METHODS FOR ESTIMATION OF BLOOD GLUCOSE : A COMPARATIVE EVALUATION

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ABSTRACT

Glucose in blood is the most frequent analyzed parameter in a clinical chemistry laboratory. In Armed Forces Laboratories, copper reduction method (Modified Folin Wu) is commonly used. Here we have compared this method as well as O-Toluidine and GOD-POD method with reference UV-Hexokinase method. Both Modified Folin Wu and O-Toluidine showed upward deviation with substantial imprecision (CV=4.9% - 3.5% and 6% - 5.8% respectively) and inaccuracy (Average deviation = 5.76% and 10.68% respectively). GOD-POD method was found linear (up to 500 mg/dl), with good precision (CV=0.7% to 1.4%) and accuracy (Average deviation= -0.97%). This method is simple, rapid, economical and sensitive, and can be adopted to a routine colorimeter.

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KEYWORDS : Blood glucose; Diabetes mellitus; Glucose oxidase.

Introduction

Diabetes mellitus is major health problem from both national and worldwide perspectives. Although there are many postulated etiologies of the different kinds of diabetes mellitus, the definition of the disease still emanates from measurement of glucose in a sample of whole blood, serum or plasma [1] emphasizing the need for its accurate estimation.

Different methods based on the different properties of glucose are described for blood glucose estimation [2-8]. Folin Wu method modified to suit the clinical laboratory conditions is being practiced by the Armed Forces Medical Services. In present study we aimed at evaluating this and two other blood glucose estimation methods with regards to their linearity, precision and accuracy vis a vis reference Hexokinase method.

Material and Methods

Fifty six samples which included normal healthy adults, hypoglycemic and hyperglycemic patients were analysed. Fasting blood samples were collected in sodium fluoride and potassium oxalate mixture in the ratio of 1:1 at concentration of 4 mg mixture per ml of whole blood. Plasma was obtained by centrifuging the blood at 2000 rpm for 10 min. We used Systronics and Hans 202 filter colorimeters; and Beckman double beam Model-25 and Shimadzu micro-flow CL-750 spectrophotometers. Hexokinase kit was obtained from Sigma Chemical Company USA and GOD-POD from Accurex, India. All other reagents were of analytical grade.

Procedures : Plasma glucose was estimated by Modified Folin Wu method [9], O-Toluidine method [5], GOD-POD and Hexokinase method as per the instructions given with the kits. Linearity of method was studied by plotting a standard curve using glucose standard in various concentrations. Precision of the method was established by determining standard deviation (SD) and co-efficient of variation (CV) in all the three types of samples (normals, hypoglycemic and hyperglycemic). Accuracy of the method was evaluated by comparing with the Hexokinase reference method [10] for glucose estimation.

Results

A) *Linearity of Modified Folin Wu, O-Toluidine, GOD-POD and Hexokinase method* :

Modified Folin Wu : Plots of absorbance (Y) vs glucose concentration (X) in the range of 40 mg/dl to 200 mg/dl showed upward deviation, (Fig 1). This method demanded reading of absorbance at zero minute as the end colour faded rapidly. We have found that on an average 31.8 per cent colour fades in first 5 minutes. To overcome this difficulty, more acidic Folin Wu colouring agent [9] was tried. The fading of colour was only marginally less with this reagent (Fig 1). Therefore in subsequent study of accuracy and precision original Folin Wu colouring reagent was used with reading of absorbance at zero minutes.

O-Toluidine : This method also showed upward deviation when absorbance (Y) is plotted against glucose concentration (X) in the range of 45 mg/dl to 360 mg/dl (Fig 2).

GOD-POD and Hexokinase : Both these methods showed linearity between absorbance (at 505 nm for GOD-POD and 340 nm for Hexokinase method) and glucose concentration up to 500 mg/dl (Fig 3).

B) *Precision* : All methods were evaluated for precision by replicate assays of hypoglycemic, normal and hyperglycemic human plasma samples of concentration 39 mg/dl, 102 mg/dl and 460 mg/dl respectively. Ten analyses were carried out on each sample and the standard deviation (SD) and co-efficient of variation (CV) was calculated for each (Table 1).

C) *Sensitivity* : The molar extinction co-efficient given by different methods was determined and detection limit was calculated for each method (Table 2).

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TABLE 1

Precision data for Modified Folin Wu, O-Toluidine, GOD-POD and Hexokinase methods

Sample	O-Toluidine			Mod. Folin Wu			GOD-POD			Hexokinase		
	Mean mg/dl	SD mg/dl	CV %	Mean mg/dl	SD mg/dl	CV %	Mean mg/dl	SD mg/dl	CV %	Mean mg/dl	SD mg/dl	CV %
Hypoglycemic (n=10 each)	60	3.6	6.0	57	2.8	4.9	38	0.52	1.4	39	0.45	1.2
Normal (n=10 each)	86	5.3	6.1	79	3.8	4.7	101	1.2	1.2	102	0.7	0.7
Hyperglycemic (n=10 each)	248	14.4	5.8	219	7.6	3.5	472	3.3	0.7	460	1.8	0.4

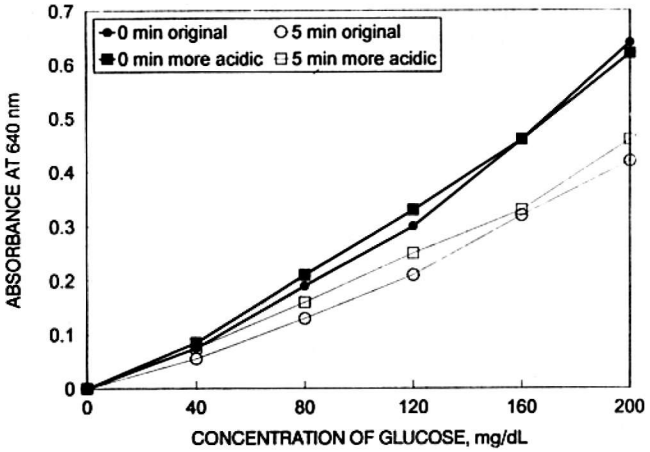

Fig. 1 : Linearity of Modified Folin Wu method (with original and more acidic Folin Wu reagent).

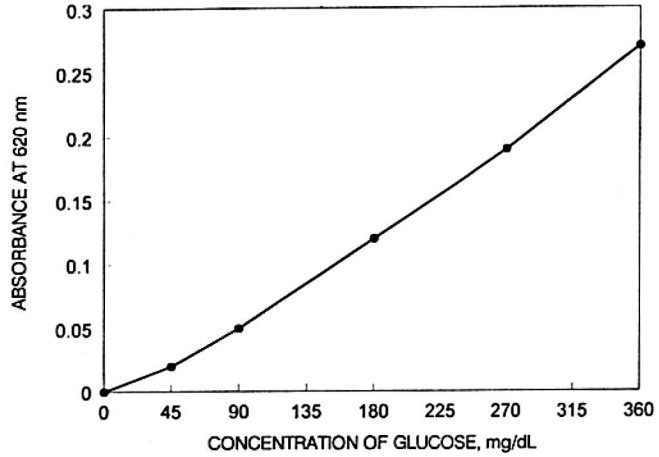

Fig. 2 : Linearity of O-Toluidine method.

TABLE 2

Molar extinction co-efficient and detection limit for O-Toluidine, Modified Folin Wu, GOD-POD and Hexokinase methods

Method	Molar ext coeff	Detection limit
O.Toluidine	1300 M^{-1} cm^{-1}	1.06 mg/dl
Mod Folin Wu	24000M^{-1} cm^{-1}	0.31 mg/dl
GOD-POD	6600 M^{-1} cm^{-1}	0.30 mg/dl
Hexokinase	6800 $M^{-1}cm^{-1}$	0.29 mg/dl

D) *Accuracy of Modified Folin Wu, O-Toluidine, and GOD-POD methods* :

We assayed 56 human plasma for glucose estimation by Folin Wu, O-Toluidine and GOD-POD methods and compared the values with Hexokinase method taken as a reference. Each sample was tested in duplicate by these methods and the deviation of the measured mean value from the target Hexokinase value was calculated. Subsequently the average deviation was calculated for each method (Table 3).

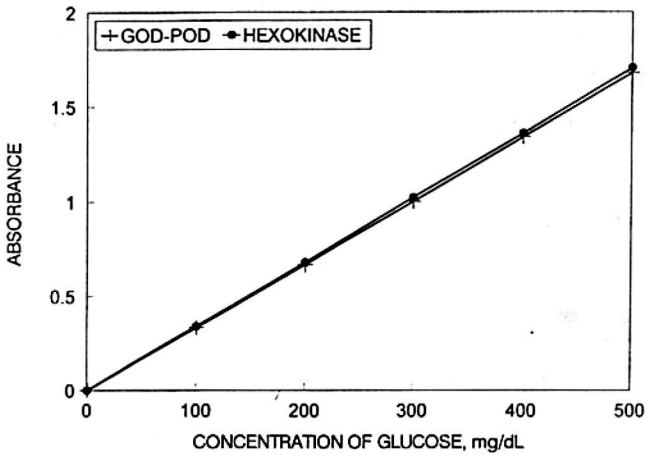

Fig. 3 : Linearity of GOD-POD and Hexokinase methods.

TABLE 3

Accuracy of Modified Folin Wu, O-Toluidine and GOD-POD methods

Method	AV. Deviation	Range
O-Tobuidine	10.68%	37% to -8.5%
Mod Folin Wu	5.76%	28% to -16%
GOD-POD	-0.97%	7.5% to -9.8%

Discussion

The Modified Folin Wu method though sensitive is nonlinear. The method shows imprecision (CV=3.5% - 4.9%, Table 1) and inaccuracy (Av.Dev = 5.76, Table 3). The fading of colour during reading of absorbance adds to this imprecision and inaccuracy. The O-Toluidine method though comparatively linear than Modified Folin Wu method and gives stable colour complex, is less sensitive (Table 2), and requires O-Toluidine reagent which is a corrosive, unstable (shelf life of one month even under dark) and carcinogenic. Moreover the method shows imprecision and inaccu-

racy higher than that of Modified Folin Wu method (Table 1 & 3). Thus O-Toluidine method does not show any advantage over Modified Folin Wu method. Both these methods require protein precipitation, centrifugation and boiling which is time consuming. Modified Folin Wu method requires special tubes (Folin Wu tubes) and accurate boiling time (6 minutes). The absorbance should be read immediately as colour fades rapidly or at fixed interval (such as 5 minutes) which is technically not feasible.

The GOD-POD method is linear (up to 500 mg/dl), sensitive (detection limit 0.3 mg/dl), simple (requires 10 microlitre of sample to be incubated for 30 minutes with single reagent at room temperature) and requires simple instrumentation (the absorbance to be read between 505 nm to 550 nm). When the results obtained by this method using Indian kit, is compared with that of Hexokinase method using a kit from Sigma Chemical Co. USA, the average deviation was found to be -0.97 per cent only (Table 3). Thus the GOD-POD method which is comparatively cheap, the kit for which is readily available and can be adopted using colorimeter, demonstrated a very good agreement with the UV-Hexokinase method.

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