APPENDIX B

REDUCING SUGAR ANALYSIS

For sugar estimation the DNS method is simple, sensitive and adoptable for large number of samples at a time (Miller, 1959).

Materials

Dissolve 3,5 dinitrosalicylic acid 2.5 g with 2 N NaOH 50 ml, add 75 g sodium potassium tartrate and final volume 250 ml. with dH_2O.

Procedure

1. Pipette out 500 µl of the sample solution in test tube.
2. Add 500 µl of DNS reagent to the test tube and vortexing then boiling for 15 min.
3. Cool down in the water.
4. Spectrophotometrically measured at 540 nm, cuvettes against a reagent blank prepared from 500 µl of DNS reagent and 1.6 ml of distilled water.
5. The reducing sugar in sample is determined by using a calibration curve of glucose standard.

Table 1B Absorbance at 540 nm of glucose standard at various concentration

Glucose concentration	Absorbance (540 nm)			
	Duplicate1	Duplicate2	Duplicate3	Mean
0	0.000	0.000	0.000	0.000
50	0.021	0.020	0.020	0.020
100	0.063	0.061	0.063	0.062
150	0.102	0.101	0.101	0.101
200	0.150	0'148	0,148	0.150
250	0.200	0.219	0.216	0.212
300	0.241	0.248	0.238	0.242
350	0.290	0.281	0.294	0.288
400	0.329	0.326	0.333	0.330
450	0.379	0.368	0.362	0.370
500	0.415	0.415	0.416	0.415
600	0.505	0.502	0.509	0.505
700	0.591	0.589	0.588	0.589
800	0.679	0.687	0.683	0.683
900	0.757	0.755	0.760	0.757
1000	0.851	0.850	0.869	0.857

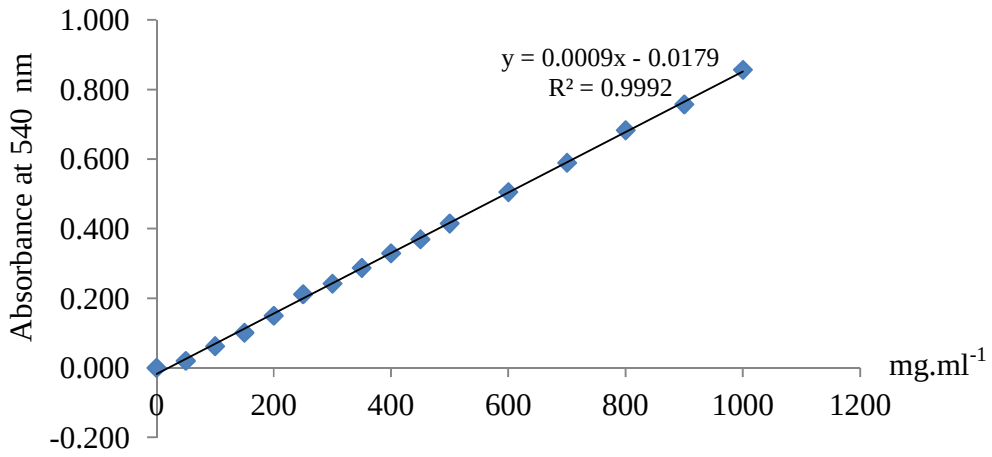

Figure 1B Standard curve of glucose concentration