SPECTROPHOTOMETRIC DETERMINATION OF HUMAN SERUM ALBUMIN BY A HOME-MADE SEMI-AUTOMATED FLOW INJECTION SYSTEM

K.H. Al-Sowdani*, Abbas D. Al-Maliki and Safaa S. N. Al-Omran
Chemistry Department - College of Education
Basrah University
ISSN –1817 –2695
((Received 27/4/2008 , Accepted 21/12/2008))

Abstract

The bromcresol green method was applied for spectrophotometric determination of albumin by using a home-made semi-automated flow injection in some pharmaceutical preparation with recoveries of 98.5-102 % and 54 serum samples of male and female patients in the range 32.48-51.25 g/L.
The method is convenient, simple and sensitive with detection limit (2x noise) 0.625 g/L. The r.s.d % is 0.74 % for 8 measurements of 40 g/L albumin.

Keywords: FIA, Spectrophotometric, bromcresol green, albumin, pharmaceutical,serum.

Introduction

In human, albumin is the most abundant plasma protein, accounting for 55-60 % of the measured serum protein [1]. The normal range for albumin concentration in blood is 32-50 g/L with optimal adult value 41 g/L [2]. Many methods have been reviewed for determining albumin in serum [3] among these various dye-binding methods such as 2-(4-hydroxyazobenzen) benzoic acid [4] methyl orange [5], bromcresol purple [6] and bromcresol green [7].
The bromcresol green method has been investigated by several authors. Its specificity is better than any another commonly used methods. This method is simple, rapid, and suitable for routine analysis for many samples in the ordinary clinical laboratory [8-12].

Flow techniques are currently regarded an attractive tool for the routine analysis in clinical chemistry [13]. Flow injection analysis has some advantages on the flow technique, such as sensitivity, speed, ease of use and use of simple instrumentation [14-15]. The aim of this work was to determine human serum albumin (HSA) with bromcresol green dye as a reagent by adapting Renoe et.al [11] method with a home-made semi-automated flow injection system and evaluated the accuracy of this system and procedure by making a comparative and recovery studies with a manual spectrophotometric method and determine the human serum albumin in 54 serum samples of male and female patients.

Experimental

Reagents and solutions

All reagents were analytical grade unless otherwise indicated. Deionized, distilled water was used throughout this work.
Human Serum Albumin (HSA) 20% (Biotest pharma Gmbh) was used as a stock solution. This solution was standardized with Bovin albumin 50 g/L (Biomaghreb kit) by following the manual spectrophotometric method [16].
The working and the standard solutions were obtained by step-wise dilution by normal saline (0.9 % w/v sodium chloride, pharmaceutical solution, Industry Ltd, KSA), also this normal saline solution was used as a carrier stream.

* Corresponding author

Bromcresol Green Reagent (BCG)

This reagent contained 0.025 g per liter of water bromcresol green (Fluka), 1 g sodium hydroxide (BDH), 4.9 g succinic acid (BDH) and 0.01 g tetrabutyl ammonium tetra flouroborate (BDH).This solution was stable for at least one month [17].

Biomaghreb Kit

The Biomaghreb kit consists of 50 g/L standard bovine albumin and BCG reagent (0.14 g/L BCG, 75 ml/L succinate buffer and 7 ml/L Brij-35)[16].

Samples Collection

54 HSA for male and female patients' samples were kindly provided from clinical laboratories in Al-Basrah General Hospital and Al-Basrah Maternity and Gynecological Hospital in Basrah province in June 2007 and all measurements were done in the same day of collection.

Instrumentation

All flow injection measurements were done by using a home-made semi-automated FIA system as shown in Fig.1, which was previously described [18].

Procedure

The flow manifold used for HSA determined is shown in Fig.2. The normal saline and BCG solutions were pumped at 2.30 ml/min and 0.23 ml/min respectively. A 20 µL of sample was injected manually in the injection valve. Two coils of 50 cm length were inserted for diluting the injected sample and reaction between the diluted sample and BCG in the flow system. The absorbance of the formed complex was measured at 600 nm by a spectrophotometer equipped with a flow cell. The recorded peak height can be related to the sample concentration.

Results and Discussion

The home made semi-automated FIA system, 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.

Effect of BCG concentration

The influence of the complexing agent concentration on the peak height increased with the concentration of BCG which thought to be due to increasing of complex formation as shown in Fig. 3. The peak height decreased above 0.025 g/L, therefore this concentration of BCG was used in subsequent experiments.

Effect of Flow Rate

Fig. 4 shows the effect of the total flow rate on the peak height. The reaction between the albumin and BCG was found to be an instant when stopped flow method was used. Fig.4 shows the peak height of 40 g/L HSA decreases with increasing flow rate which thought to be due to dispersion of the formed complex. A 2.53 ml/min total flow rate was chosen for subsequent work in order to achieve good reproducibility and rapid analysis.

Effect of Sample Volume

The effect of sample volume is shown in Fig.5. The maximum peak height was obtained by injection of 100 µL, but the peak was somewhat distorted. A small sample volume was recommended to maintain a linear working range due to the high sensitivity of BCG method [12]. Also to avoid the problems arise from using large sample volume of albumin such as blocking the micro-ports of the injection valve. So 20 µL was used to inject in subsequent work.

Effect of Dilution and Reaction Coils

Effect of inserting various lengths of dilution and reaction coils into the manifold is shown in Fig.6. The results showed that increasing these coil lengths simply leads to more dispersion in the flow systems. A 50 cm coil was chosen for subsequent work for both coils.

Effect of pH

The influence of pH by using of succinic buffer solutions [19] in the range 3.8-4.2, which is recommended for BCG method [7] on the peak height for 40 g/L HSA, is shown in Fig.7. The peak height decrease with increasing the pH value, 3.8 was used in the subsequent work. This buffer provides better linearity and sensitivity than other buffers [20].

Effect of the Surfactant type and concentration

Fig.8 shows the effect of four types of surfactants on the peak height with injection of 40 g/L HSA. There is no significant effect has been noticed between the surfactants. So, tetrabutyl ammonium tetra flouroborate was used. Fig.8 shows also the influence of the concentration of that surfactant on the peak height of 40 g/L HSA. The peak heights were increased with increasing HSA concentration up to 0.01 g/L, which thought to be due to the effect of surfactant in removing the unreacted reagent as well as reducing the non-specific background level [21].

Calibration Curve

Under the established conditions, as shown in Table1, a calibration curve for HSA was obtained (Fig.9). It is linear over the range 10-60 g/L HSA. The linear curve has a regression coefficient 0.9992, the detection limit (2 x noise) was 0.625 g/L, and the r.s.d % for eight replicates was 0.74%. The dispersion coefficient in flow system was 1.64 and the sample throughput was 120 sample / h.

Accuracy

In order to establish the validity and the accuracy of the home-made FIA system for the determination of albumin in drug and representative samples were examined by using the standard addition method listed in Table 2. The same batch of samples was analyzed by the classical manual method [16], also the recoveries and relative standard deviation were calculated as shown in Table 2.

The results have showed that satisfactory accuracy was obtained.

Application

The proposed method using the home-made flow injection system was applied for the determination of HSA in 54 serum samples of male and female patients. The obtained results are listed in Table 3 which clearly indicated that satisfactory results have been obtained by both methods. The concentrations of HSA in all samples were in normal range, except female's sample, No. 4 and 7.

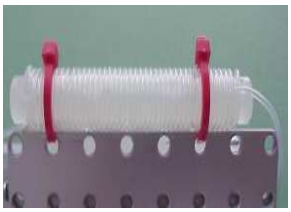
Conclusion

The determination of HSA by the home-made semi-automated flow injection system is superior compare with other conventional methods. It is simple, low cost and high samples throughput. In addition, this method decreases the possibility of the interferences of other proteins on the measurement of HSA. The total reaction time was 14 sec [11] which is minimizing the effect of reactions between the BCG and other proteins.

(a)

(b)

(c)



(d)

(e)

(f)

(g)

Fig.1 (a) Home-made semi-automated flow injection system [18].
(b) Peristaltic pump
(c) Injection valve
(d) Teflon reaction or dilution coil
(e) Spectrophotometer
(f) Flow cell with modified cell holder and top cover
(g) Recorder

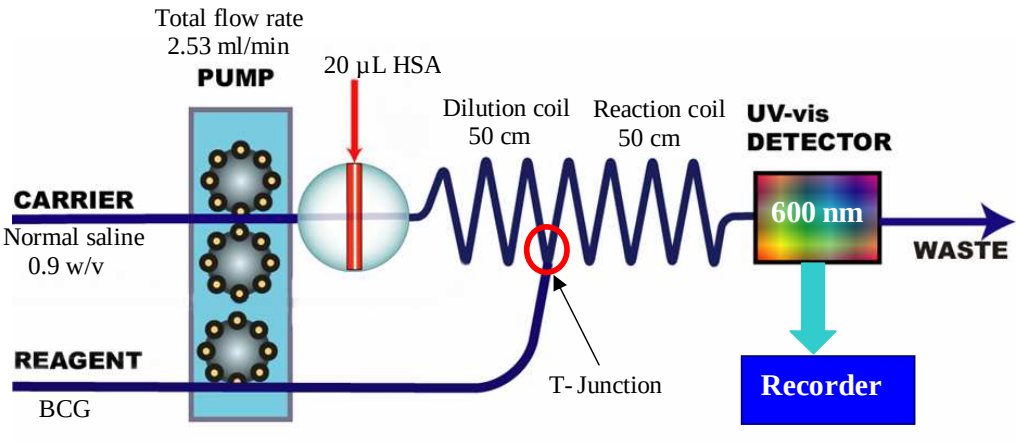


Fig.2 Dual-stream manifold for determination HSA

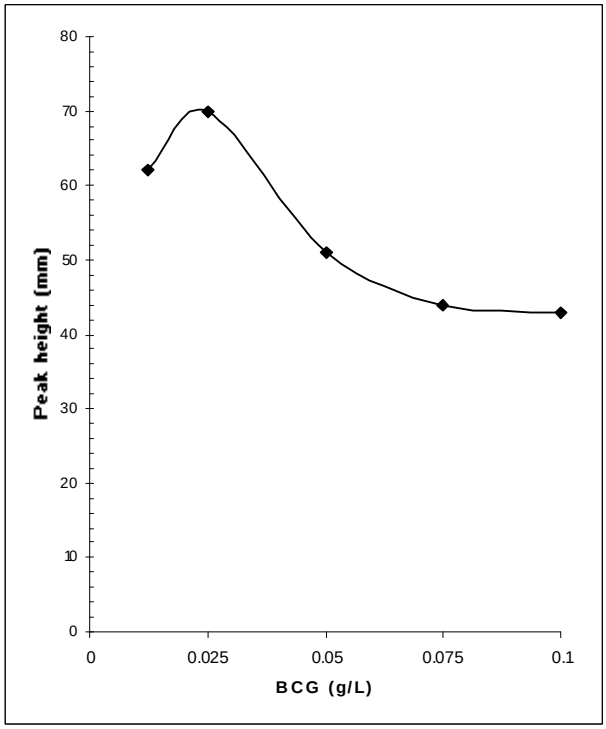


Fig.3 Effect of BCG concentration on the peak height for 40 g/L human serum albumin

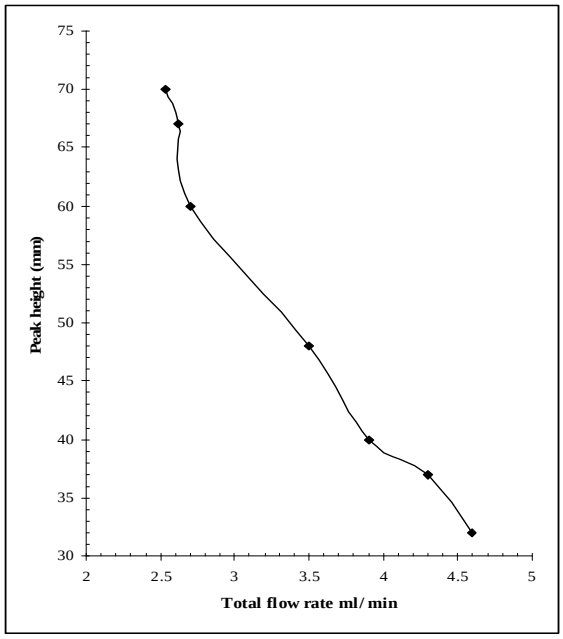


Fig. 4 Effect of the total flow rate on the peak height for 40 g/L human serum albumin

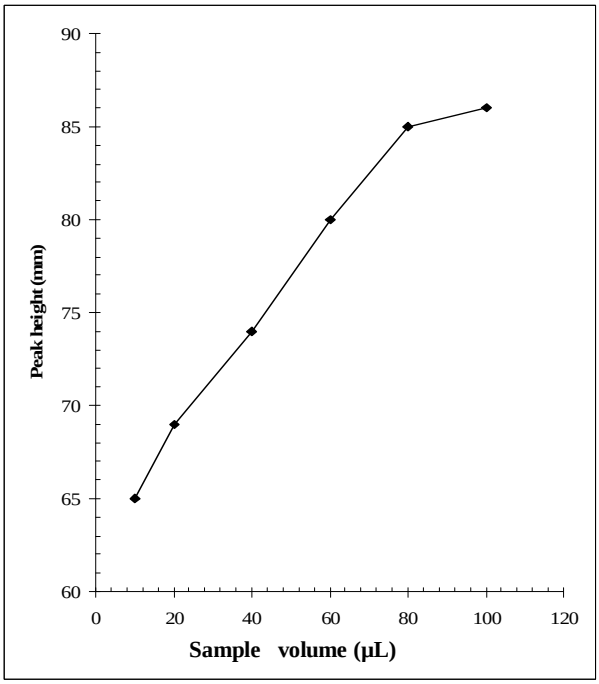


Fig. 5 Effect of sample volume on peak height for 40 g/L human serum albumin

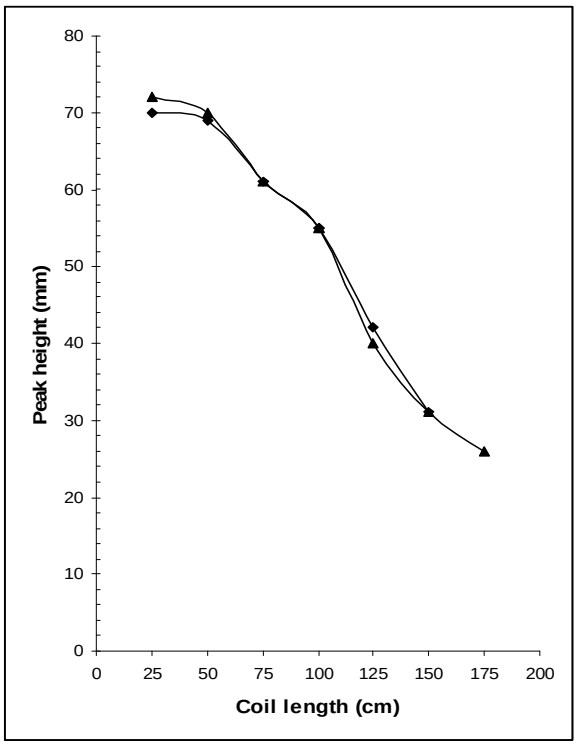


Fig. 6 ■ Effect of the dilution coil length and ▲effect of the reaction coil length on the peak height for 40 g/L human serum albumin

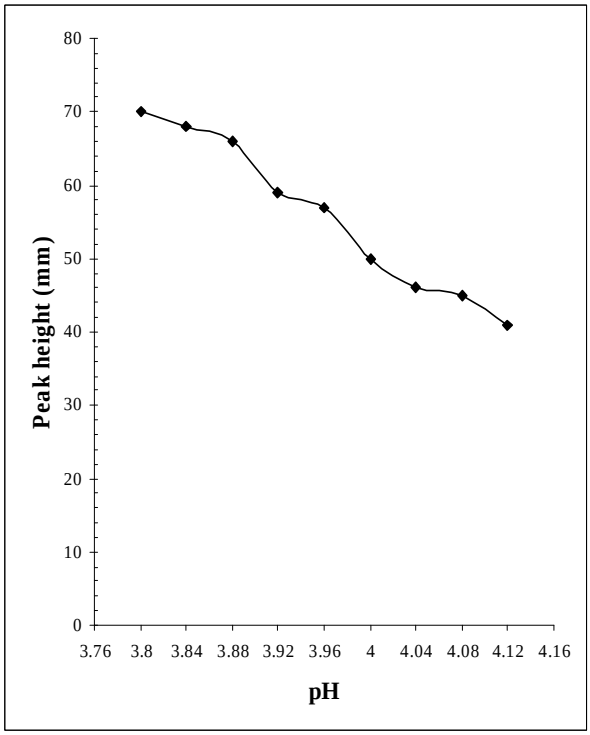


Fig. 7 Effect of pH on the peak height for 40 g/L human serum albumin

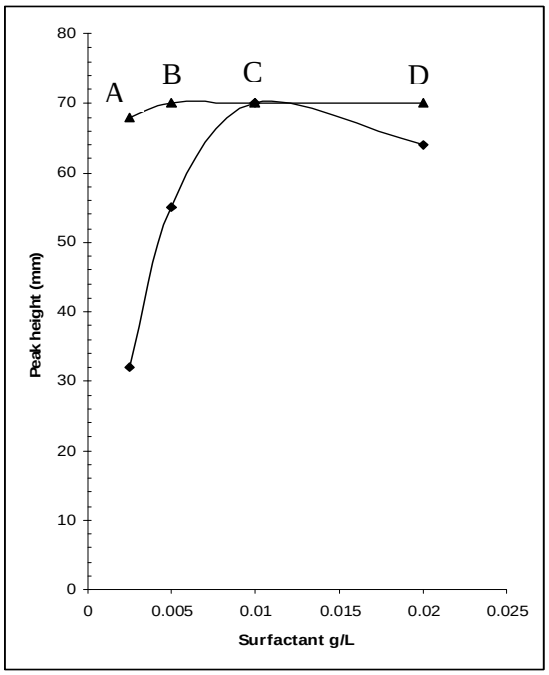


Fig. 8 ▲Effect of the surfactants type and ■ the concentration of surfactant (D) on the peak height for 40 g/L human serum albumin.

A (Tetra butyl ammonium iodate)
B (Tetra ethyl ammonium iodide)
C (Tetra butyl ammonium perchlorate)
D (Tetra butyl ammonium tetra flouro Borate)

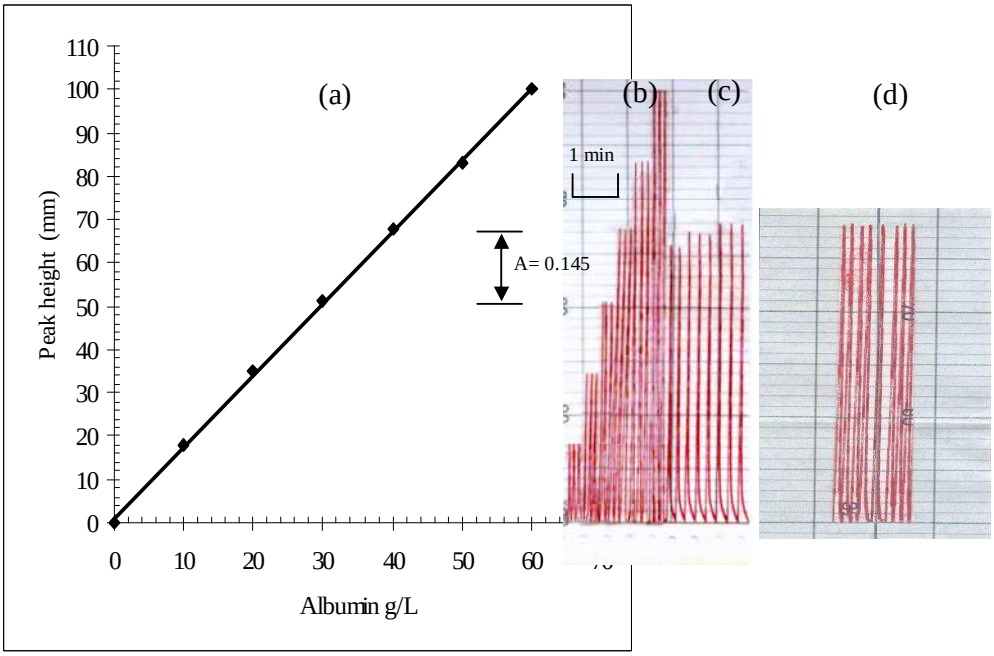


Fig. 9 (a) Corresponding calibration graph of HAS
(b) Peaks obtained by injected HSA standards in the concentration range shown above
(c) Some HSA patient peaks
(d) 8 replicate peaks of 40 g/L standard HSA

Table 1 The optimum conditions for determination of Human Serum Albumin

Parameter	Value
Total flow rate	2.53 ml/L
Sample volume	20 µL
Dilution coil length	50 cm
Reaction coil length	50 cm
λmax	600 nm
Bromcresol green concentration	0.025 g/L
Succinic acid concentration	4.9 g/L
Sodium hydroxide concentration	1 g /L
Surfactant (tetra butyl ammonium tetra flouro borate) concentration	0.01 g /L
r.s.d %	0.74 %
Sample throughput	120 sample / h
Dispersion coefficient HO / Hmax	1.2
pH	3.8
Detection limit (2 x noise)	0.625 g/L

Table 2 Determination of Albumin in drug and representative samples using the addition standard method

Sample	Claimed or added µg/ml	Found	Recovery ± r.s.d % ❖
• Sample 1	20	20	100 ± 0.74
† Sample 2	40	40.8	100.2 ± 0.72
† Sample 3	60	59.2	98.66 ± 0.70

- HSA (Biotest pharma Gmbh)

† Representative samples

❖ Three replicates

Table-3 FIA method vs. the reference method [16] for determination of HSA in serum of male and female patients

Male Patient No.	**FIA** method g/L	**Reference** method g/L
1	42.82	43.04
2	44.76	44.72
3	44.36	44.37
4	47.39	47.35
5	40.26	40.25
6	47.50	47.52
7	42.57	42.55
8	42.86	42.88
9	41.32	41.31
10	43.49	43.5
11	44.06	44.04
12	44.72	44.71
13	43.51	43.50
14	45.96	45.98
15	42.73	42.70
16	41.40	41.43
17	44.25	44.24
18	41.85	41.88
19	48.86	48.86
20	40.67	40.69
21	40.05	40.04
22	43.83	43.80
23	40.02	39.69
24	42.25	42.23
25	46.33	46.34
26	40.70	40.69
27	42.85	42.83

Female Patient No.	**FIA** method g/L	**Reference** method g/L
1	35.25	35.26
2	37.74	37.73
3	45.19	45.20
4	33.01	32.99
5	46.92	46.94
6	42.47	42.46
7	32.48	32.5
8	43.46	43.44
9	39.16	39.14
10	41.72	41.75
11	51.25	51.21
12	39.81	39.85
13	46.69	46.70
14	44.34	44.32
15	40.8	40.78
16	40.6	40.58
17	44.39	44.40
18	38.56	38.55
19	43.89	43.90
20	36.36	36.35
21	37.76	37.75
22	43.16	43.18
23	42.23	42.19
24	45.12	45.15
25	42.8	42.77
26	38.65	38.62
27	40.57	40.54

References

[1]. P. Gosling. *Albumin and the critically ill*. Care Crit III, II: 57-61(1995).

[2]. Ferret Universe (www.ferretuniverse.com), copyright (2007).

[3]. J. P. Nicholson, *M. R . Wolmarans and G. R. Park, The roles of albumin in critical illness*, British Journal of Anaesthesia 85 (4): 599-610 (2000)

[4]. A. T. Ness, H. C. Dickerson, and J. V. Pastewka, *The determination of human serum albumin by its specific binding of the anionic dye, 2-(4'- h* hydroxy benzeneazo) *-benzoic acid.* Clin. Chim. Acta 12,532 (1965).

[5]. L. V. Crowley, *An evaluation of serum protein fractionation using a methyl orange method for determination of albumin*. Clin. Chem. 10, 1131 (1964) .

[6]. F. Rocks Bernard, M. Wartel Scott, A. Sherwood Roy and C. Riley, *Determination of albumin with bromcresol purple using controlled- dispersion flow analysis*. Analyst. 110, 669-671(1985).

[7]. F. L. Rodkey, , *Direct spectrophotometric determination of albumin in human serum.* Clin. Chem. 11,478 (1965)

[8]. D. Webster, A. H. C. Bignell, and E. C. Attwood, *An assessment of the suitability of bromocresol green for the determination of serum albumin*. Clin. Chim. Acta 53,101 (1974).

[9]. J. Gustafsson, *Improved Specificity of Serum Albumin Determination andEstimation of "Acute Phase Reactants" by Use of the Bromcresol Green Reaction.* Clin. Chem. 22/5, 616-622 (1976) .

[10]. B.T. Doumas, W.A Watson and H.G. Biggs, *Albumin standards and the measurement of serum with bromcresol green*.Clin Chim Acta :31; 87-96 (1971).
[11]. B.W. Renoe, K.K. Stewart, G.R. Beecher, M.R. Wills and J. Savory, *Automated multiple flow-injection analysis in clinical chemistry: Determination of albumin with bromcresol green*. Clin. Chem. 26/2, 331-334 (1980)
[12]. H. Von Schenck, and K. Rebel, Improved continuous-flow determination of albumin with bromcresol gree.Clin.Chem., 28, 1408 (1982)
[13]. M. Miro and W. Frenzel, Encyclopedia of Analytical Science, 2 nd edn (Eds A. Townshend, P. J. Worsfold, and C. Pool, Academic press, Elsevier, Netherlands (2005).
[14]. M. Trojanowicz, *Flow Injection Analysis, Instrumentation and Applications*, World Scientific Ltd., Singapore, (2000).
[15]. J. Ruzicka, *Flow Injection Analysis*, CD-ROM tutorial, 3rd edn (2005).
[16]. Biomaghreb Kit Tunisia
[17]. O. J. Westgard and A. M. Poquette, Clin. Chem. 18, 7 (1972).
[18]. Safaa S. N. Al-Omran, MSc. Thesis, University of Basrah, (2007).
[19]. D.D. Perrin and B. Demposey, Buffer for Ph and Metal Ion Control, Chapman and Hall, London (1974).
[20]. B. T. Doumas, W.A. Watson and H.G. Biggs, *Albumin standards and the measurement of serum albumin with bromcresol green*. Clin Chem. 16, 537 (1970).
[21]. www.sigmaaldrich.com

التقدير الطيفي للآلبومين في مصل الدم البشري
بأستخدام نظام حقن جرياني محلي الصنع شبه آلي

كامل حسين السوداني ، عباس دواس المالكي ، صفاء صبري نجم العمران
قسم الكيمياء – كلية التربية
جامعة البصرة

الخلاصة:

تم التقدير الطيفي للآلبومين بطريقة بروموكريسول الأخضر بأستخدام جهاز تحليل حقن جرياني محلي الصنع في المستحضرات الصيدلانية و بأستعادية 98.5– 102% وكذلك في مصل دم الذكور و الآناث بمدى 32.85–51.25 غم/ لتر. اثبتت الطريقة بأنها مناسبة و بسيطة و حساسة و بحد كشف 0.625 غم / لتر و انحراف معياري قياسي 0.74 % لثماني قراءات من محلول 40 غم / لتر البومين.