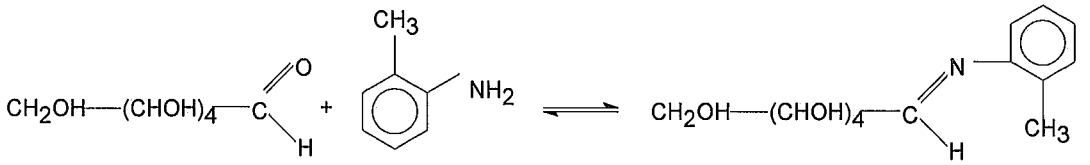
Kennedy Handout 4-4

Determination of Glucose in Blood Serum

Principles Several parameters in blood serum are routinely determined in clinical labs by spectrophotometric procedures. One of these is the determination of glucose based on its reaction with primary aromatic amines in glacial acetic acid. In the procedure described here, the blue-green complex formed with *o*-toluidine (shown below) is employed.



glucose o-toluidine blue-green complex

A calibration curve is prepared with standard glucose solutions, and the absorbance of the blood sample treated in exactly the same fashion as the standards is measured. From the absorbance, the glucose level in the blood sample may then be read directly from the calibration curve.

Directions Transfer 10 mL of *o*-toluidine reagent to each of 8 test tubes (4 calibration standards, 3 blood samples, and 1 blank), and cover with Parafilm M. Transfer 0.10 mL of calibration standards (Note 1), 0.10-mL aliquots of the blood serum sample (Note 2), and 0.10 mL of H_2O to the appropriate test tubes, using a pitpettor (obtain in the stockroom along with unknown). Cover again with Parafilm M and mix thoroughly. Place tubes in boiling water for exactly 7.5 min. Cool rapidly in ice water for 2 min. Take out of ice water, wait 5 min., and then read absorbance at 630 nm against the blank.

Prepare a calibration curve by plotting absorbance for standards versus milligrams per 100 mL. Read concentration of glucose for blood samples from the calibration curve. Report milligrams of glucose per 100 mL for the blood sample.

Notes

Preparation of calibration standards: A stock glucose solution containing 1000 mg per 100 mL be prepared by dissolving anylytically weighed ~1.000 g of glucose in H_2O and diluting to 100 mL in a volumetric flask.

Four standards are then prepared in 100ml volumetric flasks using the stock solution:

50 mg/ 100 mL: pipet 5.00 mL stock solution, dilute to the mark (100 mL)
100 mg/ 100 mL: pipet 10.00 mL stock solution, dilute to the mark (100 mL)
200 mg/ 100 mL: pipet 20.00 mL stock solution, dilute to the mark (100 mL)
300 mg/ 100 mL: pipet 30.00 mL stock solution, diluted to the mark (100 mL)

Mix all solutions well before use!