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Spectrophotometric Determination of Albumin in Human Blood Serum by a Lab Built Semi-Automated Flow Injection System

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Abstract:

Determination of albumin in human blood serum by using a lab-built semi-automated flow injection analysis system which consists of two types of microcontrollers (Arduinos) supplied with a home-made software program. First one was (UNO) type used to manipulates the home-made injection-pump stepper-motor and the other one was (Mega) type which was used as (Data Logger) to record the results as a peak height by using Microsoft Excel program. The linear calibration curve was obtained under the established conditions which was in the range (0.25-3.25 g/l) with regression coefficient of (R^2 = 0.9986) and detection limit was (0.125 g/l).The R.S.D is (0.94 %(and the sample throughput was (120 sample / h) . The system was simple, sensitive, and successfully applied to determined albumin in (100) samples of human blood serum with recoveries and concentration in the range of (95.8-104.5 %) and (21.2-49.5 g/l) respectively.

Key words: lab-built, Flow injection, Arduino, albumin and human blood serum.

I. Introduction

Many methods have been used for determining albumin in serum. Flow injection analysis (FIA)was one of these various methods. Flow injection analysis (FIA) was conceived in 1975 by (Ruzicka, Hansen) [(1)]. It is has some advantages on the flow technique, such as selectivity ,simplicity, flexibility, economical, speed , ease of use, sensitivity [(2,3,4)] . and low consumption of reagent and sample with arduino micro-controllers will offer a suitable semi-automated flow injection analysis for determination of albumin in different serum samples.The arduino micro-controllers have gained considerable attention from chemists, due do not require expert knowledge, low cost and integrated development interfaces [(5,6,7)]. Two types of arduino were supplied with suitable software. the first type was (Uno) to efficient, reproducible control to the home-made injection pump stepper motor type. the other type was (Mega) which used as a (Data logger) to record the results as a peak height by using Microsoft Excel program[(8)].

TEST Engineering & Management

May – June 2020
ISSN: 0193-4120 Page No. 16732 – 16737

The Flow injection analysis are currently regarded an attractive tool for the routine analysis in clinical chemistry [9]. This method is suitable, simple and rapid for routine analysis for many samples in the ordinary clinical laboratory [10,11]. The proteins are complex organic compounds with high molecular weight it consists of amino acids found in the composition of all creatures and viruses.

That make up proteins (50 %) of the weight of the cell. The cell contains about (3000) types of different proteins. Most proteins contain five different elements, carbon hydrogen, oxygen , nitrogen and sulfur [12] . The blood is a complex mixture of cell suspended in a fluid medium,plasma. (92 %) of this fluid medium is water and the remaining (8 %) is dissolved proteins, minerals, glucose, etc.
Plasma proteins[13,14] :

1- Albumin makes up about (32-50 g/l) of the total plasma .
2- Globulins make up about (1.5-2.5 g/l) of the total plasma .
3-Fibrinogen make up about (2-4 g/l) of the total plasma .

Aims of the study

1. Determine human serum albumin with bromocresol green dye as a reagent .
2. Procedure by making a comparative and recovery studies .
3. Determine the human serum albumin in (100) serum samples of patients.

Experimental

Reagent and solutions

During analytical application with lab-build flow system deionized distilled water was used throughout and all reagents employed were analytical grade unless otherwise stated.The value of measurement as peak height was the average of three successive measurements .

Samples Collection

(100) Human serum albumin for patients' samples were kindly provided from clinical laboratories in AL-Basrah Teaching Hospital

Bromocresol green Reagent (BCG)

Bromocresol green reagent contained (0.025 g/l) of water bromocresol green (Himedia), (1 g/l) sodium hydroxide (Thomas baker), (4.9 g/l) succinic acid (Thomas baker) and (0.01 g/l) tetrabutylammonium iodide (Fluka), The solution was stable for at least one month [15] .

Standard human albumin

Solution albumin (200 g/l) (Vienna Astria) .
Prepared the required concentrations of standard albumin with continuous dilution.

Normal saline

Solution normal saline (0.9 w/v) (pioneer company/Iraq) .

Instrumentation

The lab-build flow injection analysis (FIA) system as shown in Fig. 1

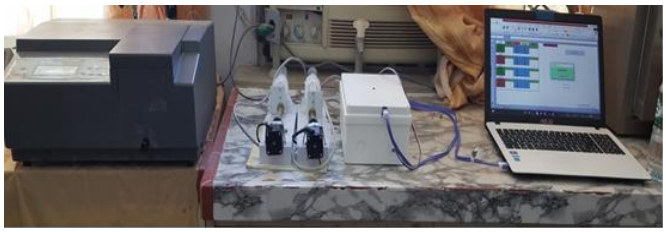

Fig.1, Shows the lab build

Procedure

The flow manifold used for determination of human serum albumin is shown in Fig.1, This method consists 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) plastic syringe with a reagent (BCG). A(100 µl) of albumin 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)

May – June 2020
ISSN: 0193-4120 Page No. 16732 – 16737

program. After that injection pump restarts to wish the flow system and restart for other samples.

Results and Discussion

The manifold of the system used for albumin determination, as shown in Fig. 2, was used to optimize the variables by carrying out a series of experiments to establish the optimum analytical conditions that influence the peak height.

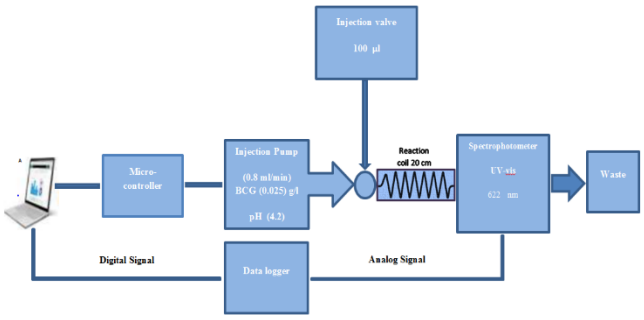

Fig. 2, Manifold of the Flow System (FIA)

Study of the complex spectrum

Complex spectrum was drawn and the highest absorption value was found at wavelength (622 nm)[16]. Which was used in all subsequent experiments.

Effect of Flow Rate

When bromocresol green reaction with albumin the peak height decreases with increasing flow rate which thought to be due to lack of complex formation [17]. A (0.8 ml/min) flow rate was chosen for subsequent work.

Effect of Bromocresol green concentration (BCG)

The peaks are distorted at concentrations less than and above (0.025 g/l) . which thought to be due to the complex is not formed at low concentrations and turbidity in high concentrations, respectively [18] .therefore this concentration of BCG was used in subsequent experiments.

Effect of Succinic acid concentration

The peak height increases with increasing acid concentration up to (4.9 g/l). after that , the peaks height begin to decline. Which thought to be due to the pH higher than the appropriate value to albumin determination (pH= 4.2)[18]. So (4.9 g/l) was used in the subsequent work .

Effect of Sodium hydroxide concentration

When increasing the concentration of sodium hydroxide, the peak height decreases . which thought to be due to the value of the pH installed (4.2) changes to base values[18] . therefore (1 g/l) was chosen for subsequent work .

Effect of Tetrabuttylammoniumiodide concentration (TBAI)

There is no effect to concentration this substance on the peak height . and this material is added for the purpose of cleaning the pipe walls[18] A (0.01 g/l) TBAI was used in the subsequent work .

Effect of the sample volume

When increasing the sample size we get distorted peaks . because the reaction between the albumin and reagent is very sensitive and requires a small size of the sample[19] so (100 µl) was used to inject in subsequent work .

Effect of the reaction coil length

The results showed that increasing these coil length simply leads to more dispersion and thus leads to decreasing the peak height [20] . therefore (20 cm) was chosen for subsequent work .

Effect of the pH

The influence of pH by adding drops of nitric acid or sodium hydroxide to the original solution (pH 4.2) for the purpose of reducing or increasing the pH respectively. where the study was within the range (3.8-4.7)[21] . the best value to pH is (4.2) . it gives clear and sharp peaks . the pH value, (4.2) was used in the subsequent work [22] .

Calibration Curve

May – June 2020
ISSN: 0193-4120 Page No. 16732 – 16737

Under the established conditions , as shown in Table 1, It is linear over the range (0.25-3.25 g/l) . the linear curve has a regression coefficient (R^2 =0.9986) and detection limit was (0.125 g/l) . the R.S.D. was (0.94 %) and the sample throughput was (120 sample / h) . the value of R.S.D. for reproducibility obtained was (0.55 %) .Fig. 3

Recovery study

Table 2. shows the recovery to the determination of human serum albumin . the flow injection analysis (FIA) used to the determination albumin in serum was good.

Application

The proposed method using the home-made flow injection system was applied for the determination of human serum albumin in (100) serum samples of patients . the obtained results are listed in Table 3, this is results obtained by using the addition standard method[23] . the linear correlation (R^2= 0.940) Fig.4, Which clearly indicated that satisfactory results have been obtained by both methods .

Conclusion

1. The results can be self-obtained through the use of micro -controllers, an injection pump , and self-control to stop the reaction and restart .
2. The determination of human serum albumin by the home-made flow injection system is superior compare with other conventional methods .
3. It is low cost , simple , sensitive and high samples throughput .
4. This method decreases the possibility of the interferences of other proteins on the measurement of human serum albumin.

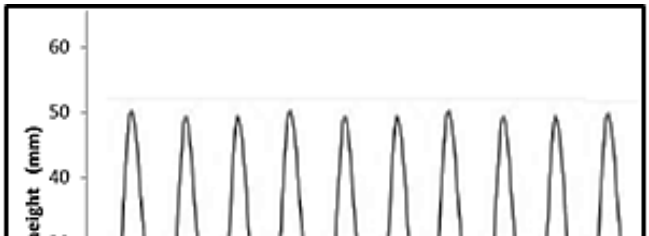

Fig. 3,The reproducibility of height peak of (1.25 g/l)

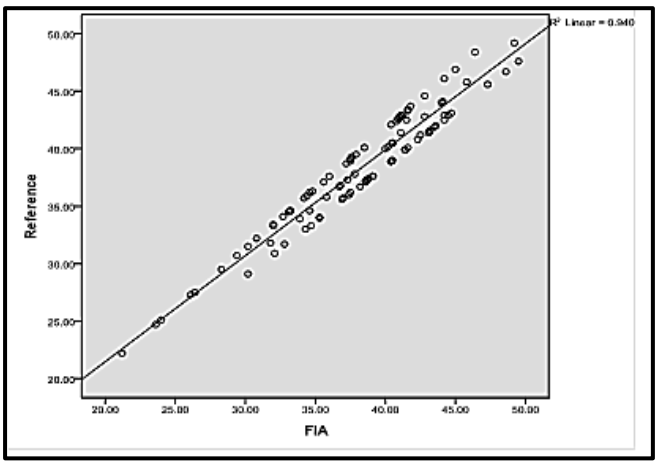

Fig. 4, The linear correlation between FIA technique and reference (hospital) for albumindetermination.

Table 1, The optimum conditions for determination of Human Serum Albumin

Parameter	Value
Wavelength	622 nm
Flow rate	0.8 ml/min
Bromocresol green concentration	0.025 g/l
Succinic acid concentration	4.9 g/l
Sodium hydroxide concentration	1.0 g/l
Tetrabutylammonium iodide concentration	0.01 g/l
Sample volume	100 µl
Cell volume	450 µl
Reaction coil length	20 cm
pH	4.2

May – June 2020
ISSN: 0193-4120 Page No. 16732 – 16737

Table 2, The recovery for determination of HumanSerum Albumin

Albumin conc. (g/l)	Peak height (mm)			Mean	Recovery %	R.S.D %
	1	2	3			
0.25	8	8	8	8	100	0.00
0.75	30	31.5	31.5	31	98.4	2.79
1.25	50	50	50	50	100	0.00
1.75	78	78	78	78	100	0.00
2.25	101	101	104	102	100.9	1.69
2.75	121.5	121.5	126	123	97.6	2.11
3.25	145	145	145	145	100	0.00

Table 3, FIA method and the reference method of determining Human Serum Albumin for patients

NO.	Reference Method g/l	FIA method g/l	Recovery %
1	39.9	41.4	96.3
2	42.9	44.2	97
3	44.1	44.1	100
4	33.3	32	103.9
5	37.4	38.8	96.3
6	29.5	28.3	104
7	36.3	34.8	104.1
8	40.2	40.2	100
9	31.7	32.8	96.6
10	34.6	33.2	104
11	43.3	41.6	103.9
12	39.5	37.9	104
13	42.8	42.8	100
14	38.9	37.5	103.5
15	47.6	49.5	96.1
16	41.4	43.1	95.9
17	41.2	42.5	96.9
18	35.7	34.2	104.2
19	34.1	32.7	104.1
20	27.3	26.1	104.3
21	34.6	33.2	104
22	38.7	37.2	103.8
23	37.6	36	104.2
24	31.8	31.8	100
25	39.9	41.4	96.3
26	34.5	33.1	96
27	45.8	45.8	100
28	46.7	48.6	96
29	45.6	47.3	96.3
30	36.2	37.5	96.5
31	41.5	43.1	96.2
32	40.1	41.6	96.3
33	33.9	33.9	100
34	43.1	44.7	96.3
35	33.4	32	104.1
36	42.5	41.5	102.3
37	35.8	35.8	100
38	34	35.3	96.2
39	35.6	36.9	96.4
40	42.9	44.5	103.7

NO.	Reference Method g/l	FIA Method g/l	Recovery %
41	24.7	23.6	104.4
42	38.9	40.4	96.2
43	46.9	45	104
44	41.6	43.2	96.2
45	36.7	36.7	100
46	22.2	21.2	104.5
47	36	37.4	96.2
48	41.9	43.5	96.2
49	37.1	35.6	104
50	34.6	34.6	100
51	46.1	44.2	104.1
52	32.2	30.8	104.3
53	40.1	38.5	103.9
54	27.5	26.4	104
55	42.1	40.4	104
56	42.7	41	103.9
57	42	43.6	96.2
58	48.4	46.4	104.1
59	37.3	37.3	100
60	42.5	44.2	96
61	42.6	40.9	103.9
62	49.2	49.2	100
63	44.6	42.8	104
64	37.8	37.8	96.1
65	40.8	42.3	96.4
66	30.9	32.1	96.2
67	40	40	100
68	35.9	34.4	104.1
69	33	34.3	96.1
70	41.4	43.1	95.9
71	42.4	40.8	103.7
72	43.7	41.8	104.3
73	37.6	39.1	96.1
74	37.2	38.6	96.3
75	42.9	41.1	104.1
76	37.2	38.6	96.3
77	40.5	40.5	100
78	37.6	39.1	96.1
79	39.1	37.5	104
80	31.5	30.2	104.1

May – June 2020
ISSN: 0193-4120 Page No. 16732 – 16737

81	42.9	41.2	103.9
82	42.8	41	104.2
83	39.3	37.6	104.3
84	41.4	41.1	100
85	25.1	24	104.3
86	35.7	37	96.4
87	36.7	38.2	96
88	37.3	38.7	96.3
89	37.1	38.6	96
90	40.5	40.5	100
91	29.1	30.2	96.3
92	34	35.3	96.2
93	39	40.5	96.2
94	44	44	100
95	43.4	41.6	104
96	36.2	34.6	104.4
97	38.9	40.4	96.2
98	33.3	34.7	95.8
99	30.7	29.4	104.2
100	36.8	36.8	100

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