

in order to be compared with the new method (SLS).

B) SLS METHOD^[10]

Twenty microliter of fresh blood with EDTA was added into 3 ml of SLS reagent & read the absorbance within 20 second on spectrophotometer at 535nm wavelength.

Thus for preparing a calibration graph or standard graph it is necessary to adopt a two-stage procedure using fresh blood as an intermediate reference preparation, the Hb value of which must first be established by the HiCN method. For this purpose blood collected into APD or ACD anticoagulant is suitable for 2-3 weeks if stored at 4 c providing it remain sterile. A standard sample will maintain its assigned value for several months, especially if stored frozen.

After established of Hb value by HiCN method we make dilution by SLS

reagent 1 in 2, 1 in 3, 1 in 4.... etc. Plot the reading into linear graph paper using arithmetical scale, with absorbance as ordinates (vertical scale). The points should fit a straight line that pass through the origin. From the standard curve, we calculate the Hb value and plot in table.

Results

1. Spectrophotometry:

a. Conversion Time:

Total conversion to Hb-SLS was extremely rapid-less than 15 seconds required to obtain an initial measurement on the spectrophotometer.

b. Stability:

In freshly prepared solution of Hb-SLS kept at room temperature no significant difference were seen in absorbance at 535nm on repeated measurements during the first 120 minutes after dilution (Figure 1).

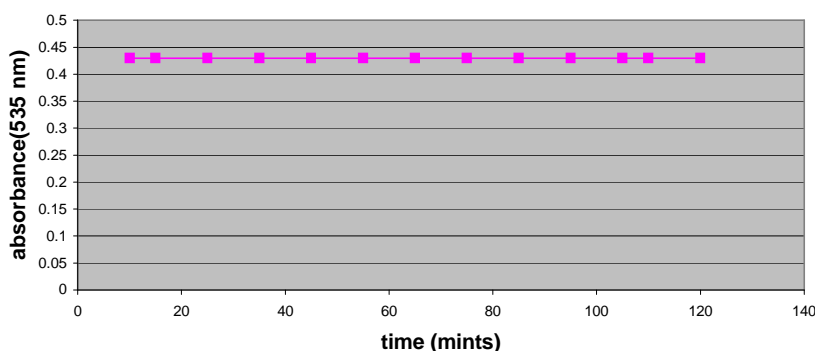


Figure 1: Haemoglobin SLS colorization

2. Technical Assessment:

a. Linearity: