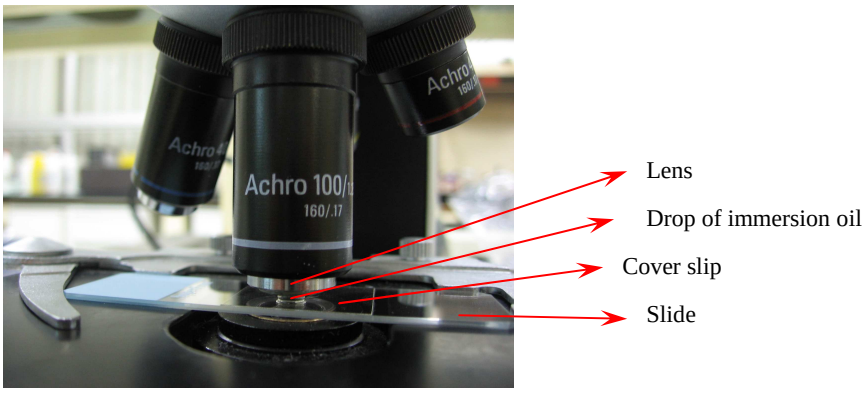
Material needed

- 3 problem samples in eppendorf tubes (A, B and C)
- microscope slides and cover slips
- microscope
- immersion oil
- plastic Pasteur pipettes

Experimental Procedure

To visualise the microbial cultures (A, B and C), place a drop of one of them on a microscope slide, using a Pasteur pipette and carefully place a cover slip over the drop.

Observe this preparation through the microscope. First try with a 40x lens and if it is not possible to observe the microorganism, use the 100x lens. In the latter case, you will need a drop of immersion oil between the cover slip and the objective of the microscope, in contact with both of them (see figure).



Repeat with the other two cultures.

When you finish all three observations, answer the questions in the Answer Sheet.

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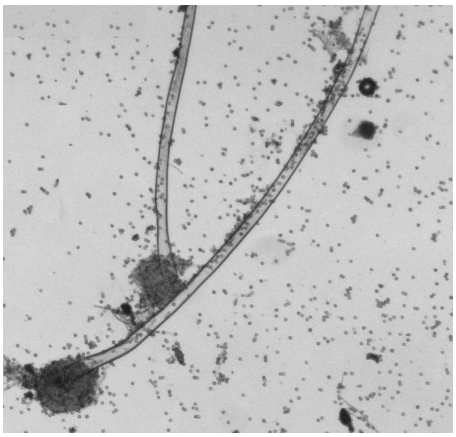
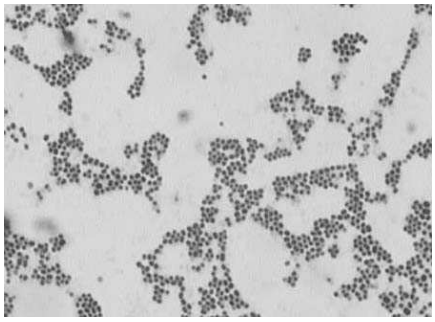
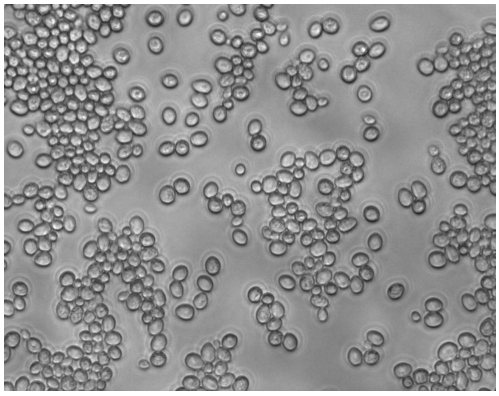
B1.1. Based on the photographs shown on the next page and your microscope observations, relate each preparation observed with one of the photographs. Establish the relation by connecting the sample (culture) and the picture number.



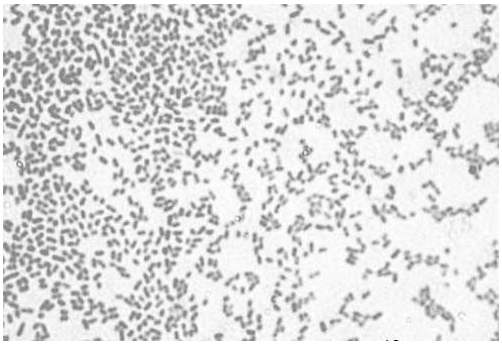
1. Aspergillus



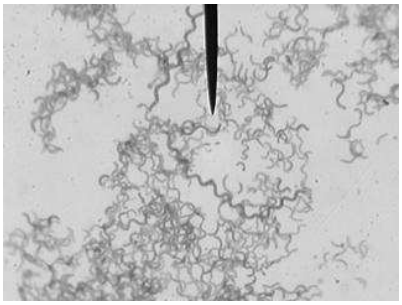
2. *Staphylococcus*



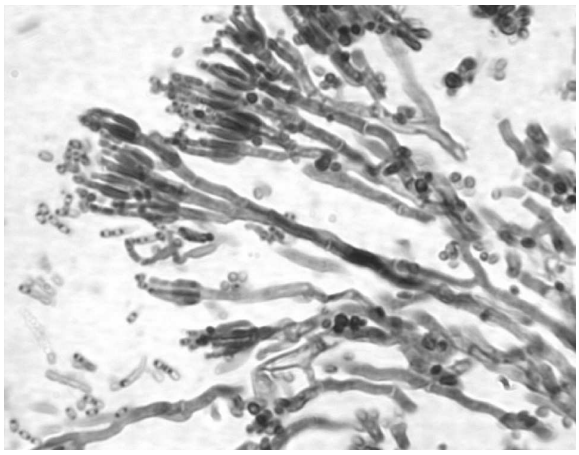
3. Saccharomyces



4. Escherichia coli



5. Spirillum



6. Penicillium

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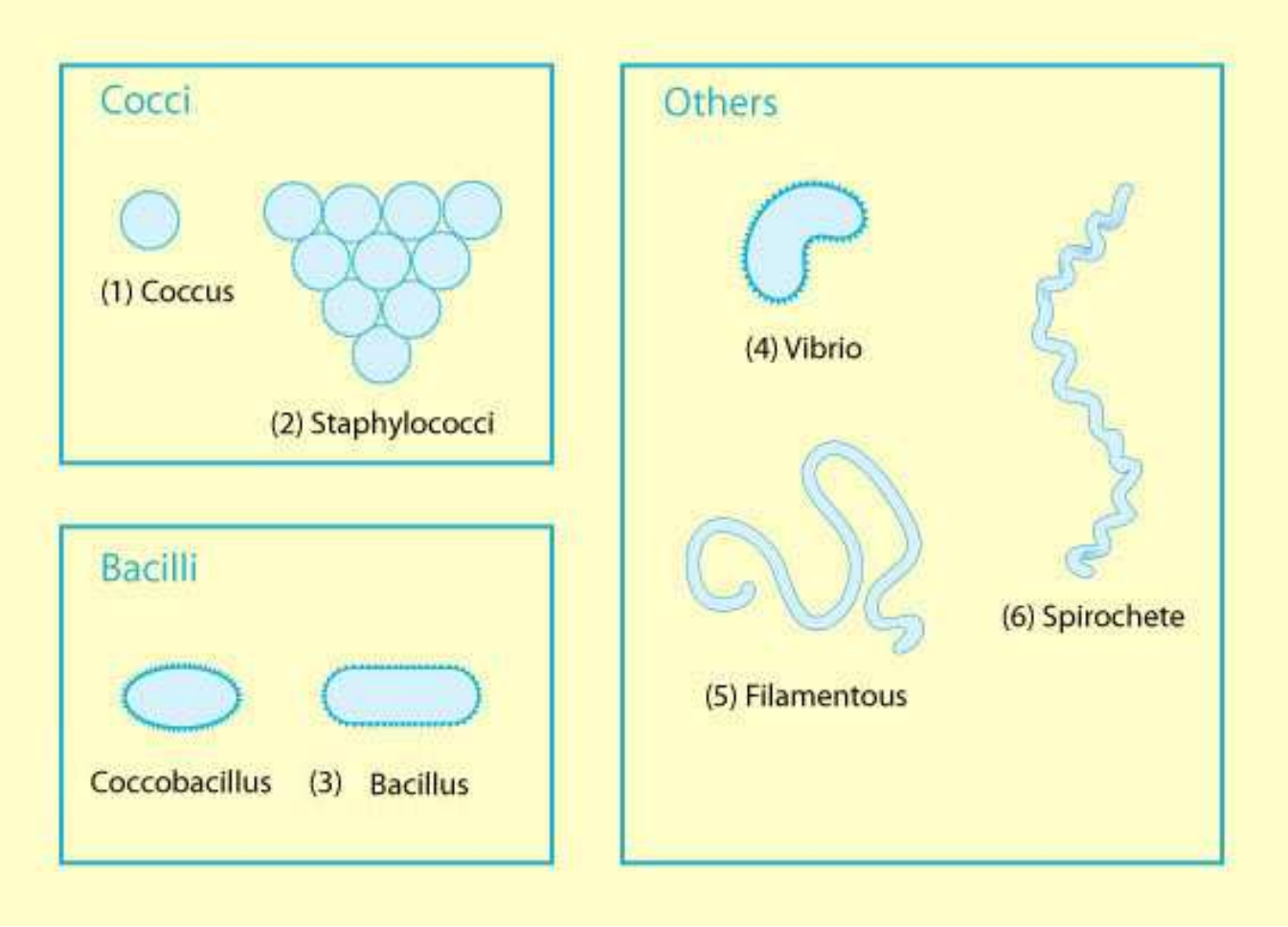
B.1.2. Mark with an (X) in the adequate box of the Answer Sheet the type of microorganism (bacterium, mould or yeast), observed in the microscope for cultures A, B and C.

B.1.3. According to the following schema, which shows the most common morphologies in bacteria, and the photographs in B1.1., identify the morphologies of



Escherichia coli, *Staphylococcus aureus* and *Spirillum sp.*, assigning the corresponding number in the Answer Sheet (B.1.3).

B.1.4. Mark with an X if the statements in the Answer Sheet are true or false.

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Task B2. Determination of pectinase production in microbial cultures

Material needed

- viscometer
- oven at 37 ºC
- juice sample
- 6 screw-topped test tubes
- a plastic container for tubes
- distilled water
- eppendorf-tube rack containing following samples:
    1.- Water
    2.- Pectinase disolved in water at 0.005 Units/mL
    3.- Pectinase disolved in water at 0.02 Units/mL
    4.- Pectinase disolved in water at 0.06 Units/mL
    5.- Supernatant of culture A
    6.- Supernatant of culture B
- chronometer
- pipette pump
- micropipette
- tips for micropipette
- marker pen
- calculator
- plastic beaker to be used as water bath
- plastic funnel

Experimental procedure

a) Place 10 mL of the juice sample into each of the test tubes.
b) Label the tubes 1-6.
c) Add 0.5 mL of the sample from eppendorf tube nº 1 to test tube nº 1; 0.5 mL of the sample from eppendorf tube nº 2 to test tube nº 2, and so on.
d) Shake to mix the contents of each tube.
e) Place the tubes in the test-tube rack and ask the supervisor about the location of the oven.
f) Incubate the tubes at 37 ºC for 40 minutes.
Go back to the task B.1.
g) After the incubation time, cool the tubes with the tap water for 30 seconds.

Viscosity is a property associated with internal friction or drag of substances that flow. Viscosity can be measured easily in laminar flow conditions, which can be

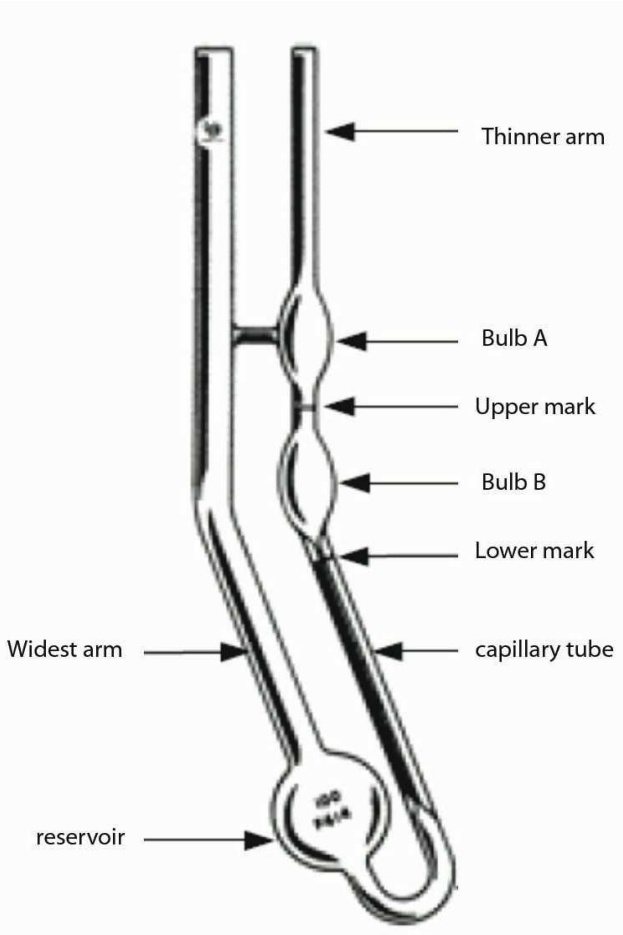
considered to take place when thin layers of a liquid flow over others at a different velocity.

Laminar flow also takes place when liquids flow through tubes at a moderate speed: the thin layer of liquid in contact with the test tube walls is probably stationary, the next layer moves slowly and the following more rapidly, etc. The liquid can therefore be considered as a series of concentric cylinders, each moving at a constant velocity, which increases towards the centre. Poiseuille studied the movement of liquids inside capillaries and found a direct relation between the volume of liquid passing through the capillary per time unit and the viscosity of the liquid. For a given viscometer, the viscosity could be expressed as

$$\nu = k_\nu \cdot t_c$$

where ν is the dynamic viscosity, k_ν is a constant and t_c the falling time. The most commonly unit used for viscosity is the centipoise, cP, which is the hundredth part of a poise, P, (1 g cm^{-1} s^{-1}).

The device used to determine the viscosity of a fluid is known as a viscometer. Some simple devices, such as the Cannon-Fenske viscometer depicted in the figure are based on the measurement of the time require for the fluid to pass by gravity through a capillary tube.



To measure the viscosity of the products obtained after incubation with a viscometer of this type, proceed as follows:

- With the viscometer in the water bath, introduce 10 mL of the fluid of tube number 1, obtained after incubation, through the widest arm using the plastic funnel from previous task.
- Place the viscometer as vertical as possible and allow sufficient time to reach the temperature of the bath.
- Place the pipette pump over the mouth of the thinner arm and suck the fluid until bulb A is half filled.
- Remove the pipette pump and allow the liquid to fall in order the device to be rinsed.
- Suck again in the same way and measure, using the chronometer,

the time the liquid needs to fall from the upper mark of bulb B to the lower mark.

- Remove the viscometer from the water bath (loosen the rubber supporting it), add some distilled water and empty the contents into the sink. Rinse twice with distilled water before placing in water bath to measure other sample.
- Measure in the same way the viscosity of the liquids of the other tubes.

B2.1. Write the falling times for each liquid.

B2.2. Plot in a graph the times needed by the samples treated with commercial pectinase *vs.* the enzyme concentration. DON'T FORGET TO INCLUDE THE GRAPH IN THE ANSWER SHEET.

B2.3. Identify the microbial culture, A or B, which produces pectinases and calculate the pectinase activity in it (expressed as units of activity per millilitre of supernatant).

B2.4. Calculate the change in viscosity (in cP) caused by the treatment with the pectinase-producing culture, knowing that:

$$\text{viscosity (cP)} = 0.25\ (\text{cP s}^{-1}) \times \text{time (s)}$$

B2.5. What will be the viscosity unit in the International System (SI)? What is its equivalence in centipoises?

B2.6. A company wants to produce a juice with a viscosity of 16 cP. What is the concentration of pectinase necessary to treat 10 mL of fruit preparation according to the treatment previously described? How many pectinase units are needed to treat 5000 L of fruit preparation?

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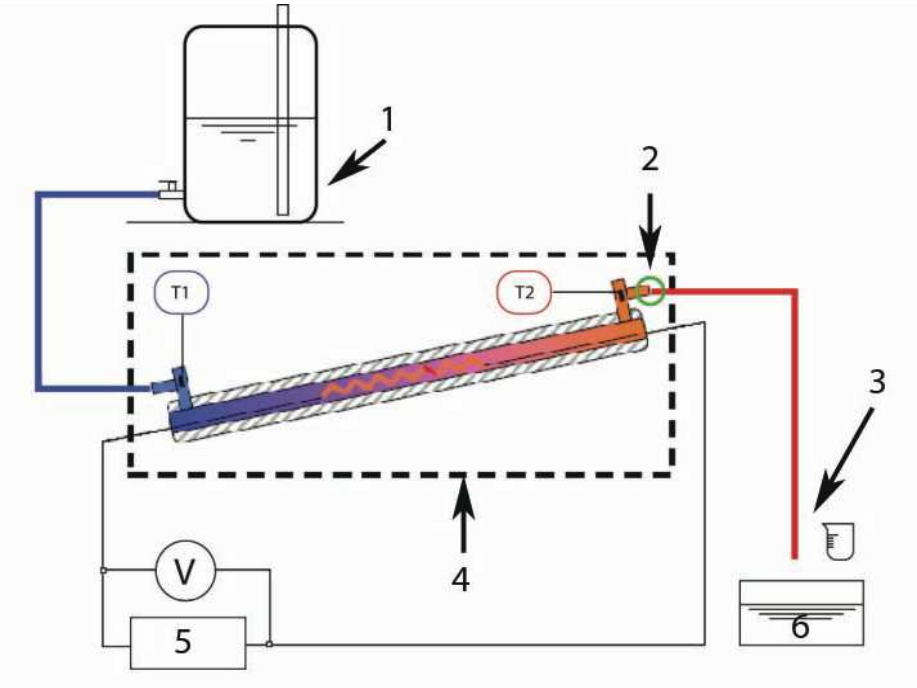
TASK C

Now, Mr. Hero only needs to find out about the thermal properties of juices in order to know the energy required to cool juice tanks in which the juice is transported.

In the Department of Physics, experiments are carried out to estimate the specific heat of liquids based on Callendar and Barnes' method. The EUSO students are engaged in this task, too, and, after trying several designs, have managed to construct a suitable calorimeter to carry out the measurement. Knowing that he will learn as much as before, Mr. Hero joins the team which is about to measure the approximate specific heat of one of the juices that they have analysed.

You will need:

* A continuous flow calorimeter. The device is thermally insulated and equipped with an internal heater (resistor, R = 100 Ω) and a flow regulator.
* Two temperature sensors.
* Chronometer.
* Juice container.
* Voltage regulator to vary the potential (*V*) applied to heater.
* Digital multimeter
* A plastic 500 mL beaker.
* Balance



1.- Container

2.- Flow regulator

3.- Sampling

4.- Insulating shield

5.- Voltage regulator

6.- Sink

T1 and T2.- Temperature sensors.

The figure represents the experimental set-up.

The procedure consists of circulating the juice through the insulated tube in which a resistor heats the liquid (juice). Knowing the energy supplied and the increase in temperature produced at a given mass flow, the specific heat of the juice at a constant pressure can be obtained from an energy balance.

The following assumptions must be made:

- the liquid is uncompressible,
- the density of the liquid remains constant despite the rise in temperature;
- the heat released by friction between the liquid and the walls of the tubing can be considered negligible;
- the value of the electrical resistor remains constant despite changes in temperature;
- the steady state is reached when the entrance and exit temperatures do not vary with time;
- the variation of the kinetic energy and of the potential energy, from the entrance to the exit, of the liquid is negligible; and
- the entrance and exit cross-sections are the same.

If we carry out an energy balance of the system with a given mass of liquid circulating through (entering and leaving) the tubing and heated by the resistor as it passes over it, assuming the above, the following should be fulfilled:

$$Energy_{supplied} = Energy_{gained\ by\ the\ liquid} + losses$$

The energy supplied during time t is the heat supplied by the resistor R due to the Joule effect

$$Energy_{supplied} = (V^2/R)\cdot t$$

where V is the electrical potential difference applied to the resistor.

The energy gained by the liquid is

$$Energy_{gained\ by\ the\ liquid} = m\cdot c_p\cdot \Delta T$$

where m is the mass of liquid, c_p is its specific heat, and ΔT is the increase in temperature that the liquid undergoes between the entrance (T_1) and exit (T_2) in the figure.

Although the calorimeter is insulated, there will inevitably be a small loss of the energy supplied towards the surroundings. We can represent this term by *losses.*

Therefore, the energy balance can be expressed by the equation

$$(V^2/R)\cdot t = m\cdot c_p\cdot \Delta T + losses$$

If we refer this balance to the unit of time, we obtain:

$$\frac{V^2}{R} = \dot{m} \cdot c_P \cdot \Delta T + K \qquad (1)$$

where $\dot{m}$ is the mass flow (mass per unit of time) flowing through the tube for a given setting of the flow regulator, and K is the energy lost (*losses*) per unit of time. The first member of the equation represents the electrical power supplied.

Since the term *losses* is unknown, the experiment has to be done at least twice. By adjusting the flow ($\dot{m}$) at different voltages (V) steady states can be reached for which the entrance and exit temperatures remain the same as in the first array. By doing so we may assume that the term *losses* (K) is the same in every array.

EXPERIMENTAL PROCEDURE

Before switching on the apparatus, ask the supervisor to check that all the connections are correct and that the calorimeter is full of juice. Ask him/her to sign your Answer Sheet. (C.1). IF YOU DO NOT OBTAIN THE SUPERVISOR'S SIGNATURE YOU WILL NOT GET ANY MARKS FOR THIS PART OF THE TEST.

ASSAY 1

- Open the valve regulating the flow to ensure that juice flows to the calorimeter. Check that the juice exiting the system goes to the sink.
- Switch on the calorimeter and adjust the voltage using the voltage regulator to a low voltage, e.g. 100 V.
- Notice that the temperature of T_2 gradually increases to reach a practically constant value, while T_1 remains constant or varies only by a few decimals throughout the assay. Try to obtain a low flow rate in the first experiment, adjusting the flow regulator very gently, but enough for the exit temperature not to exceed 37 ºC.
- Check the temperature of the juice as it enters and exits the calorimeter. (the recommended exit temperature is around 37 ºC). There is no need to note the temperatures at this stage; simply bear them in mind. When you see that the exit temperature change very slowly (less than 0,2 ºC in one minute) it can be considered that the steady state has been reached.

- Once the steady state has been reached, collect a certain amount of juice in the beaker during a time that you must measure accurately with the chronometer (e.g. during one minute, although any other time is acceptable). Write in the Answer Sheet (C.2) both the mentioned temperatures and the voltage values at the start (S) of the collection, at half way through (M) and at the end (E). Write the time you take to collect the juice in the beaker.
- Obtain the mean values of T_1, T_2 and V for the experiment and write them in Table C.2.
- Using the balance, obtain the mass of the juice M_{juice}, and calculate the mass flow taking into account the time required to collect it (**WARNING: If the weight exceed the capacity of the balance, use two beakers to weigh the juice collected**). Write these values in Table C.2 in the Answer Sheet.

ASSAY 2

- Move the flow regulator approximately half a turn and observe how the exit temperature falls. Adjust the voltage to approximately 140 V.
- After a while, the exit temperature (T_2) will begin to increase. By adjusting the flow, you should obtain the same temperatures in this assay as in Assay 1.
- When the new steady state is reached (same entrance and exit temperatures of the juice as in Assay 1), collect the juice that leaves the system after a given time (this can be the same as in Assay 1), which must be measured using the chronometer. Write the results in Table C.2, as you did before.

ASSAY 3

- Repeat the procedure with a new voltage (approximately 180 V) and a new mass flow. Write the values in the Answer Sheet (Table C.2).

Draw a graph and plot V^2/R versus flow $\dot{m}$ (C.3). Determine from this plot the specific heat C_p, express it in SI units (C.4).

How much energy the heater has supplied during the collecting time used in the assay 2? (C.5).

Assuming that 10 000 L of juice have to be cooled from 15 °C to 4 °C (the common temperature in tankers), how much energy has to be withdrawn? (C.6)

The basic requirement in all these experiments is to ensure that, when calculating the mean values, the steady state has been reached and that the entrance and exit temperatures remain constant. The factor that most affects is the mass flow. To ensure a uniform mass flow the apparatus known as Mariotte flask is used (CONTAINER in the figure). As can be appreciated, this consists of a closed recipient

with a narrow tube, the bottom of which is immersed in the liquid while the top is exposed to the atmosphere.

In C.7 state which of following statements is INCORRECT.

a) The Mariotte flask ensures that the exit flow rate of the liquid through the tubing is constant.

b) The Mariotte flask ensures that the pressure on the free surface of a liquid in the deposit increases as it empties.

c) The Mariotte flask ensures that the pressure on the free surface of the liquid decreases as the deposit empties.

d) The Mariotte flask enables the atmospheric air to reach the upper part of the deposit.

CONGRATULATIONS!

YOU'VE FINISHED!

DON'T FORGET TO COLLECT THE MEDALS AND DIPLOMAS ON SATURDAY, APRIL 4th.