24th of April 2012

Experiment 1

Tasks

$Country

Amber

General instructions

Wear the supplied plastic coat and protective goggles at all times in the laboratory. Eating and drinking is prohibited in the laboratory.

It is highly advisable to wear disposable gloves and protective eyewear when handling chemicals.

All paper used, including rough work paper, must be handed in at the end of the experiment.

All results must be entered into your Answer Book.

Your graphs must be handed in along with the Answer Book.

Only the final Answer Book, and the attached graphs, will be marked.

The experiment consists of 4 Tasks and can be completed either individually or as a team.

Task 1: 25 credit points
Task 2: 25 credit points
Task 3: 25 credit points
Task 4: 5 credit points

Introduction:

Baltic amber is known as gold of the Baltic States. It is unique as it is only found in the Baltic Sea region. One of the most valuable examples of Baltic amber is "Sun Stone", which is extraordinary, almost circular and is as big as a human head. In 2002 an amber bar weighing 3526.32 g was stolen from the Lithuania National Amber Museum, luckily it was mysteriously returned during the investigation. Today "Sun Stone" is one of the most valuable ambers of the Baltic States and is estimated at over 250 000 €.

The Phoenicians were the great merchants of ancient times and it is well known that amber had a high value during that period (16^{th} century BC). The Baltic Lithuanian term for amber is Gintaras. The top rated amber bars were those that had inclusions. Even the smallest amber bars with inclusions were traded for 120 swords or 1200 spears. The Lithuanian Historical Association has set the task for young scientists from the European Union to investigate the possible exchange rate, if "Sun Stone" was sold in the ancient Phoenicians' market.

Your task is to determine the hypothetical value of "Sun Stone". There are five main categories that determine the value of amber: mass, colour, intensity of colour, density and inclusions. Referring to the catalogue in **Task 4**, please suggest the possible profit that merchant Gintaras (Amber) could get in the Phoenicians' market.

TASK 1: Identification of arthropods

The type of animals and plants found in Baltic amber is influenced by the distribution of resin-producing trees and is dependent on the conditions that prevailed in the amber forests over time. Insects are the most abundant among Baltic amber inclusions. They make up 86-92% of all inclusions; arachnids comprise 7.5-13%, other species 0.1-1.7%, plants 0.4%, and other phyla, such as worms, mollusks and vertebrates are extremely rare. An amber forest was an array of different habitats. Over time groups of insects were entrapped in the amber found in forests, swamps, meadows, lakes and rivers.

You will have to identify seven arthropods found in different amber pieces identical to those found by archaeologists. Based on your results you will determine which arthropod gives the best added value to the final price of the amber. The results of your experiments will allow you to calculate how many swords, spears and arrows Lithuanian merchant Gintaras would have received for "Sun stone" in the Phoenician market.

Equipment and materials:

- 7x different arthropods in numbered plates
- 7x microscope slides
- 18 mm cover slips
- 1x preparation needle
- 1x forceps
- 1x vial with glycerol
- 1x stereoscopic microscope
- 1x identification key (which is found in the envelope)

How to make a temporal slide of the arthropod body parts

- Very carefully tear out the wing, antenna or leg of the arthropod as close to the body as possible.
- Put a drop of glycerol onto a microscope slide.
- Put the detached body part into the drop of glycerol.
- Put a glass cover slip onto the drop of glycerol.
- Inspect the temporal slide with the microscope.

You can put two or three drops of glycerol on the same microscope slide and make preparations of several different body parts of the same arthropod on one slide.

NOTE: arthropods should be put under the microscope ONLY on the microscope slide or in plate.

Task 1.1. Putting a correct name

In biology, an identification key is a printed or computer-aided device that aids the identification of biological entities, such as plants, animals, fossils, microorganisms, and pollen grains. It is a

modelling method used for categorising species using logical choices. A **dichotomous key** is a key where the sequence and structure of identification steps is fixed. At each point in the decision process, multiple alternatives are offered, each leading to a result or a further choice to eventually arrive at the correct identification. Following instructions in the key, you must answer a number of questions about one or more features until you have arrived at the final decision about a particular arthropod name. Do this with all the seven specimens that have been provided to you on plates. If the feature in the key is not visible, prepare a microscope slide of the body part in question.

Write down your steps following the guidelines in the key (1.1.1) and the respective names (1.1.2) onto the Answer Sheet.

General insect morphology

Insect wing. Insect wings have rigid veins that support the wing in flight (Fig.1). However, the wing veins may look different in different insect groups (Fig. 2 A-B). Scientists have tracked that all different insect wings have evolved from the same ancestor, i.e. wings have evolved only once in the particular species' history.

Different modifications of wings can be found in insects: two pairs of equally developed wings; the first pair larger than the second; the first pair hardened (wing cases) and the second membrane-like; the first pair membrane-like and the second pair reduced into club-like structure (haltere) and so on.

Fossil records show that the wings of a primitive insect had 8 pairs of main veins. Each pair diverges from the wing base into the **anterior** convex and **posterior** concave sectors (e.g., MA and MP). Due to evolution, insect wings, in most cases, have a reduction in the number of veins.

Costa (C) – the vein at the leading edge of the wing, strong and marginal, extends unbranched to the apex of the wing.

Precosta (PC) – the first longitudinal vein is fused with costa in all extant (currently living) insects and is barely recognisable.

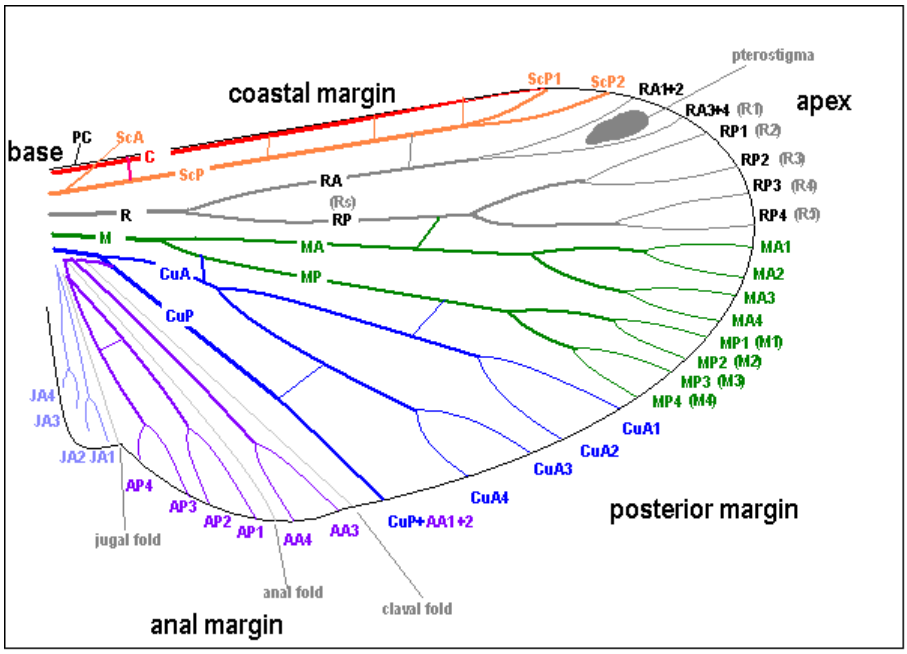


Figure 1. Typical insect wing venation

Subcosta (Sc) - the second longitudinal vein, is mainly found in the subcosta posterior sector **(ScP)**.

Radius (R) -is the third longitudinal vein, usually the strongest vein on the wing, with branches (**RA** and **RP**) usually covering the largest area of wing apex. **RP** and **RA** are often referred to as the radial sector **(Rs)** and the end branches are numbered **R1-5**.

Media (M) - the fourth longitudinal vein, **MA** and **MP** usually have 4 branches each. **Cubitus (Cu)** – the fifth longitudinal vein, **CuA** may have up to 4 branches, while **CuP** is unbranched and lies near the claval fold and ends at the wing posterior margin.

Anal veins (A) are veins located behind the cubitus. **The anal fold usually separates AA and AP**.

Jugal (J) - small veins located in the jugal area are only found in some insects.

The black **pterostigma** is located near the wing tip, between **RA1+2** and **RA3+4**.

Cross-veins are transverse veins that join longitudinal veins. Their names are based on the position relative to longitudinal veins, e.g., **r-m** is the cross-vein between the radius and media longitudinal veins.

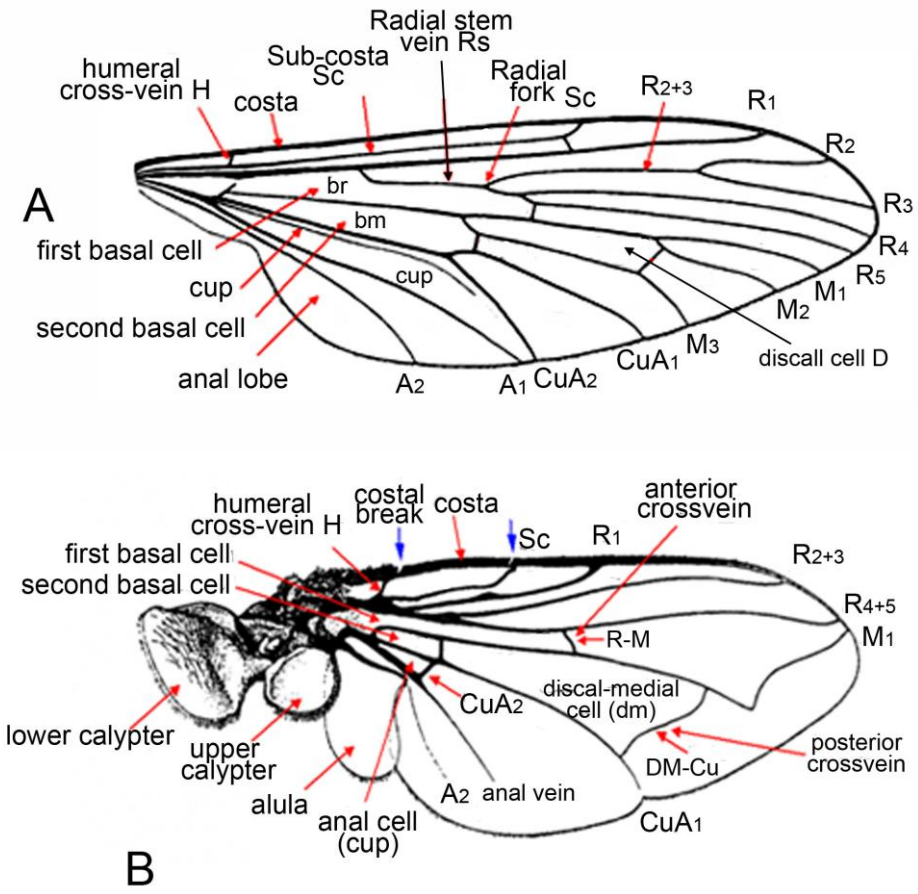


Figure 2. Wings of true flies: A. more primitive (craneflies), B. more advanced (house flies, blow flies)

Insect antennae: Antennae are the primary olfactory sensors of insects and many other arthropods. Antennae are located between the eyes on the forehead and are well-equipped with a wide variety of sensilla, which are paired, mobile, or segmented. The three basic segments of the typical insect antenna are the *scape* (base), the *pedicel* (stem), and finally the *flagellum*. The flagellum often comprises many units known as *flagellomeres* and can have feathery or filamentary outgrowths called *arista* (Fig. 3B). The number of flagellomeres can vary greatly, and is often of diagnostic importance.

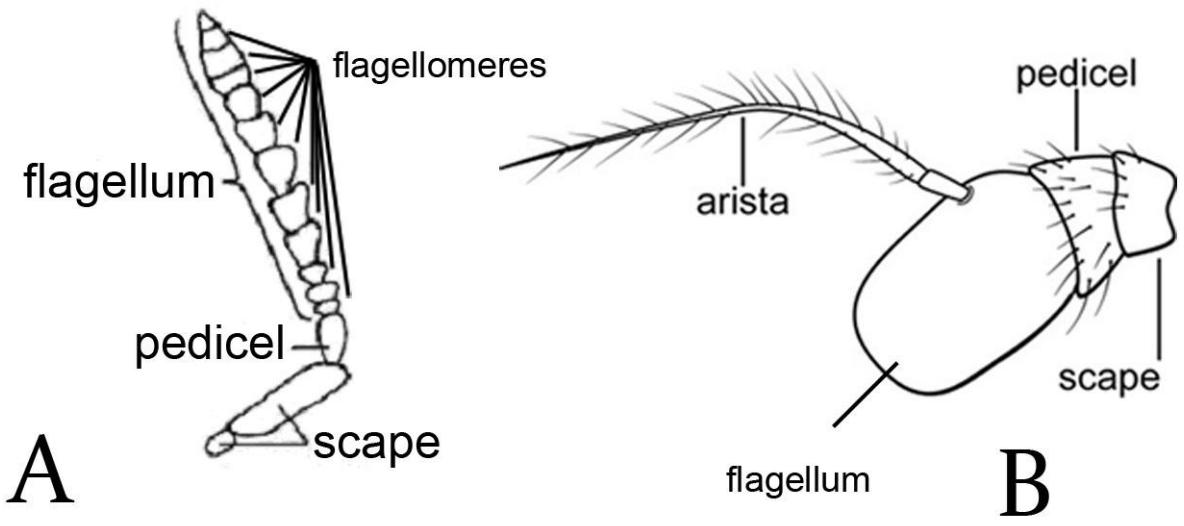


Figure 3. Insect antenna A - more primitive (beetle), B. - more advanced (true fly)

Insect leg: Insects and their relatives are hexapods, having six legs, each with five components, starting from the body to the end: the coxa, trochanter, femur, tibia, and tarsus (Fig.4). Each is a single segment, except the tarsus, which can have three to seven segments, and each is referred to as a *tarsomere* (*t1, t2*, … *t7*). At the end of the tars, claws or other similar structures are found. These help insects to cling to surfaces.

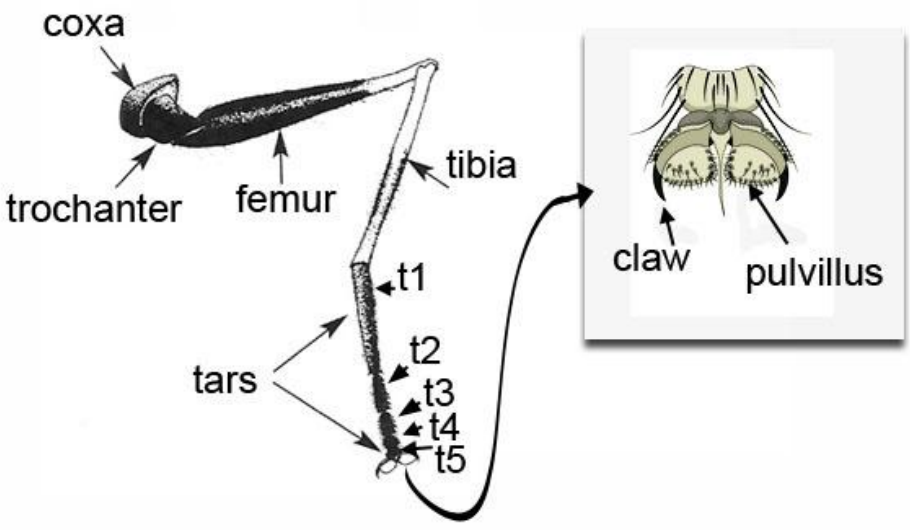


Figure 4. Insect leg parts, example of a fly leg.

Task. 1.2.: Finding characters and drawing a phylogenetic tree

In order to know which arthropod would be the most expensive, you will have to find out which inclusions were the rarest. To do this, you will need to construct a character matrix and draw a phylogenetic tree.

1.2.1. Finding the character states

Character states are usually marked by two symbols: **0** if the character state is different from the one in the statement; **1** if the character state is exactly the same as the one in the statement.

How does it work? Let's say you have a statement: *arthropod has eyes.* If the one you are observing has eyes, you write 1, if it doesn't, you write 0. If the statement describes characteristics of the structures that your arthropod doesn't have at all, you should write 0 (e.g., if the statement is "*legs have long claws*" and your object doesn't have legs at all, you should write 0). It is like saying YES and NO in the identification key.

You will need to answer 10 questions about the characters (A-J shown below) for each of the seven samples of arthropods you have by indicating **0** or **1** until all the cells of the table in the Answer Sheet are completed. You will be required to prepare and inspect some body parts using a microscope to help you answer the 10 questions. There is representation of at least one "1" character state in every column.

The statements are as follows:

A. Eyes are present and are made of many facets.
B. Well-developed wings are present, some might be solid.
C. Only two well-developed wings are present, the second pair is transformed into a club-like (haltere) structure.
D. The entire surface of all wings is covered in hairs.
E. Four developed wings are present.
F. Two wings are present, 7 or more veins clearly reach the wing margin.
G. Two wings are present, large pterostigma is present over the end of the first radial vein.
H. Two wings are present, first medial vein does not go straight to the wing tip, instead, it ends at the radial vein or very close to it at the wing apex.
I. Two wings are present, t4 of hind leg has a deep depression and looks like the letter "v".
J. Two wings are present; the first basal cell is about three times longer than the second basal cell.

Write down the respective character states onto the <u>Answer Sheet. (1.2.1.)</u>

1.2.2. Completing a phylogenetic tree

All arthropods share common ancestry. The similarities and dissimilarities among groups of organisms are the result of a branching process in the phylogenetic tree. Construction of a phylogenetic tree of studied species relies on the basic idea of comparing specific features of the species. These features are respective character states in the table you have completed in **Task 1.2.1**. In the base of the tree (root) the most primitive species will be found and they will have a **0** in most of the character states. New characters in other species will be presented as branches of the tree. If several species have the same feature, they must be connected. Characters specific to only one species form a new group and in the tree they will be presented farthest from the root.

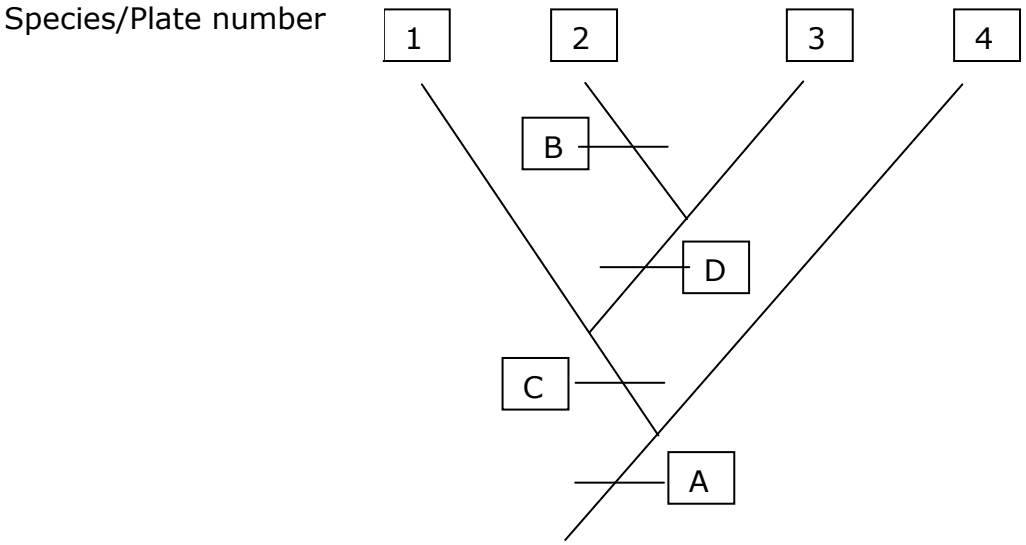


Figure 5. An <u>example</u> of a phylogenetic tree (A-D characters, 1-4 species/plate number).

Complete a phylogenetic tree onto the <u>Answer sheet. (Task 1.2.2.)</u>

1.2.3. The value of amber pieces

The most expensive piece of amber is the one that has the most primitive arthropod in it. This arthropod is put onto the first branch of your phylogenetic tree.

Write the number of the plate with the most primitive arthropod onto the <u>Answer Sheet. (1.2.3.)</u>

End of **TASK 1**.

Blank paper.

TASK 2: Colour and intensity of colour measurements

Phoenician merchants valued pieces of amber according to visual aesthetics of colour and colour intensity. The amber colour depends on the ratio of two dye components (colourants) – red and yellow, while the colour intensity is dependent on the total amount of colourant mixture in the material. It is very difficult to qualitatively measure the colour and colour intensity of a piece of amber, therefore the following scientific method will be used.

You will use Thin Layer Chromatography (TLC), Column Chromatography (CC) and Colourimetric analysis techniques. TLC helps to identify the components in a colourant mixture and to choose the solvent for CC. CC is a technique used to separate different substances (colourants in our case). Finally, colourimetric analysis allows you to identify the quantity values of the components in the colourant sample.

"Sun Stone" is an invaluable property. To evaluate the colour and colour intensity a dye mimicking the Amber colour is used instead of real amber pieces. Today you will investigate the dye which has analogous colourimetric properties as "Sun Stone".

Equipment and material:

- 1 x burette (filled with silica gel and fixed to the stand)
- 1 x funnel
- Tweezers
- 1 x 50 mL Erlenmeyer flask "TLC Eluent"
- 4 x Pasteur pipette with a bulb
- 2 X 5mL graduated pipettes
- 4 x TLC plate
- 1 x beaker "Organic waste"
- 4 x capillary
- 4 x 50 mL measuring cylinder
- 1 x 50 mL beaker
- 1 x Petri dish
- 1 x 50 mL Erlenmeyer flask containing "Yellow colourant standard solution"
- 1 x 50 mL Erlenmeyer flask containing "Red colourant standard solution"
- 1 x 100 mL Erlenmeyer flask containing eluent and labeled "Eluent"
- 1 x 5 mL vial containing colourant mixture for column chromatography.
- 1 x 2 mL vial containing colourant mixture for thin layer chromatography "TLC sample"
- 1 x 20 mL vial containing Ethyl Acetate
- 1 x 20 mL vial containing Petroleum Ether

- 2 x Stickers "Isolated colourant"
- Slips of paper

2.1. Thin Layer Chromatography

Thin Layer Chromatography (TLC) is a technique used to separate mixtures. It is performed on a plate, which is coated with a thin layer of silica gel – the stationary phase. A glass capillary is used to transfer a minute amount of the analyzed mixture and mark a spot on the "Start" line on the TLC plate (Fig. 6). A developing chamber (a beaker with a Petri dish on top of it) is filled with a desired eluent (the mobile phase) so that the level of the solution is 2-3 mm high from the bottom. A spotted TLC plate is then placed vertically into the TLC chamber using tweezers and covered with the Petri dish (Fig. 7).

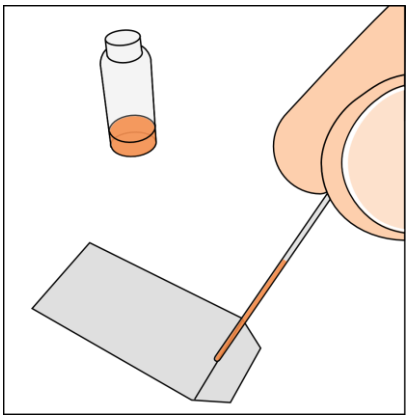


Figure 6. Spotting of a TLC plate

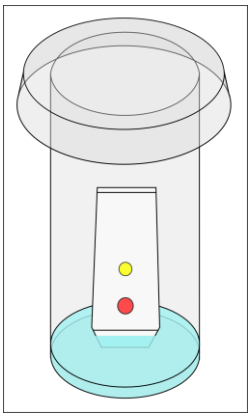


Figure 7. Development of a TLC plate

During the experiment the eluent will travel up the plate. When the eluent reaches the "Finish" line the plate must be removed from the chamber. The retardation factor (R_f) for every spot on the TLC plate can then be calculated.

R_f is the ratio of the distance travelled by the center of the spot to the distance travelled by the solvent front. For example, if a particular substance travels 2.5 cm and the solvent front travels 5.0 cm, the R_f would be 0.50. Every chemical compound has a unique R_f value which is dependent on the eluent.

For effective separation, an optimal eluent must be chosen. To separate a mixture of two coloured compounds you will try three eluents, differing in the volume ratio of petroleum ether and ethyl acetate. Notice that you have one extra TLC plate and capillary. You can use this in case something goes wrong. Follow these instructions:

1. Calculate the volume you need to prepare 5.0 mL of petroleum ether and ethyl acetate mixtures: 9:1 and 3:1 (v.v.). Show calculations on the Answer Sheet (**Task 2.1.1.**).
2. To prepare 5 mL 9:1 ratio mixture, pour the necessary volume of petroleum ether and ethyl acetate, using the graduated pipettes, into an empty 50 mL Erlenmeyer flask labeled "TLC eluent". Share the bulb with teammates who are using it in Task 3.
3. Pour the prepared eluent from the flask "TLC eluent" into the TLC chamber so that the height of the liquid layer is approximately 2 -3 mm. Place the Petri dish on top of the chamber to prevent any volatile solvents from evaporating.
4. Use the capillary to spot the colourant sample on the mark on the TLC plate. Use the tweezers to place the TLC plate vertically in the TLC chamber and cover the chamber with the lid.
5. Wait until the eluent reaches the finish line, then, using the tweezers, take the TLC plate out and wait until it dries. Dispose of the eluent into the organic waste container. Keep the organic waste container covered with a slip of paper to minimize evaporation.
6. Calculate the R_f value. Show calculations on the Answer Sheet (**Task 2.1.2**).
7. Repeat the experiment (steps 2 to 6) with another eluent (mobile phase from petroleum ether and ethyl acetate with a ratio 3:1 (v:v)). Be aware that you have to use a new TLC plate for every new experiment.
8. Once again repeat thin layer chromatography using the given eluent from the flask "Eluent" and show calculations on the Answer Sheet (**Task 2.1.2).**
9. Complete the Answer Sheet by answering (**Task 2.1.3 – Task 2.1.6.**)
10. Place the TLC plates back into the plastic bag.

2.2. Column Chromatography

Although the TLC is mainly used to analyze mixtures qualitatively, the same principle can be applied to separate amounts from micrograms up to kilograms. This process is called Column Chromatography (CC). In this case, the flow of the eluent (mobile phase) slowly moves the compounds down the column. Different compounds move at different speeds, depending on their R_f values. When one fraction a compound starts to drip out of the column, the collection container is replaced with an empty one. In such manner multiple different fractions containing different chemicals are collected in separate containers.

In this part you will use Column Chromatography to separate different colourant from the given sample. Follow the instructions:

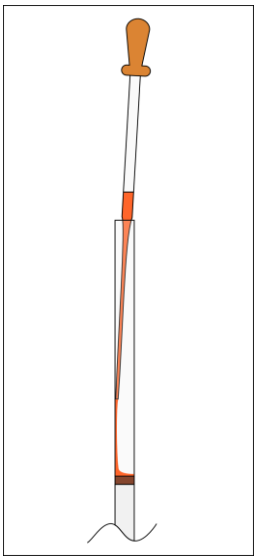


Figure 8. Transferring the sample to the burette

1. The burette is already filled with silica gel and the eluent used in TLC analysis. Place the organic waste container under the burette and use the tap to equalize the levels of eluent and sand layer.
2. Use the Pasteur pipette to transfer the whole colourant sample on the very top of the sand layer. Pour the sample slowly down the burette wall and avoid disturbing the uniform sand layer (Fig. 8).
3. Carefully open the tap for the sample to soak. Close the tap immediately when the whole sample is soaked into the sand layer.
4. Use a new Pasteur pipette and pour a small amount of the eluent into the burette to wash down any remaining sample on the walls.
5. Repeat step 3.
6. Use the recent pipette to fill the burette with eluent slowly. Remembering not to disturb the sand layer, fill the burette to the very top. When there is enough eluent in the burette to avoid disturbing the sand layer, you can pour the remaining eluent directly from the flask using a funnel.
7. Chromatography starts when the burette tap is opened. Observe the colourant separation, but be aware – ***the top level of the eluent should not reach the sand layer and do***

not let the sand layer get dry. Therefore, from time to time you will need to add some more eluent to the burette.

8. When the first colourant-containing fraction is about to flow from the burette, replace the organic waste container with the measuring cylinder.
9. Once the entire coloured fraction has been collected, place the organic waste container back under the burette and cover the measuring cylinder with a slip of paper. Write down the volume of the collected fraction on the Answer Sheet (**Task 2.3.1)**.
10. When the fraction containing the second colourant starts to flow from the burette, again replace the organic waste container with another empty measuring cylinder.
11. Close the burette tap after collecting the second fraction. Write down the volume of the collected fraction on the Answer Sheet (**Task 2.3.3).** Stick the labels "Isolated colourant" to both cylinders containing the collected solutions. **They will be used for marking**.

2.3. Colourimetric analysis

Colourimetric analysis is a method for determining a concentration of a coloured compound in a solution. In this task you will use your eyes as an analytical instrument to measure the intensity of the colour and thus deduce the quantities of your previously separated colourants. Follow the instructions:

1. If the volume of the colourant-containing solution in the cylinder is lower than 20 mL, pour in some eluent until the total volume is at least 20 mL.

2. Take two cylinders out of their stands, the first with the colourant solution (labeled "Isolated colourant") and the second is empty. Hold them both above a sheet of white paper and while looking vertically from the top (Fig. 9), add the standard solution of the same colour using a Pasteur pipette dropwise into the empty cylinder until the colours in both cylinders are equal in colour intensity. Write down the volume V_{min} of the standard solution in the cylinder on the Answer Sheet (**Task 2.3.1**).
3. Continue increasing the volume of the standard solution in the second cylinder until you can see a difference in the intensity of colour in both cylinders. Write down the volume V_{max} of the standard solution in the cylinder on the Answer Sheet (**Task 2.3.1**).
4. Pour the standard solution in the cylinder back into its original container.

5. It is recommended that you repeat the analysis with a single colourant at least 3 times. If you find it hard to notice the difference in colour intensities you can ask your teammates for help.
6. Repeat the analysis for the other colourant solution and complete the table on the Answer Sheet in **Task 2.3.3**.
7. Calculate the amount of each colourant in the given mixture using the law $c_1V_1=c_2V_2$ where, c – concentration (g/L), V – volume. The colourant concentrations of both standard solutions are 0.10 g/L.

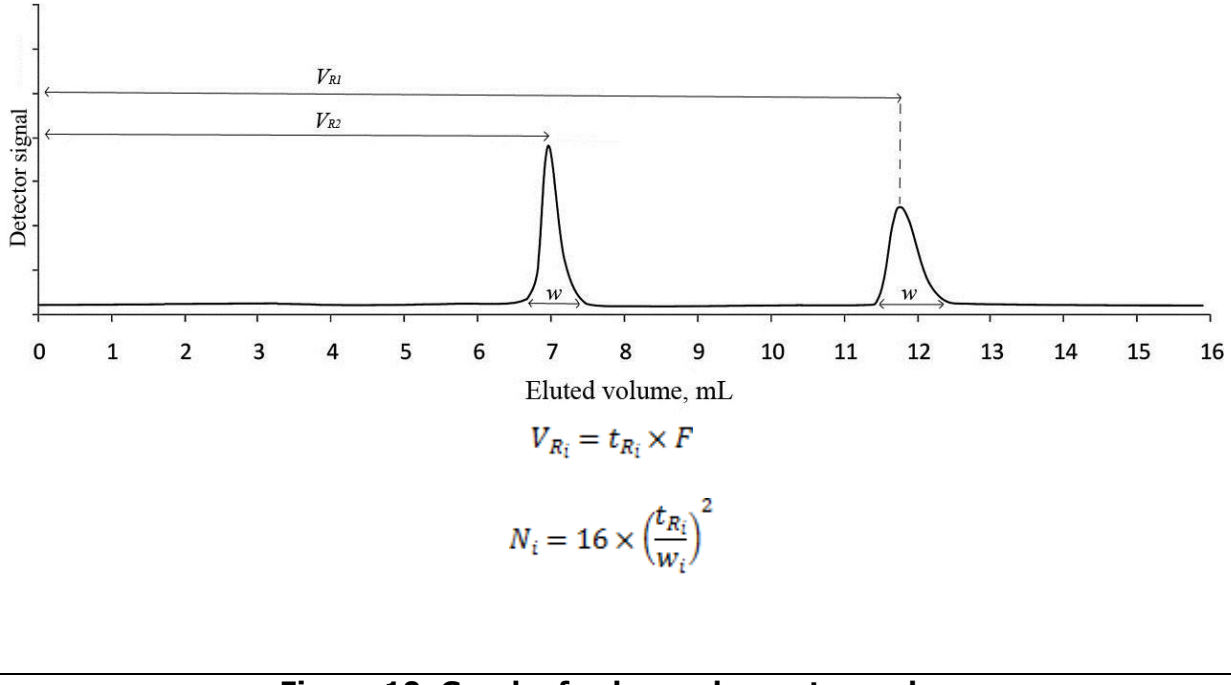
Write your answers on the Answer Sheet in task 2.3.2. and 2.3.4.

Figure 9. Colour comparison during colourimetric analysis

2.4. Column Chromatography Efficiency

The given figure represents chromatographic separation of two colourants. Eluted mobile phase volume is plotted on the X axis and the Y represents detector`s signal. The ***retention volume*** $\boldsymbol{V_R}$ is the volume of mobile phase passed through the column between the start point and the peak maximum. The ***retention time*** $\boldsymbol{t_R}$ of a solute is taken as the elapsed time between the start time and the time of the peak maximum of the solute elution. ***Theoretical plate N*** is the value which describes the efficiency of column chromatography. ***W*** is the width of the peak base in mL. ***F*** is the volumetric mobile phase rate and in this particular case it is 1 mL/min.

Use the information given in Fig. 10 to calculate the N value (efficiency of the column) for each colourant. (2 pts)



$$V_{R_i} = t_{R_i} \times F$$

$$N_i = 16 \times \left(\frac{t_{R_i}}{w_i}\right)^2$$

Figure 10. Graph of column chromatography

Answer the question on the Answer Sheet Task 2.4.1.

End of **Task 2**.

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Task 3: Density distribution of amber

The third task of the experiment will enable you to determine the value of amber using densities.

Traditionally, Phoenician merchants valued pieces of amber according to density. Firstly, you will have to measure the density distribution of amber. Later you will determine the value of "Sun Stone" due to its density.

Task 3.1. Density distribution of amber

Measure the range of the distribution of density of the amber pieces and draw a bar chart to represent this distribution.

Equipment and material:

- ~200 x small pieces of amber (size 2-5 mm)
- ~1L x 13% NaCl solution
- ~1L x distilled water
- 1 x 250 mL glassware
- 1 x 50 mL measuring cylinder
- 1 x 25 mL measuring cylinder
- 1 x 10 mL pipette
- 1 x Pipette bulb (share between your teammates)
- 1x spoon
- 1 x percolator
- Tissue

Experiment

It is known that the density of NaCl solution in water depends on the NaCl concentration by mass c (concentration by mass is the ratio of the mass of NaCl to the total mass of the solution, expressed as a percentage, %). This relation is shown in Fig. 11.

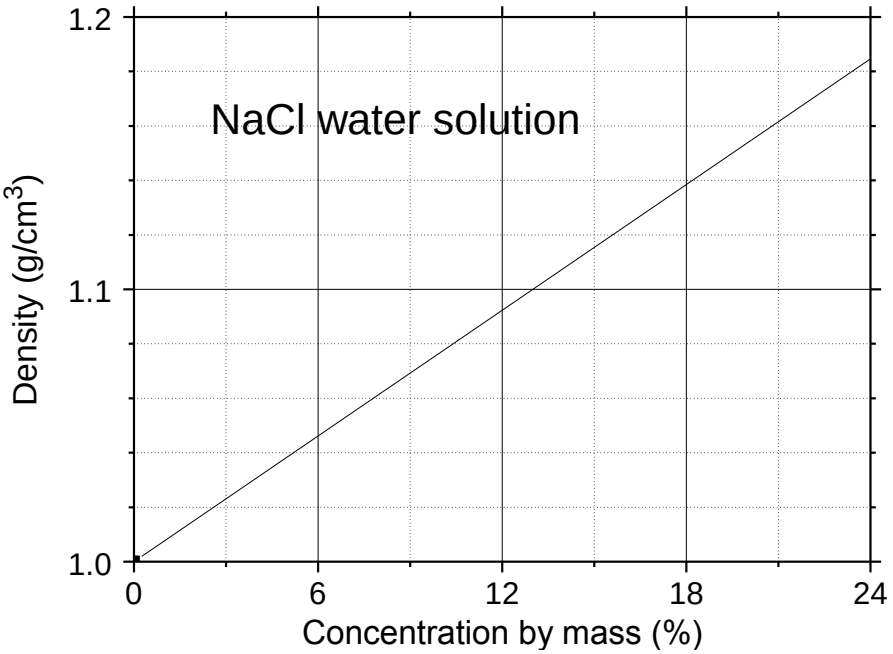

Figure 11. Density of NaCl solution in water versus concentration by mass

In order to determine the distribution of the density of the pieces of amber, it is necessary to prepare different solutions of NaCl with known densities (Table 3.1., ρ) and analyze the behavior of amber samples in the solution using Archimedes' principle. These solutions can be made by mixing V_1 (ml) of concentration by mass c_1 = 13.0% NaCl solution (its density ρ_1 = 1.10 g/cm^3, see Fig. 11) with V_0 (ml) amount of distilled water. Recommended values of V_0 are given in the 2nd column of Table 3.1. You will then calculate the corresponding V_1.

Table 3.1.

Density of solution, g/cm^3 (ρ)	Recommended volume of distilled water, mL (V_0)	Volume of NaCl 13% solution, mL (V_1)	The number of newly surfaced pieces of amber (n)	The percentage of newly surfaced pieces of amber of the total number (%)
1.030	80			
1.035	70			
1.040	6			
1.045	60			
…	…	…	…	

Use your calculations to fill in Table 3.1 on the Answer Sheet (3.1.7)

The density of distilled water is $\rho_0 = 1.00 \frac{\text{g}}{\text{cm}^3}$ and concentration by mass is $c_0 = 0$.

Task 3.1.1. Write an expression for the total mass of NaCl m_{NaCl} in the initial solution (c_1 = 13%) in terms of V_1, ρ_1, and c_1.

Task 3.1.2. Write an expression for the mass of water m_w in the same solution in terms of V_1, ρ_1, and c_1.

Task 3.1.3. Write an expression for the total mass m of mixed fluids of V_1 and V_0 in terms of V_0, V_1, ρ_0, and ρ_1.

Task 3.1.4. Write an expression for the mass concentration c in mixed solution of fluids V_1 and V_0 in terms of V_0, V_1, ρ_0, ρ_1, and c_1.

Task 3.1.5. What is the relation of the ratio V_0/V_1 with c? Use ρ_0, ρ_1, and c_1.

Task 3.1.6. What is the relation of the ratio V_0/V_1 with ρ? In the final expression, use the densities ρ_0, ρ_1, and ρ. Express V_1 in terms of V_0, ρ_0, ρ_1, and ρ.

Note: use the fact that density linearly depends on concentration, see Fig. 11

Use the equation from **Task 3.1.6.** to fill in the 3rd column of Table 3.1. on the Answer Sheet.

Task 3.1.7. Make the corresponding measurements: prepare solutions using the values calculated in Table 3.1. (solution with density ρ can be made by mixing V_1 with V_0), stow all amber pieces into the solution (V_0+V_1) and count the number of newly surfaced pieces of amber (n). Fill in the remaining columns in Table 3.1. The newly surfaced pieces of amber should be placed on the tissue. Every next measurement should be done after having removed the batch of newly surfaced pieces of amber. Repeat the experiment until you have completed Table 3.1.

Task 3.1.8. Draw a bar chart $n(\rho)$ in percentage assuming that the 1st bar corresponds to the density interval from 1.030 g/cm^3 to 1.035 g/cm^3, the 2nd bar corresponds to the density interval from 1.035 g/cm^3 to 1.040 g/cm^3 and so on. The bar height should be equal to the number of corresponding newly surfaced amber pieces expressed as a percentage of the total number of amber pieces (the height of the 1st bar should correspond to the value in the cell of the last column in the 2nd measurement data row of Table 3.1., the height of the 2nd bar should correspond to the last column of the 3rd measurement data row and so on).

Task 3.1.9. Of the investigated pieces of amber**,** determine the density interval within which the number of the newly surfaced pieces of amber was the highest.

Task 3.1.10. Assume that "Sun stone" is very close to a spherical shaped body with a diameter equal to 18.50 cm and a weight of 3526.32 g. Calculate its volume and density.

Task 3.1.11. According to the experiment, please, explain: "Why does amber found in the sea usually have an irregular shape, compared to other stones which look more rounded and polished?"

Write the answers of tasks (3.1.1. – 3.1.11.) on the Answer Sheet.

End of **Task 3**

Task 4: "Sun Stone" evaluation catalogue

Phoenician historical papers prove that amber evaluation was a very precise, accurate and an important process. Firstly, mass and density were determined using ancient weighing techniques, then colour tone and colour intensity and, finally, price was determined by the presence of inclusions.

Your task is to use the evaluation catalogue, based on the information presented below, to determine how many swords, spears and arrows Lithuanian merchant Gintaras would have received for "Sun Stone" in the Phoenician market.

- The main currency used was weapons: swords, spears and arrows.
- One sword was worth 10 spears or 100 arrows.
- Initial price was established based on the mass, then other factors were assessed in the following order: colour tone, colour intensity, density and inclusions.
- The initial value was multiplied by a coefficient based on the referred properties.
- Red amber was twice as expensive as yellow amber (there is a linear correlation, and the coefficient for pure red amber is 2).
- In addition, inclusions are evaluated in terms of their rarity: The rarer the type of the inclusion in the piece of amber the more valuable it was.

Instructions for the determination of the value of amber:

Task 4.1.1. Derive the formula for the initial amber value (in swords).

Task 4.1.2. According to your result, obtained in **Task 2**, calculate "Sun Stone" coefficient value with regards to colour tone.

Task 4.1.3. Assuming that the colorant mixture for Column Chromatography was obtained from a 1 g sample of "Sun Stone", calculate the coefficient value with regards to colour intensity. You will need to use your data from **Task 2** and the data below.

Task 4.1.4. What is the percentage value loss of the "Sun Stone" according to its density distribution?

Use the density value of the "Sun Stone" from **Task 3.1.10** and find the corresponding percentage according to the density distribution in **Table 3.1** of **Task 3.1.7** or the chart from **Task 3.1.8**. This percentage would be the reduction in the value of the "Sun Stone". (0.25 pts)

Task 4.1.5 It was found that "Sun Stone" contains inclusion, the same that you determined to be the most valuable in **Task 1**. Calculate what additional value (in swords, spears and arrows) the most valuable inclusion adds to the total price.

Task 4.1.6. Calculate the hypothetical value of "Sun Stone" in the Phoenician market (in swords, spears and arrows).

End of **Task 4**.

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