09/04/18

- Spectrophotometer was calibrated using 5% CuSO4 solution and 0.01% KMnO4 solutions.
  However, expected results of calibration as standard graph were not observed and therefore, the experiment needs to be repeated.
- The experiment to check intensity of colour developed using variable concentration of slaked lime and fixed concentration of catechu was performed using the protocol-Link

Fig 1: Before Boiling

Fig 2: After Boiling

As it can be observed that the pigment was not extracted considerably, therefore the experiment needs to be repeated using the same protocol.

10/04/18

- Calibration of UV-Vis spectrophotometer was repeated using 5% CuSO4 solution and 0.01% KMnO4 solutions. Expected results were obtained for 0.01% KMnO4 solution as follows :-

Fig 3: Absorbance spectrum for 0.01% KMnO4 Solution

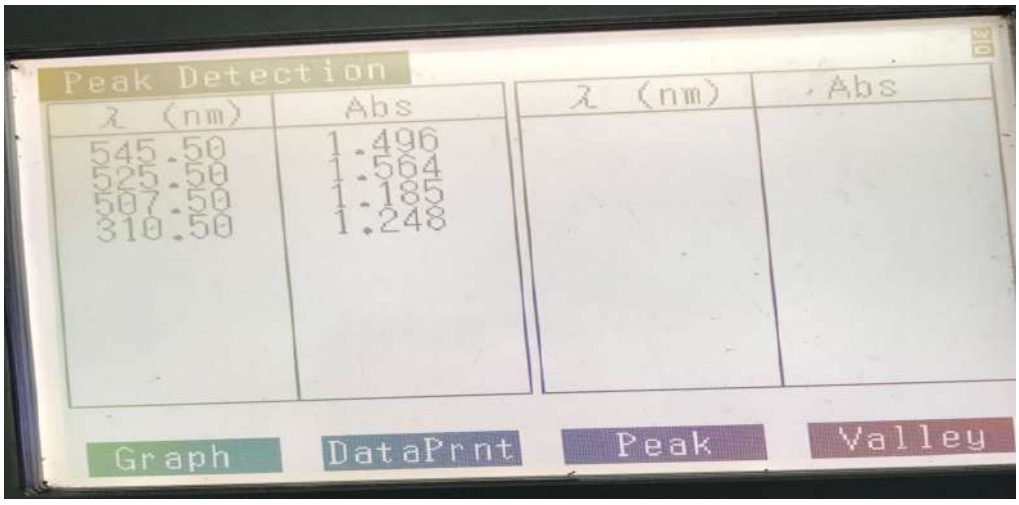

Fig 4: Absorbance Maxima Values for 0.01% KMnO4 Solution

However, 5% CuSO4 solution did not give expected absorbance values. Hence,it is predicted that the CuSO4 solution consists of some impurities and needs to be replaced in the lab immediately.

- The relative amounts of Catechu and slake lime required in media preparation was determined by following the protocol - Click here.
- It was observed that the solution containing 0.05g of slaked lime and 0.1g of catechu showed the maximum pigment extraction.
- Wavelength of maximum absorbance (λ_{max}) for the paan stain pigment was determined. The results obtained were as follows :

i) λ_{max} for slaked lime blank (when blanked with D/W): 279nm (A=1.166).
ii) λ_{max} for 1:0.05 ratio (when blanked with SL blank): 254nm (A=0.544).
iii) λ_{max} for 1:1 ratio (when blanked with SL blank): 330.5nm (A=0.044).
iv) λ_{max} for 1:2 ratio (when blanked with SL blank): Not determined.

Following are the respective pictures of the same:

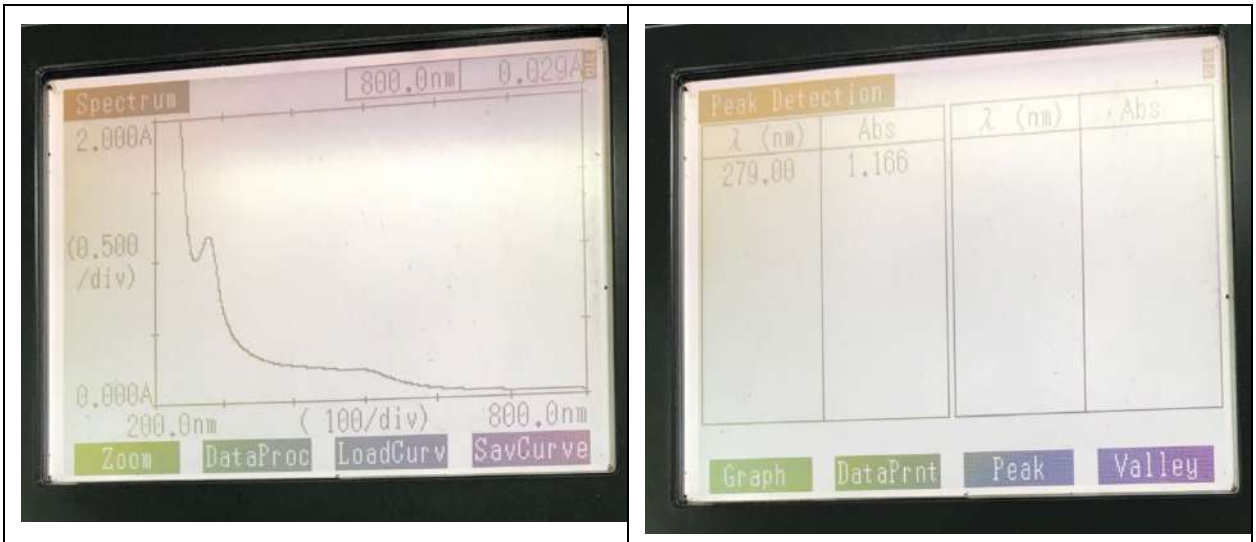

Fig 5 i): λ_{max} for slaked lime blank (when blanked with D/W): 279nm (A=1.166)

Fig 5 ii): λ_{max} for 1:0.05 ratio (when blanked with SL blank): 254nm (A=0.544).

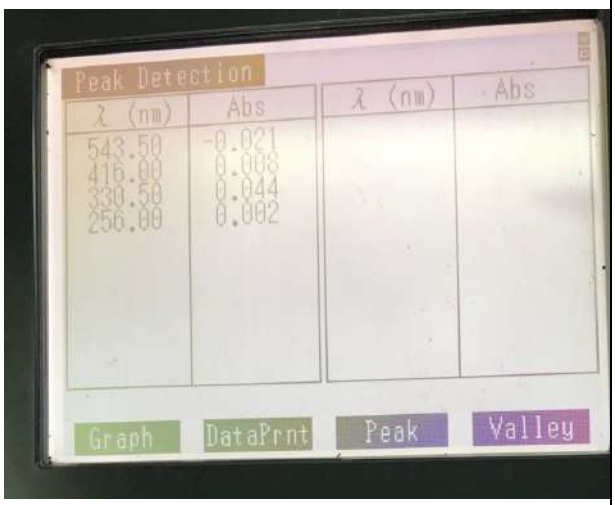

Fig 5 iii): λ_{max} for 1:1 ratio (when blanked with SL blank): 330.5nm (A=0.044).

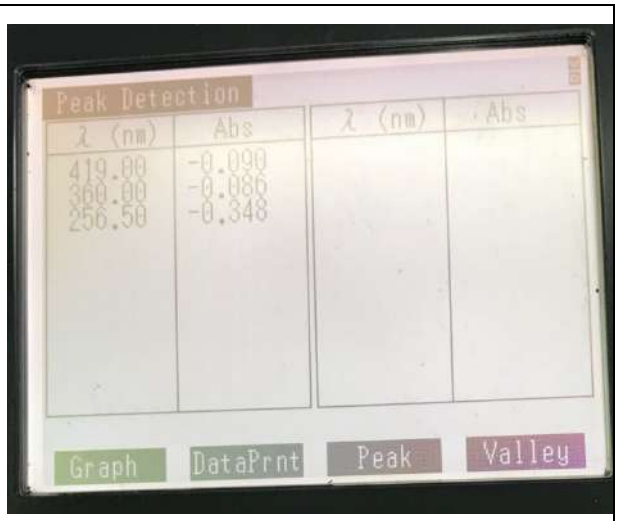

Fig 5 iv): λ_{max} for 1:2 ratio (when blanked with SL blank): Not determined

λmax of paan stain pigment from spectrophotometer showed to be 254nm but colorimeter observations showed 505nm. Therefore, we need to carry out a literature survey again of what could be the possible absorption maximas of the different constituents of paan & later again carry out a spectral analysis.

11/04/18

- The effect of varying concentration of catechu extracted and constant amount of slake lime added *(0.01 g - from the experimentation done on 10/4/18 it was determined that maximum intensity of red colour solution was obtained in solution of 1:0.1 catechu:slake lime conc.)* was determined. The solution obtained in the tube with 50:1 catechu:slake lime conc. was observed to have maximum intensity of red colored soln.Further ratios of conc. from 60:1 to 100:1 is to be determined further till we observe a constant intensity of colour which does not increase further with increase in catechu conc.

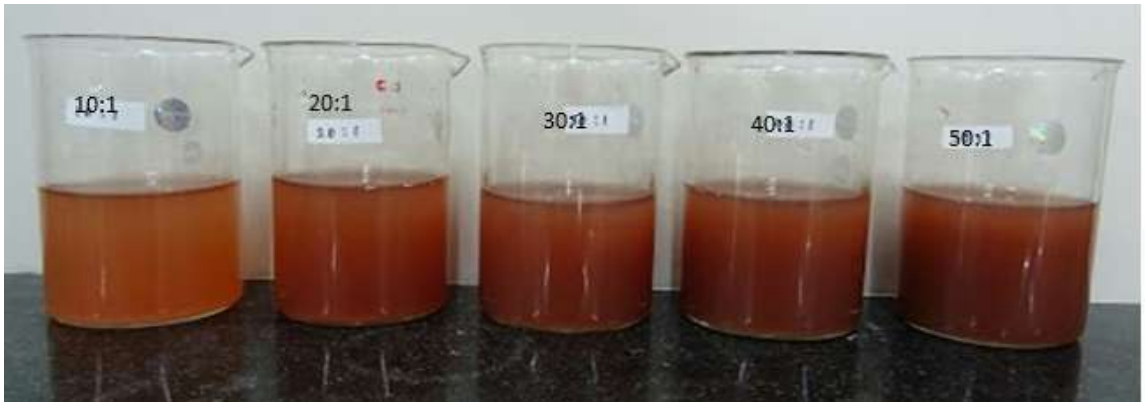

Fig 6 (i): Mixtures prepared after addition of slake lime (Before centrifugation)

Fig 6 (ii): The solution with extracted pigment and slake lime complex (After centrifugation)

- Spectral analysis of these solutions is to be determined (with 0.01g SL in 100ml water as blank) for confirmed data analysis.

- Nutrient media for the enrichment and isolation of desired paan degrading microorganisms was formulated. 4 different media are to be prepared for enrichment process:
    (1) M9 medium without any C source (to identify if microbial growth is due to the nutrients coming with sample-negative control)
    (2) M9 medium with 1% glucose
    (3) M9 medium with extracted catechu+slake lime complex
    (4) M9 medium with 1% glucose+catechu+slake lime complex
- An experiment to determine whether autoclaving change the intensity of red colouration of extracted pigment solution obtained was performed.
    Since no change in the intensity of red coloration of solution was observed, it can be predicted that media utilising this solution can be sterilized by autoclaving.

12/04/18

- Samples for microbial growth were collected in order to isolate paan stain degrading microorganisms.The samples were collected in accordance with iGEM safety and security principles,

Sample	**Location**	**Date**	**Time**	**Collected by**
Soil with paan stain	BKC Bridge	12/04/18	11:30am	MP,AR,NP
Mithi river water	Below BKC bridge	12/04/18	11:15am	MP,AR,NP
Paan Stain on bridge	BKC Bridge	12/04/18	11:30am	MP,AR,NP
Mithi river water	Near dead end	12/04/18	11:40am	MP,AR,NP
Soil	Ulhas river	12/04/18	3:45pm	SS,AV
Water from Ulhas river	Ulhas river	12/04/18	3:45pm	SS,AV
Soil with paan stain	Ulhas river	12/04/18	3:45pm	SS,AV
Paan stain from footpath	Anushakti nagar	12/04/18	10:45am	MS

Paan stain from bus stop	Anushakti nagar Bus stop	12/04/18	10:45am	MS
Paan stain from bus stop	Panjabwadi bus stop	12/04/18	11:30am	MS

Fig 7: Collected samples

- Requirements for M9 have been autoclaved and are to be reconstituted next day.
- Catechu extract was prepared to be added to the media. Pigment extracted from 10g catechu and 1g slake lime in 100ml boiling distilled water has given enough intensity to serve as a stock solution to be added in our media.