Paan colour standardization

Protocol:1) To standardize the quantitative assay of colour produced due to mixing of catechu and slaked lime.

Goal: To have a standard graph of absorbance vs concentration of our coloured compound.
The graph obtained shall serve as a standard while doing the enzyme assays to check the efficiency of color degradation.

(KEEPING SLAKE LIME CONSTANT)

Stock: 0.1g% catechu solution
Diluent: Distilled water
Range: 0.01g% - 0.1g%
Absorbance maxima: Needs to be determined.
Ca(OH)2 :0.1g% aqueous solution

Tube no.	Conc. (g%/ml)	Stock (ml)	Diluent (ml)	Ca(OH)2 (ml)	Total Vol.(ml)	Absorbance
1	0.01	0.5	4.5	1.0	6.0	
2	0.02	1.0	4.0	1.0	6.0	
3	0.03	1.5	3.5	1.0	6.0	
4	0.04	2.0	3.0	1.0	6.0	
5	0.05	2.5	2.5	1.0	6.0	
6	0.06	3.0	2.0	1.0	6.0	
7	0.07	3.5	1.5	1.0	6.0	
8	0.08	4.0	1.0	1.0	6.0	
9	0.09	4.5	0.5	1.0	6.0	
10	0.10	5.0	0.0	1.0	6.0	

11	BLANK	----	5.0	1.0	6.0	

In the above table tube no. 2, 4 and 6 will have a catechu: slake lime ratio of 1:1 , 1:2 and 1:3 respectively. (By mass since we are using 0.1g% of the solutions)

(KEEPING CATECHU CONSTANT)

Stock: 0.1g% Slake lime solution
Diluent: Distilled water
Range: 0.01g% - 0.1g%
Absorbance maxima: Needs to be determined. (will be same as above)
Catechu :0.1g% aqueous solution

Tube no.	Conc. (g%/ml)	Stock (ml)	Diluent (ml)	Catechu (ml)	Total Vol.(ml)	Absorbance
1	0.01	0.5	4.5	1.0	6.0	
2	0.02	1.0	4.0	1.0	6.0	
3	0.03	1.5	3.5	1.0	6.0	
4	0.04	2.0	3.0	1.0	6.0	
5	0.05	2.5	2.5	1.0	6.0	
6	0.06	3.0	2.0	1.0	6.0	
7	0.07	3.5	1.5	1.0	6.0	
8	0.08	4.0	1.0	1.0	6.0	
9	0.09	4.5	0.5	1.0	6.0	
10	0.10	5.0	0.0	1.0	6.0	
11	BLANK	---	5.0	1.0	6.0	

Protocol:2) To measure the optical density of the colour produced due to mixing of catechu and slaked lime colorimetrically.

(KEEPING CATECHU CONSTANT)

1) Weigh fixed amount of sieved catechu powder and slaked lime (in grams) (amount needs to be standardised)
2) Mix the two components in various ratios.(1:2,1:3,1:4,1:5)
   since catechu is constant the amount of slaked lime must be varied)
3) Add a fixed volume of boiling water to the mixtures with 1:2,1:3,1:4 and 1:5.

Ratio	Amt of catechu (in g)	Amt of slaked lime (in g)	Boiling water (in ml)	Absorbance
1:1	0.1	0.1	100	
1:2	0.1	0.2	100	
1:3	0.1	0.3	100	
1:4	0.1	0.4	100	
1:5	0.1	0.5	100	

(KEEPING SLAKE LIME CONSTANT)

1) Weigh fixed amount of sieved catechu powder and slaked lime (in grams) (amount needs to be standardised)
2) Mix the two components in various ratios.(1:2,1:3,1:4,1:5)
   (since slaked lime is constant the amount of catechu must be varied)
3) Add a fixed volume of water to the mixtures with 1:2,1:3,1:4 and 1:5.

Ratio	Amt of slaked lime (in g)	Amt of catechu (in g)	Boiling water (in ml)	Absorbance
1:1	0.1	0.1	100	
1:2	0.1	0.2	100	

1:3	0.1	0.3	100	
1:4	0.1	0.4	100	
1:5	0.1	0.5	100	

Protocol:3) To determine the relative amounts of Catechu and slake lime required in media preparation.

For Catechu+slaked lime:

1) Take six 100 ml beakers and add 100 ml of boiling distilled water to it.
2) To each of the beakers add 0.1g gram of catechu and let it boil again for 10 minutes to extract the catechu pigment.
3) Cool these solutions and then add slake lime paste to it in the following manner:

Ratio (Catechu:slake lime)	Amount of catechu(grams)	Boiling water (ml)		Slake lime (grams)	Absorbance
1:0.1	0.1	100		0.01	
1:0.2	0.1	100		0.02	
1:0.3	0.1	100		0.03	
1:0.4	0.1	100		0.04	
1:0.5	0.1	100	Cool the	0.05	
1:0	0.1	100	solution	0.0	
1:1	0.1	100	For 15-20	0.1	
1:1.5	0.1	100	mins	0.15	
1:2	0.1	100		0.2	

Protocol:4) To determine the effect of varying concentration of catechu extracted and constant amount of slake lime added.

From the experimentation done with protocol 4 it was determined that maximum intensity of red colour solution was obtained in solution of 1:0.1 catechu:slake lime concentration.

1) Boil around 500 ml of distilled water and using a measuring flask transfer 100 ml to 5 different beakers.
2) Add 0.1, 0.2, 0.3, 0.4, 0.5 grams of fine catechu powder to different beakers.
3) Hence ratios would now be, 10:1, 20:1, 30:1, 40:1, 50:1 catechu:slake lime conc.
4) Heat these mixtures again separately till boiling and let cool at RT then ice water bath.
5) Add 0.01 g slake lime in each beaker and then stir till the white powder is completely dissolved.
6) Centrifuge the mixtures and collect the supernatant.
7) Discard the pellet (which may be white ppt or slightly reddish brown)

Protocol: 5) To determine the relative amounts of Catechu and slake lime required in media preparation.

Goal: To have a standard protocol for extraction of catechu pigment for the purpose of media preparation.

1) Take six 100 ml beakers and add 100 ml of boiling distilled water to it.
2) To each of the beakers add 0.1g gram of catechu and let it boil again for 10 minutes to extract the catechu pigment.
3) Cool these solutions and then add slake lime paste to it in the following manner:

Ratio (Catechu:slake lime)	Amount of catechu(grams)	Boiling water (ml)		Slake lime (grams)	Absorbance
1:0.1	0.1	100		0.01	
1:0.2	0.1	100		0.02	
1:0.3	0.1	100		0.03	
1:0.4	0.1	100		0.04	
1:0.5	0.1	100	Cool the	0.05	
1:0	0.1	100	solution	0.0	
1:1	0.1	100	For 15-20	0.1	
1:1.5	0.1	100	mins	0.15	
1:2	0.1	100		0.2	