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Determination of Total Proteins in Human Blood Serum by A Lab- Built semi-automated Flow Injection Analysis (FIA)

Najah Z. H. AL-Hisnawy, K. H. A. AL-Sowdani

Basrah University, College of Education for Pure Science, Chemistry Dept.

E-mail : najah19691969@gmail.com

Abstract

Determination of total proteins in human blood serum(HBS) by using a lab built semi-automated flow injection (FI) system. This technique consists of two types of microcontrollers (Arduinos) supplied with a home-made software program. The first one was (UNO) type used to manipulates the home-made injection-pump stopper-motor type and the other one was (Mega) type which was used as a data logger to record the results as a peak height by using Microsoft Excel program. The maximum absorption obtained at a wavelength of (551 nm). The linear calibration curve was applied in the range (2-8 g/l) with a regression coefficient of (R^2 =0.9986) and the detection limit was (1.0 g/l). The R.S.D is (0.29 %) for 12 successive measurements of 4 g/L of albumin and the sample throughput was (120 sample / h). The recoveries of (95.2-104.7 %) and (74) serum samples of patients in range (40-78 g/l). This method is simple, sensitive, and convenient which was successfully applied to the determination of total proteins in different samples of human blood serum.

Key words : Lab-built , FIA, Total proteins, Adriano, Biuret reagent, , HBS.

Introduction

A variety of techniques and methods have been reviewed for the estimation of the concentration of total proteins in human serum blood [1-5]. The spectrophotometric technique is frequently employed in Biochemistry and Clinical laboratory chemical analysis [5-7]. It offers compensation in terms of the accessibility of instruments, ease of procedures, rapidity, precision, accuracy, and validity to a wide range of bio-medically vital substances Many conventional spectrophotometric methods have been reported for total proteins in human blood serum which was suffering from many drawbacks[7-11]. The most widely used is a method based on the Biuret

protein assay, in which an alkaline copper (II) solution reacts with peptide linkages to form a complex that absorbs light at wavelength (540 nm)[12- 14].

This method was available to estimated protein concentration since the 1940s [15-17] and still in use because it is so suitable and low-cost to prepare and simple to use[18,19].

Flow injection analysis(FIA) [20-22], from its beginning found a smart place and wide applications among other flow analysis techniques in the clinical lab. It is currently regarded as a superior tool for routine analysis which can be mainly attributed to its versatility, high sample throughput, easy to use, low cost and the low consummation of reagents, and samples, easy to automation, and minimization[23- 27]

Recently, a Lab-built semi-automated FI system equipped with microcontrollers was developed in our laboratory [28 – 30] .It was used for the determination of Albumin in Human Blood Serum[31] , So thought that lab- Built emi-automated (FI) system can be used in this work to determine total proteins by Biuret spectrophotometric method.

This combination can be providing a fast, simple, reproducible, low cost and low consumption of samples and reagents. To evaluate the proposed system a recoveries study was carried out on three representative samples(Table 1) by using the addition standards method. Also, correlation studies were established between the results obtained by our lab-built system, and the official method used by the clinical laboratory at Teaching Hospital- Basrah – Iraq (Biuret method by Cobas instrument which)which were used..

Experimental

Reagent and solutions

During the determination of total proteins by lab-build flow system deionized distilled water and normal saline solution (0.9 w/v) (pioneer company/Iraq) were used throughout this work. The working and the standard solutions were obtained by step-wise dilution by normal saline

All reagents employed were analytical grade unless otherwise stated. Human serum albumin is acceptable as an alternative standard to total proteins and it was used during this work for total protein**s** studies.

The value of measurement as peak height was the average of three successive measurements.

Samples collection

(74) total proteins for patients samples were kindly provided from clinical laboratories in Basrah Teaching Hospital provided in June 2019, blood samples transferred in July tube and all samples keep in (Blow Kings/India) cooling box to keep and all measurement was done in the same day of collection and the excess samples were kept freezing.

Biuret reagent [32]

Consists of copper sulphate (1.0 % w/v) with sodium hydroxide (40 % w/v). (1.0 % w/v) copper sulphate (Merck) was prepared by dissolving (1.0 gm) in (100 ml) of water. (40 % w/v) sodium hydroxide (Thomas baker)

was set up and prepared by melting (40 gm) in (100 ml) of some water. The required concentrations were prepared of biuret with continuous dilution .

Standard human albumin

Human Serum Albumin (HSA) (200 g/l) (Vienna – Austria)was used as a stock solution. This solution was standardized with Bovine albumin (50 g/L) [33] by following the manual spectrophotometric method.The working and the standard solutions were obtained by step-wise dilution by normal saline (0.9 **% w/v** sodium chloride).

Instrumentation

Fig.1, (a and b) shows the lab-build (FI) system and its components which described in details previously [31] .

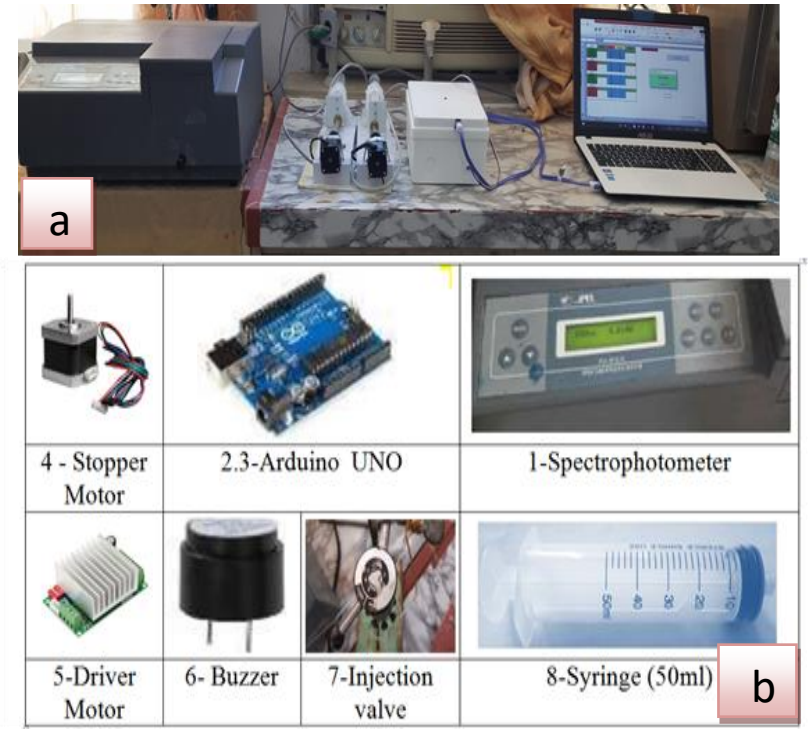

Fig. 1, (a and b) the lab-build FI system and its components

Procedure

The flow manifold used for determination of total proteins is shown in Fig.2, This method consist of two microcontrollers (Arduino). The Arduino type (Uno) used to control the injection pump; first of all, wash the manifold spontaneously for (20 min).Then refilled the (50 ml) first plastic syringe with a copper sulfate and the other with sodium hydroxide. A (200 µl) of total proteins sample injected manually through injection valve into mixed reagent stream. The other Arduino type (Mega) to recording the signals as peak height with aid (Microsoft Excel 2010) program. After that injection pumps restarts to wish the flow system and restart for other sample.

Results and Discussion

The manifold of the system used for total proteins determination , as shown in Fig. 3, was used to optimum conditions are found and appropriate which is, wavelength (551 nm), flow rate for every stream (copper sulfate, sodium hydroxide)(2.0 ml/min), total flow rate (4.0 ml/min), sodium hydroxide concentration (2.5 g/100 ml), copper sulfate concentration (0.25 g/100 ml),sample volume (200 µl), cell volume (450 µl) and Reaction coil length (50 cm) .

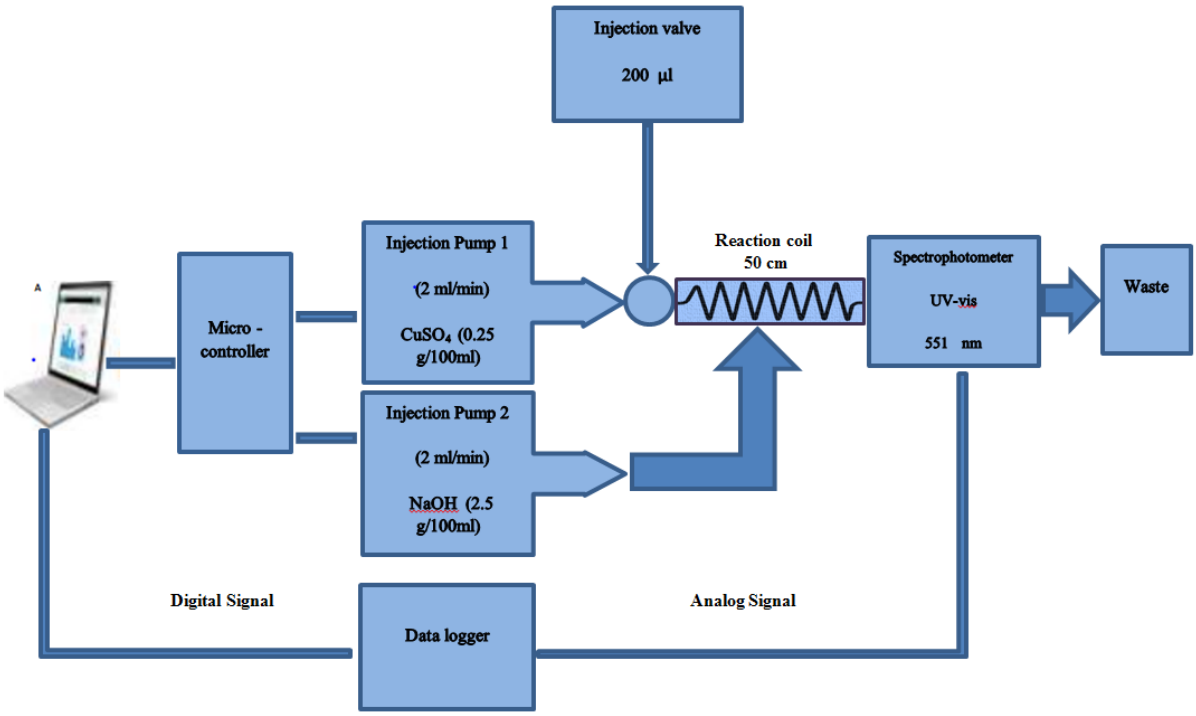

Fig. 2, Manifold of the Lab-Build FA System

Study of the complex spectrum

Complex spectrum was drawn within the range (350-750 nm) by(CECIL Instruments/England) and the highest absorption value was found at wavelength (551 nm) . It was used in all subsequent experiments . as shown in Fig. 3,

Calibration Curve

Under the established conditions and appropriate, a calibration curve for total proteins was obtained Fig. 4, it is linear over the range (2-8 g/l) . the linear curve has a regression coefficient (R^2 = 0.9986) . and detection limit was (1.0 g/l) . the R.S.D. was (0.29 %) and the sample throughput was (120 sample / h) . the value of R.S.D. for reproducibility obtained was (0.009 %) Fig.5

Recovery study

Table 1. shows the recovery to the determination of total proteins . The Home-made semi-automated (FIA) used to the determination total proteins in serum was of good recoveries' . It can be used to determination total proteins in the blood .

Application

All results for (74) samples were obtained by using the additions standard method for determination total proteins in human blood serum , Fig.6 shows one of these samples. Fig.7 shows the linear correlation (R^2 = 0.983) which clearly indicates that the results of both methods were close to each other and the results are acceptable. Fig 8, shows the result of the t-test with a P-value equal to (0.97) which is acceptable statistic results.

Table 2 list the values ranged (43-78 g/l) and it demonstrate s that the values of some of these results are out-off the normal range for the value of total proteins (60-80 g/l) and may be attributed to different related causes which are outside our scope.

The results can be self-obtained through the use of micro -controllers, an injection pump , and self-control to stop the reaction and restart .

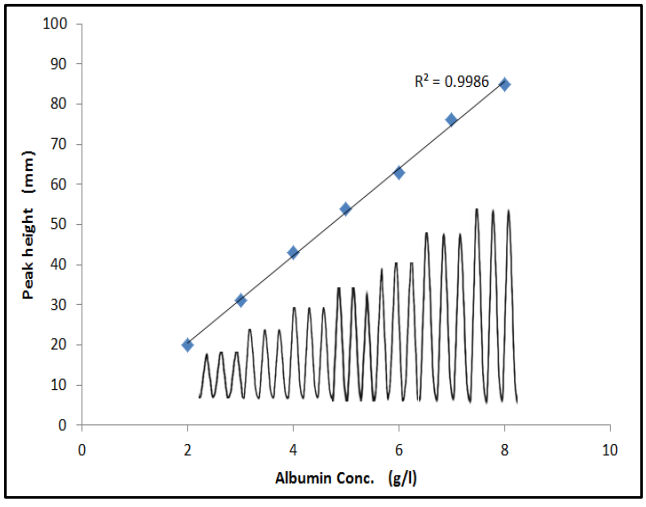

Fig.4, The calibration curve for total proteins

1. The determination of total proteins by the home-made flow injection system is superior compare with other conventional methods .
2. It is low cost , simple , sensitive and high samples throughput .
3. This method decreases the possibility of the interferences of other proteins on the measurement of total protein.

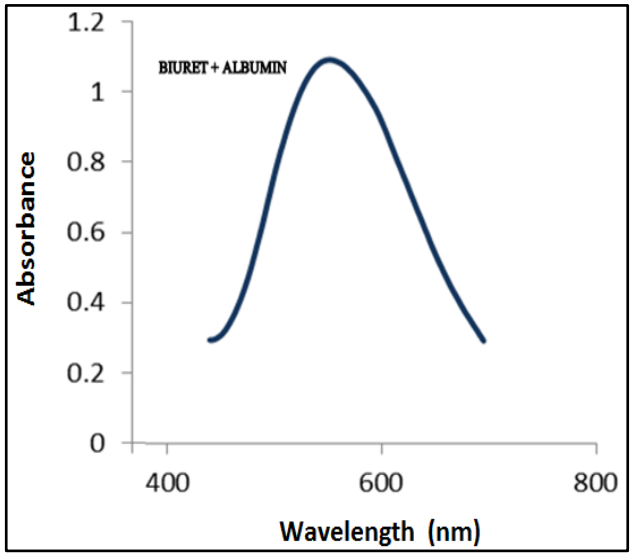

Fig.3, the spectrum of albumin with Biuret

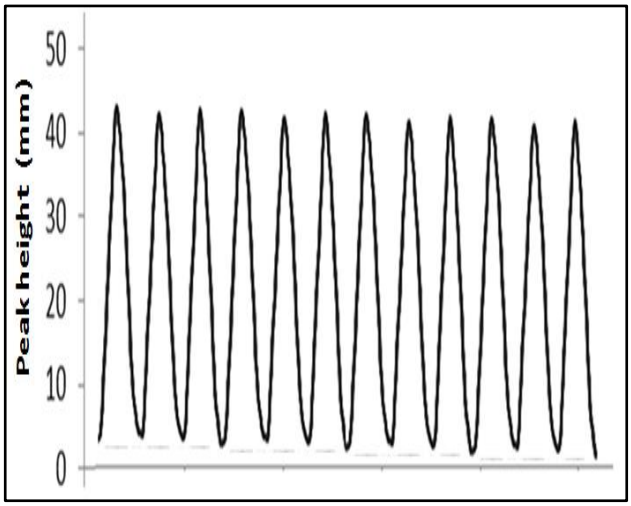

Fig.5, The reproducibility of height peak for (4 g/l) albumin

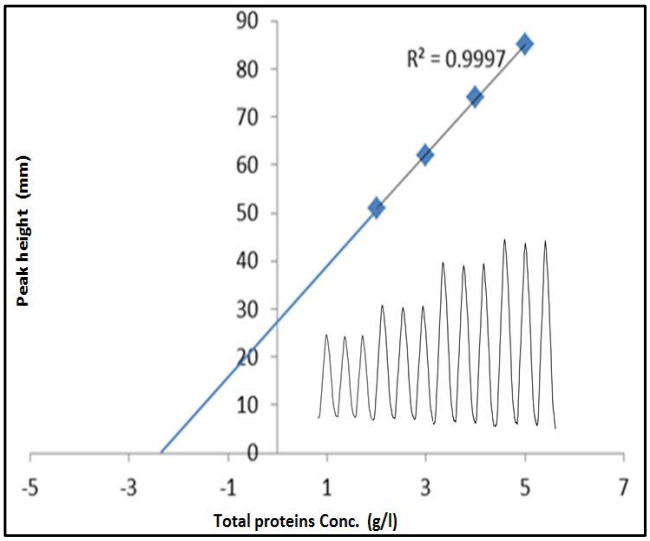

Fig.6, Determination of total proteins in sample (60 g/l) by the standard addition method

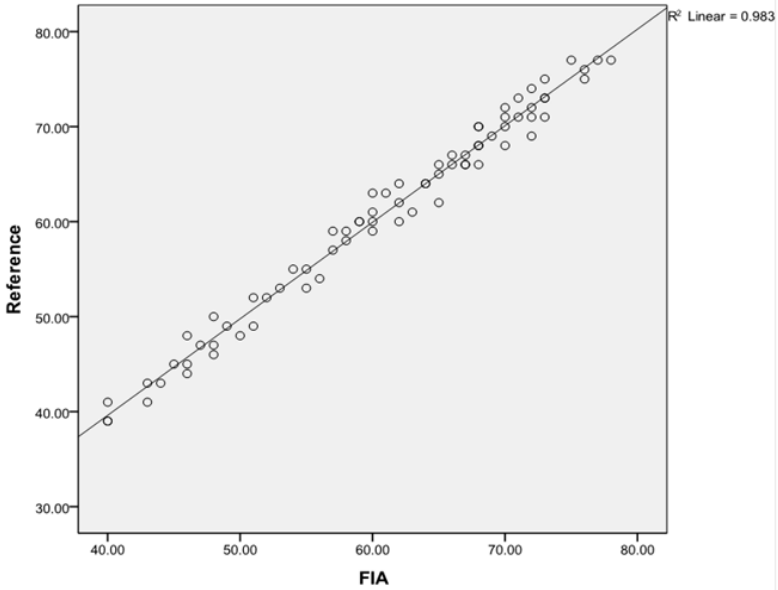

Fig.7, The linear correlation between FIA technique and reference (hospital) for total proteins determination

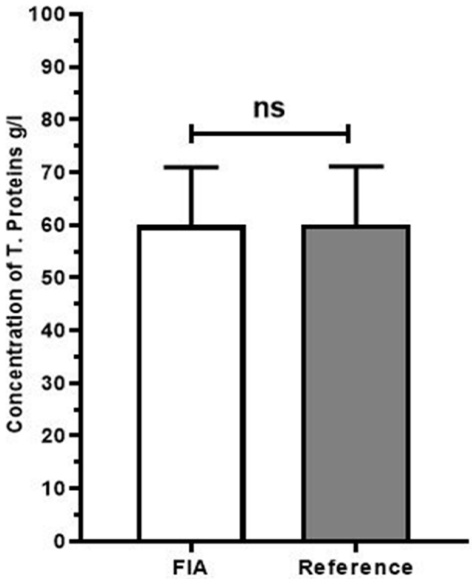

Fig.8, T-test (ns = non significantly different)

Table 1, The recovery for determination of total proteins

Albumin Conc.(g/l)	Peak height (mm)			Mean	Recovery %	R.S.D %
	1	2	3			
2	19.8	20.1	20.1	20	99.5	0.86
3	31	31	31	31	100	0.00
4	43	43	43	43	100	0.00
5	54.2	54.2	53.6	54	100.7	0.64
6	62.6	63.2	63.2	63	99.7	0.54
7	76	76	76	76	100	0.00
8	85	85	85	85	100	0.00

Table 2, FIA method and the reference method of determining Total proteins for patients

NO.	Reference Method g/l	FIA Method g/l	Recovery %	NO.	Reference Method g/l	FIA Method g/l	Recovery %
1	62	65	95.2	38	77	75	102.5
2	52	52	100	39	45	45	100
3	43	44	97.7	40	47	48	97.9
4	61	60	101.6	41	49	51	96
5	39	40	97.5	42	53	53	100
6	64	64	100	43	63	60	104.7
7	70	68	102.8	44	71	71	100
8	58	58	100	45	74	72	102.7
9	59	58	101.6	46	60	59	101.6
10	39	40	97.5	47	44	46	95.5
11	66	67	98.5	48	62	62	100
12	63	61	103.1	49	41	43	95.2
13	67	67	100	50	48	50	95.9
14	65	65	100	51	59	57	103.3
15	60	59	101.6	52	66	67	98.5
16	55	54	101.8	53	70	68	102.8
17	49	49	100	54	73	73	100
18	71	73	97.2	55	76	76	100
19	68	68	100	56	71	72	98.6
20	59	60	98.4	57	60	62	96.7
21	60	60	100	58	52	51	101.9
22	66	68	97	59	66	66	100
23	72	70	102.7	60	43	43	100
24	77	77	100	61	69	72	95.7
25	69	69	100	62	64	62	103.1
26	54	56	98.2	63	50	48	104
27	46	48	95.7	64	73	73	100
28	57	57	100	65	45	46	97.8
29	73	71	102.7	66	41	40	102.4
30	64	64	100	67	53	55	96.3
31	68	70	97.1	68	47	47	100
32	75	76	99.7	69	66	65	101.5
33	48	46	104.1	70	72	72	100
34	55	55	100	71	75	73	102.6
35	67	66	101.4	72	71	70	101.4
36	61	63	96.8	73	77	78	98.8
37	70	70	100	74	68	68	100

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تقدير البروتينات الكلية في مصل الدم البشري باستخدام نظام شبه ذاتي للجريان المستمر والمصنع محلياً

نجاح زياد حسين الحسناوي , كامل حسين علوان السوداني

جامعة البصرة , كلية التربية للعلوم الصرفة , قسم الكيمياء

الخلاصة

تم في هذا البحث تقدير البروتينات الكلية في مصل الدم البشري باستخدام نظام الحقن الجرياني المستمر المصنع محلياً . تتكون هذه التقنية من نوعين من المتحكمات الدقيقة (اردوينو) (Arduinos) المجهز ببرنامج محلي الصنع اعد لهذا الغرض . الاول نوع (يونو) (Uno) يستخدم للتحكم بتشغيل وايقاف مضخة الحقن اما الثاني نوع (ميكا) (Mega) يستخدم كمسجل بيانات (Data Logger) لتسجيل النتائج على شكل قمم بواسطة برنامج الاكسيل . تم الحصول على اعلى امتصاص للمعقد (551 nm) والخطية لمنحنى المعايرة القياسي تتراوح بين (2-8 g/l) مـــع معامل ارتباط خطي مقداره (R^2 =9986) وحد الكشف (1.0 g/l)ومعامل الانحراف القياسي (R.S.D. = 0.29 %) وكان حاصل النمذجة (120 sample/h) . الاسترجاعية (Recovery) كانت تتراوح بين (95.2- 104.7 %) للعينات التـي تم دراستها (74) عينة لمرضى المستشفى التعليمي في البصرة وكان تركيز البروتينات الكلية فيها يتراوح بيـن (40-78 g/l) وبمعدل ثـــلاث قراءات متتاليـــة لكـــل تركيز او عينـة. اثبتت الطريقة بانها بسيطة وحساسة ومناسبة وتم تطبيقها بنجاح لتقدير البروتينات الكلية في العينات المختلفة لمصل الدم البشري .

الكلمات المفتاحية : الحقن الجرياني المستمر , البروتينات الكلية , اردوينو , كاشف بايوريت ومصل الدم البشري .