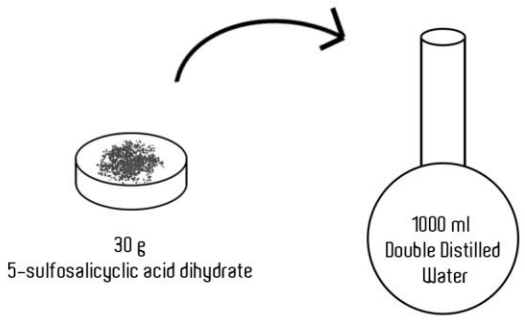
PROLINE ANALYSIS

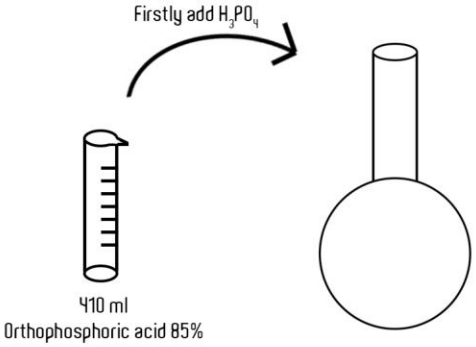
PRE – PREPARATION

Sulfosalicylic acid solution (SAS)



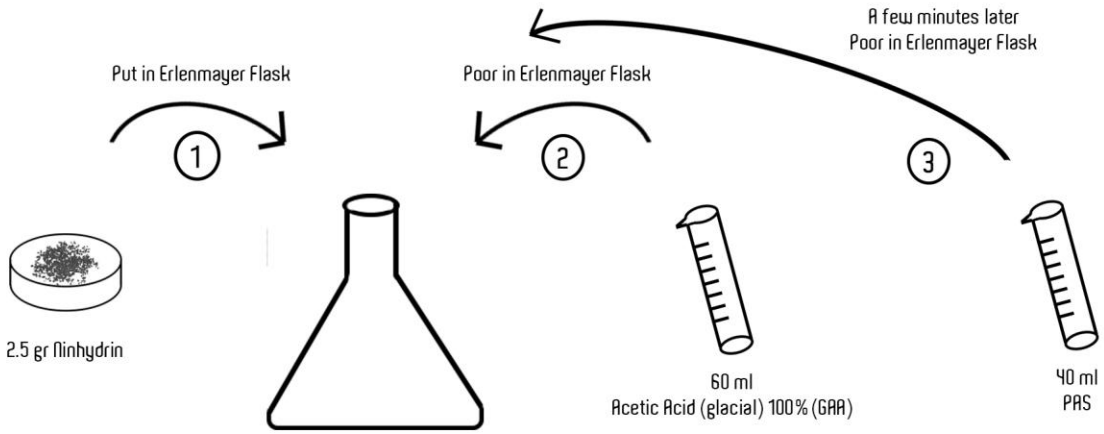
**Hints;
Not put all of the sulfosalicyclic acid (SA) into the balloon joje in one time. Firstly add some SA and double distilled water into balloon, then shake it kindly. When you sure it is dissolved in water, fill in with double distilled water.

H_3PO_4 solution (PAS)



**Hints;
Firstly pour 410 ml H_3PO_4 into balloon joje and fill with 590 ml double distilled water. After mixing, the amount of solution may decrease. Add water again up to 1000 ml.

Ninhydrin Solution (NNS)



**Hints;
Ninhydrin dissolving is hard. It needs a magnetic mixer with heater. Firstly add 2.5 gr ninhydrin (1) and 60 ml GAA (2) into erlenmayer flask. Start magnetic mixer and just a little bit open heater. A few minutes later pour 40 ml PAS (3) into the erlenmayer. Then approximately 20-30 minutes later, make sure all particules are melted and take it off from magnetic mixer.

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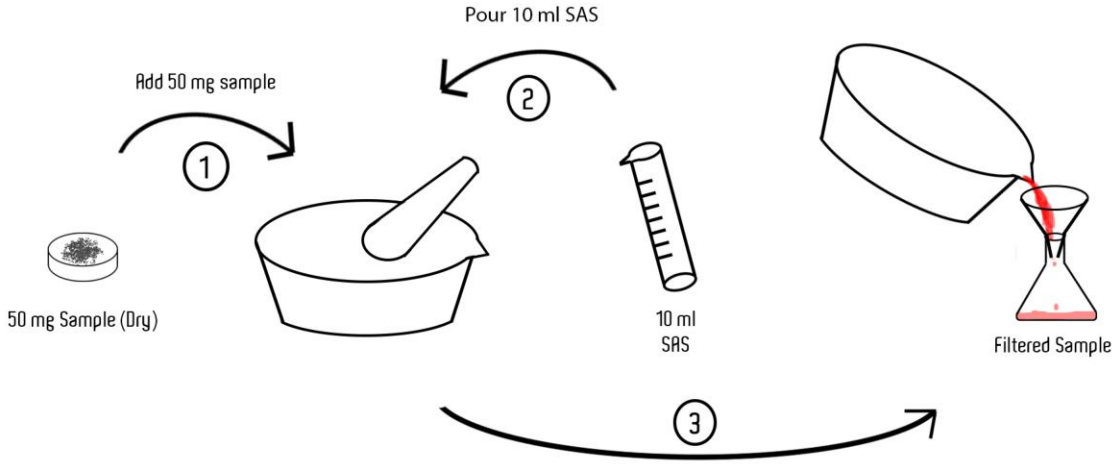
PROLINE ANALYSIS

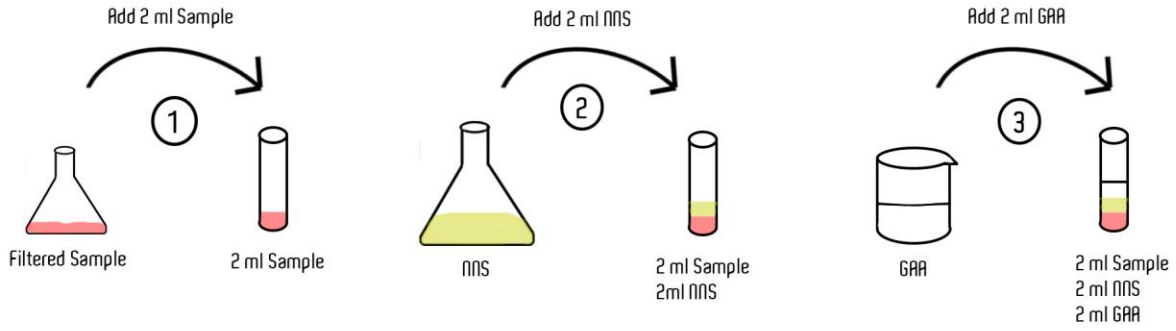
FIRST STEP

Sample Extraction with SAS



**Hints;
Put all of the sample in mortar (1). Firstly add 0.5-1 ml SAS and grind it until there is no big particules. When you see it, pour all of the SAS and kindly mix it (2). Cut your filtration paper shaped like funnel, combine with glass funnel and put it on erlenmayer flask. After extraction of sample, pour all of the sample which is extracted with SAS into the funnel and wait it until it's finish (3).

Make Solution Before Water Bath



**Hints;
Put 2 ml filtered sample into the tube (1). Add 2 ml NNS into the tube and shake it kindly (2). Ultimately, add 2 ml GAA and shake it kindly again. If you use micropipette while add solutions, your analysis would be more healthier. Finally, we have 6 ml solution in tube, close all tube with cap which is have a hole and now it is ready to use in water-bath. Put your tubes into water-bath which is boiling at 90-100°C and wait 1 hour.

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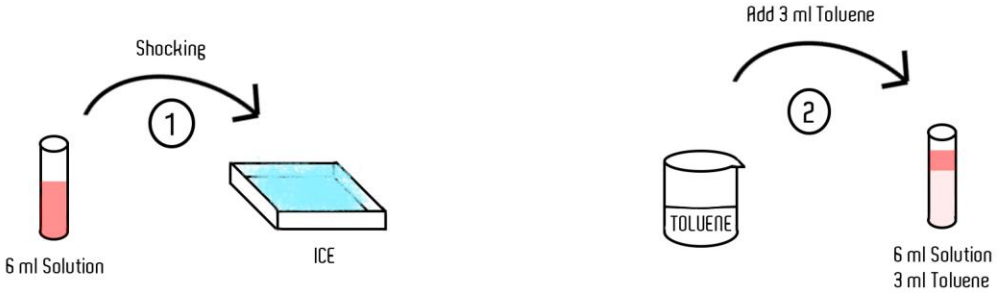
PROLINE ANALYSIS

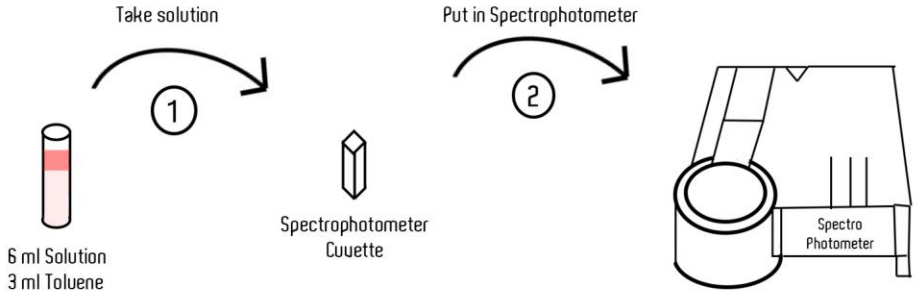
SECOND STEP

Prepare Last Solution Before Reading with Spectrophotometer



**Hints;
After water-bath, take your sample and put in ICE container immediately (1). Make sure your solution level get in touch with ice. Wait a few minutes, when it is shocked, take off tubes and wait under room temperature about 5 minutes. Last step before reading your proline values is that adding 3 ml toluene and and shake it well (2). After shaking, you would see a seperation in tube and you will use upper part of solution while reading with spectrophotometer.

Reading With Spectrophotometer



**Hints;
Firstly make your spectrophotometer settings. Use simple reads and set 518 nm. Make your zero with toluene. Take 2 ml solution from upper part of tube and pour it in cuvette. Then make sure cuvette is clean and read absorbance value.

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