Integration of Folin Ciocalteu reagent on a paper-based sensor for phenolic compound determination by using the external calibration curve

Aim:

Determination of total phenolic content in herbal infuses by using Folin Ciocalteu-based assay.
Integration of a conventional photometric assay to a paper to develop a paper-based device.
Compare the classical photometric assay with the one performed in the lab.

Lab utilities and reagents overview:

Reagents: Folin-Ciocalteu; buffer pH 10; gallic acid; MilliQ water (in Squeeze bottle), herbal infuse (green tea; Antioxidant tea; Slimming herbal tea).
Materials: Squeeze bottle; fiberglass; eppendorf; rak; Gilson pipette; tips; falcon (15 mL and 50 mL); syringe, and syringe filter (PTFE), Petri dish, volumetric flask; vials.
Apparatus: analytical balance; heated plate; own smartphone; please download the app RGB Color Detector from the Google Play Store (Icon:).

PROCEDURE:

1) **Colorimetric strip manufacturing (PRE-CEV INTERVIEW)**

- Cut the fiberglass ribbon into 20 squares (named strips) sized 1 cm x 1 cm.
- Drop-cast 10 µL of Folin Ciocalteu reagents on each strip and let it dry for 20 minutes at room temperature.

2) **Standard solution preparation (PRE-CEV INTERVIEW)**

- Prepare 10 mL of 10 mM gallic acid standard solution in methanol. Calculate the grams of gallic acid needed for the solution preparation (M.W.: 170.12 g/mol).
- Prepare an intermediate dilution of 1 mM gallic acid in 2 mL of methanol from the mother solution at 10 mM.
- Perform the calculation to prepare the solutions described below:
  From the 1 mM gallic acid solution, prepare gallic acid solutions concentrated at 500, 250, 200, 150, 125, 100, 75, 50, 25, and 10 µM. Each solution should be prepared in 1 mL of final volume with buffer pH 10 as solvent, using **serial dilution** methods.
  PAY ATTENTION. **PREPARE THE SOLUTION AFTER THE CEV INTERVIEW**
  Before starting: (i) understand the concentrations to be diluted serially; (ii) get the dilution factor among the concentration set; (iii) fill the table.

C (µM)	500	250	200	150	125	100	75	50	25	10	BLANK
	µL	µL	µL	µL	µL	µL	µL	µL	µL	µL	µL
Gallic acid											//////////
Buffer pH 10											

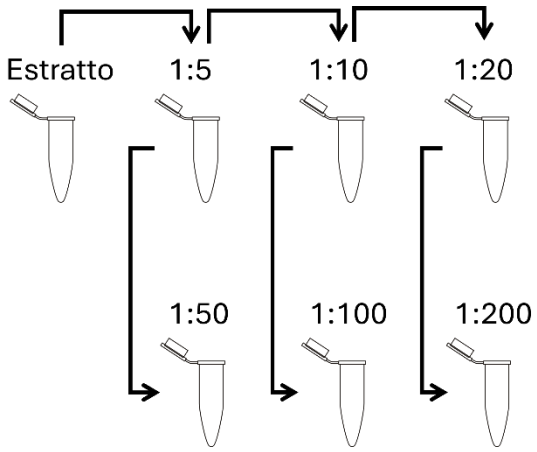
3) Sample extraction and preparation (PRE-CEV INTERVIEW)

- Weight 500 mg of the chosen infuse directly in Falcon by using the analytical balance. Record the weight performed.
- Add to the sample 10 mL of MillQ water, stir for 1 minute, then let it extract statically (*Which kind of extraction is?*).
- Filter the extract by using a syringe filter (PTFE): (i) screw the filter to the syringe; (ii) place the syringe filter on the 50 mL falcon; (iii) load the extract in the syringe; (iv) perform the filtration.
- Perform the calculation to prepare the dilutions described below:

  Dilute the extract 1:5, 1:10, 1:20, 1:50, 1:100, and 1:200 in a final volume of 1000 µL by using buffer pH 10 as solvent, using **serial dilution** methods.

  See the figure and the suggestion below:

  - Directly form the extract prepare serially the dilutions 1:5, 1:10, and 1:20
  - From the solution 1:5, prepare the solution diluted 1:50
  - From the solution 1:10, prepare the solution diluted 1:100
  - From the solution 1:20, prepare the solution diluted 1:200



PAY ATTENTION: **PREPARE THE SOLUTIONS AFTER THE CEV INTERVIEW**.

Before starting: (i) understand the concentrations to be diluted serially; (ii) get the dilution factor among the concentration set and sign it in the figure; (iii) see the suggestion below; (iv) fill the table with the calculated volume considering the **final volume of 1 mL**.

DILUTIONS	**1:5**	**1:10**	**1:20**	**1:50**	**1:100**	**1:200**
	µL	**µL**	**µL**	**µL**	**µL**	**µL**
Extract						
Buffer pH 10						

4) External calibration curve building-up and sample analysis (POST-CEV INTERVIEW)

- Prepare the standard solutions according to **point 2)**. Mix each solution by pipetting to ensure a homogenous solution.
- Prepare the sample dilutions according to **point 3)**. Mix each solution by pipetting to ensure a homogenous solution.
- Align the n= 17 colorimetric strips on the white support including also the blank of reaction (*What does it mean?*).
- Onto each colorimetric strip load 100 µL of standard solutions (5 - 200 µM) and diluted extract (1:5-1:200) according to increasing concentration and dilution degree and let them react for 20 minutes at 50°C on the heated plate (up to strip drying). PAY ATTENTION! YOU SHOULD RUN THE BLANK OF REACTION (Buffer pH 10 only) TOGETHER TO THE STANDARDs AND SAMPLEs.
- At the end of the reaction, recover the reacted colorimetric strips.
- Open the RGB color detector and collect pictures ensuring a good and uniform lighting condition.
- Using the RGB color detector record the Red (R) and Blue (B) hues for each reacted colorimetric strip. Before collecting the value adjust the pointer/sampler size (*Why we don't collect the Green hue?*).
- Report the collected data in an Excel file.

COMPARISON CHART FOR DIRECT QUANTIFICATION: