New Approach for the On-Line Spectrophotometric Determination of Folic Acid in Pure and Pharmaceutical Preparation via Oxidation by Cerium(IV) Sulphate Using Ayah 3Sx3-3D-Solar Cell CFI Spectrophotometer Analyzer

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Abstract

A newly photometric analytical method characterized by its speed and sensitivity was developed for the determination of folic acid in pure and pharmaceutical samples via its oxidation to reddish-orange coloured complex through oxidation by cerium (IV) sulphate in aqua medium using homemade Ayah 3Sx3-3D-solar cell CFI photometer. The colored species were determined using supper bright green light emitting diode (LED) as a source. A 100µl was taken as a best sample volume for the determination of folic acid. The linearity of calibration curve for the instrument response versus folic acid concentration was 0.005-20 mmol.L^{-1} while the L.O.D. was 0.5 µ mol.L^{-1} from the stepwise dilution for the minimum concentration of lowest concentration in the linear dynamic range of the calibration graph. The correlation coefficient (r) was 0.9994 while the percentage linearity (r^2%) was 99.88%. The method was applied successfully for the determination of folic acid in pharmaceutical preparation. RSD% for 8 mmo.L^{-1} (n=6) less than 1% . Using paired t-test it was shown that there was no significant difference between the proposed method and official method (Quoted value- 5mg) and on that basis the new method can be accepted as an alternative analytical method.

keywords: Flow injection analysis , Folic acid , Spectrophotometer , Colorimetry

نمط جديد للتقدير الاني الطيفي لحامض الفوليك في المستحضرات الصيدلانية والنقية من خلال الاكسدة بكبريتات السيريوم (IV) بأستخدام المحلل الطيفي للحقن الجوياني المستمر Ayah 3Sx3-3D-solar cell

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الخلاصة:

طورت طريقة تحليلية طيفية سريعة وحساس ة لتقدير حامض الفوليك في المستحضرات الصيدلانية والنقية من خلال الاكسدة إلى معقد ملون برتقالي –محمر بوساطة اكسدته بكبريتات السيريوم (IV) في الوسط المائي بأستخدام منظومة الحقن الجرياني المستمر والمصممة محليا Ayah 3Sx3-3D-solar cell .قدرت القطع الملونة باستخدام ثنائي وصلة باعث للضوء الاخضر الساطع كمصدر تم اخذ 100 مايكروليتر كأفضل حجم لانموذج محقن لتقدير حامض الفوليك . الخطية لمنحني المعايرة للاستجابة الالية ضد تركيز حامض الفوليك 0.005–20 مللي مول.لتر$^{-1}$ بينما حد الكشف 0.5 مايكرومول.لتر$^{-1}$ من التخفيف التدريجي

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لاقل تركيز في منحني المعايرة . معامل الارتباط (r) 0.9994 بينما نسبة الخطية (%r^2) 99.88% . طبقت الطريقة بنجاح لتقدير حامض الفوليك في المستحضرات الصيدلانية . الانحراف القياسي النسبي المئوي لتركيز 8 مللي مول. لتر−1 (n=6) اقل من 1% . بأستخدام اختبار −t المزدوج تبين انه لايوجد فرق جوهري بين الطريقة المقترحة والطريقة الرسمية (القيمة الموثقة − 5 ملغم) وعلى هذا الاساس بالامكان قبول استخدام الطريقة المستحدثة كطريقة تحليلية بديلة.

1-Introduction :

Folic acid (FA) chemically: (s)-2-(2-((2-amino-4-hydroxy pteridine-6-yl) methyl amino) benzamido) pentandioic acid [1] one of the water soluble B vitamins. It is degraded in aqueous solution by sunlight, ultraviolet and visible light. In aqueous solution folic acid is stable at 100°C for 10h in a PH range of (5-12) when protected from light, but becomes increasing unstable as the PH decreases below 5. Under neutral to alkaline conditions, folic acid displays the increased stability of alkali hydroxides and carbonates as well as a limited number of organic solvents. It has little native fluorescence, it can be turned into a strongly fluorescene compound by oxidation [2].

Folic acid is made up of bicyclic pterine linked by a methylene bridge to para-amino benzoic acid, which is joined by peptide linkage to a single molecule of L-glutamic acid as shown in figure-1. [3]

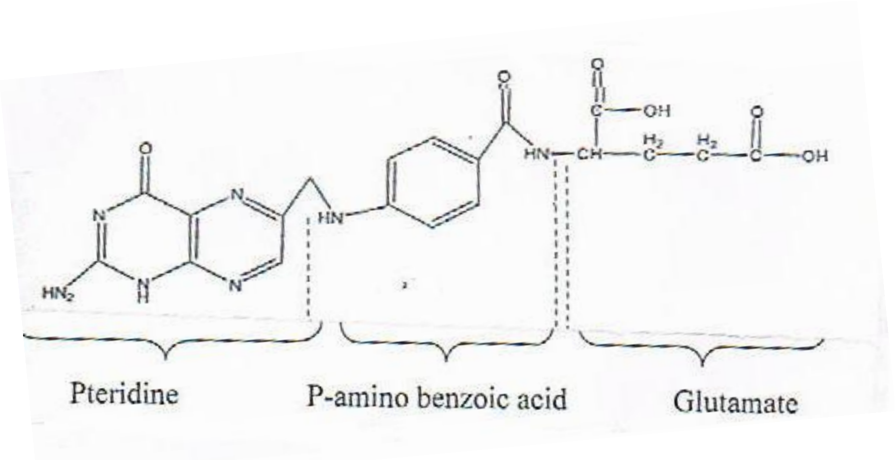


Figure 1- The structure formula of folic acid

The various coenzyme of folic acid facilitate the transfer of one-carbon units from donor molecule into important biosynthic pathways leading to methnionine, purine and pyrimidine biosynthesis [4].Folic acid is added to many food products to prevent folate deficiency in individuals. A deficiency of folate in the diet is closely linked to the presence of neural tube defects in new borns and to an increase rise of megaloblastic anemia, cancer, alzheimer's disease and cardiobascular disease in adults [5].Many methods have been reported for the determination of folic acid in pharmaceuticul formulations and biological fluids; including:Spectrophotometry by coupling reaction [6], liquid chromatography [7], reversed-phase liquid chromatography [8], electrodeposited gold nanoparticles [9], Ion-pair RP-HPLC[10], HPLC [11], and Chemiluminescence using Luminol-OH^- -folic acid system [12].

In this present work, used a FI spectrophotometric procedure for the determination of folic acid based on it is oxidation with cerium (IV) sulphate in acidic media is described. This procedure is involved using a homemade [13] FI photometer which is equipped with three different light emitting diode: [blue (470 nm), green (525 nm) and red (635nm) as a sources and solar cell as a detector .The proposed method was checked by analyzing commercial pharmaceutical preparations.

2- Experimental:

- Chemicals

All chemicals used were of analytical reagent grade. Distilled water was used to prepare all the solutions. Standard solution of folic acid ($C_{19}H_{19}O_6N_7$), Hopkins & Williams, M.wt= 441.403g.mol^{-1}, 10 mmol.L^{-1}) was prepared by dissolving 2.20710 gm of folic acid in a small quantity of sodium carbonate solution to affect complete dissolution and the volume was transferred to 500ml from distilled water. A stock solution of Ce (IV) sulphate (0.1 mol.L^{-1}, Hopkins & Williams) was prepared by dissolving 16.614 gm from $Ce(SO_4)_2$ in 500 ml of sulphuric acid . 1mol L^{-1} from H_2SO_4 (18 mol.L^{-1}, BDH, 1.84 g.ml^{-1}, 96%) was prepared by diluting 27.7ml of H_2SO_4 in 500 ml distilled water (standarized against Na_2CO_3 solution). Sodium carbonate (anhydrous): 10.599gm of Na_2CO_3 in 1L distilled water.

- Apparatus and Manifold:

The flow injection system used for the determination of folic acid as shown in figure-2.

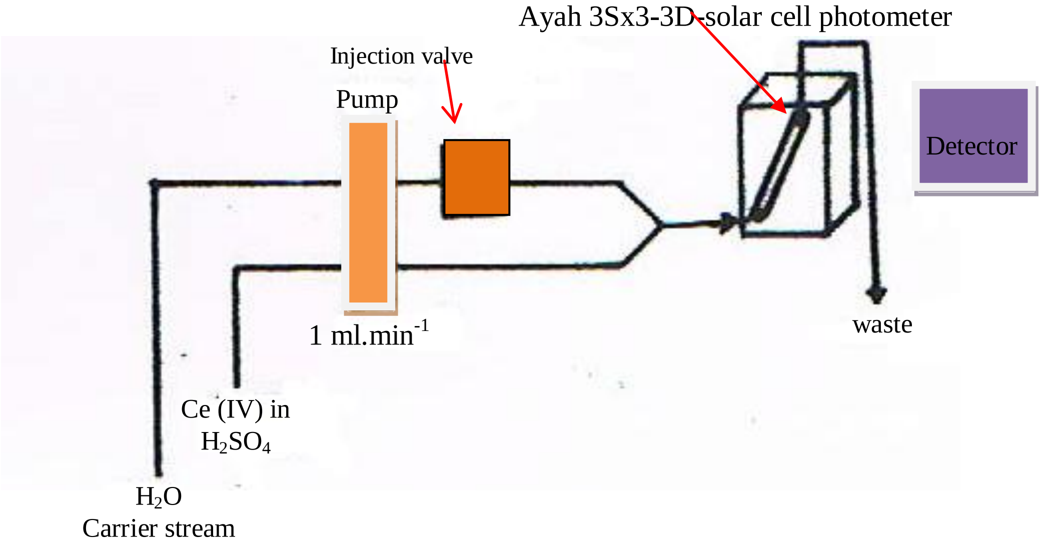
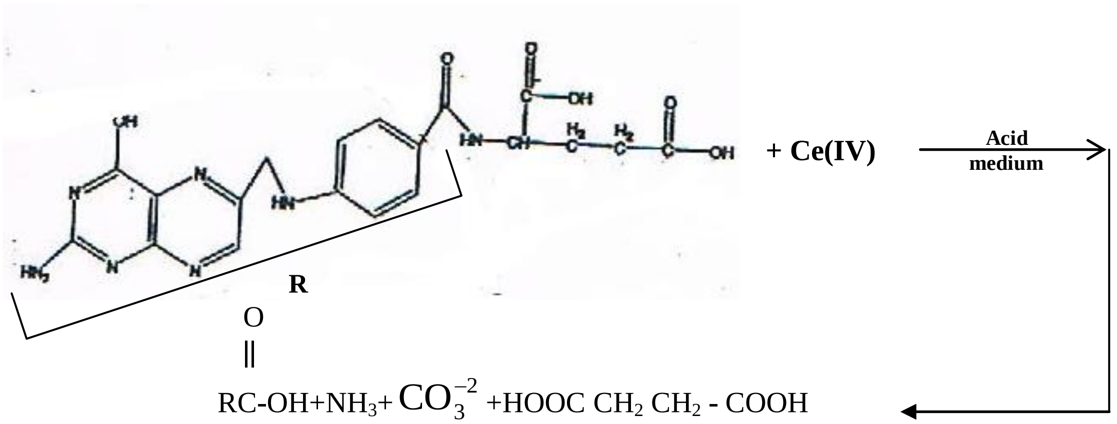


Figure 2- Schematic diagram of flow injection analysis system for determination of folic acid.

Which composed from a peristaltic pump: four channels, variable speed (Ismatic, USA), 6-port medium pressure injection valve (IDEX corporation, USA) with a sample loop (0.7 and 0.5 mm I.D., teflon, variable lengths). The instrument response was measured by Ayah 3Sx3-3D solar cell FI photometer (homemade) using super bright blue, green and red light emitted diode (LED) as a source with three solar cell as a detector. The out put signals was recorded by Potentiometric recorder (kompensograph-model C-1032-siemens, Germany), using the range of 1-500 mV or 1-500mvolt. Peak height was measured for each signals. UV-Vis spectrophotometer digital double beam type (UV-1800, shimadzu, Japan) was also used to scan the spectrum of product of the reactants using 1 cm glass cell.

- Methodology:

The whole reaction manifold system for determination of folic acid via its oxidation in acidic medium is shown in figure-2. The manifold is composed of two lines: first line supplied with Ce(IV) sulphate (0.5 mmol.L^{-1}) in H_2SO_4 (0.5 mol. L^{-1}) at 1ml.min^{-1}, while the second line is a carrier stream which leading to the injection valve to carry folic acid sample of 100µl at 1ml.min^{-1} flow rate. Both line meet at junction (y-junction) with an outlet for reactants product (reddish- orange species). The response peak of the resulting colour species is measured using Ayah 3Sx3-3D solar cell FI photometer while the variation in response was monitored using super bright green light emitting diode. Each solution was assayed in triplicate. A proposed mechanism of oxidation of folic acid in acidic medium is presented in scheme-1. [14].



$$\overset{O}{\overset{\|}{RC}}\text{-OH}+NH_3+CO_3^{-2}+HOOC\ CH_2\ CH_2\text{ - }COOH$$

Scheme 1: Proposed mechanism of oxidation folic acid by Ce(IV) sulphate

3- Results and discussion

3-1- Spectroscopic study :

A dilute aqueous solution of folic acid (0.2 mmol.L^{-1}) with Ce(IV) sulphate (0.5 mmol.L^{-1}) (in 0.5 mol.L^{-1} H_2SO_4) as oxidizing a gent an intense reddish-orange coloured product was formed immediately, the product shows a maximum absorbance at 535 nm against reagent blank as shown in figure-3.

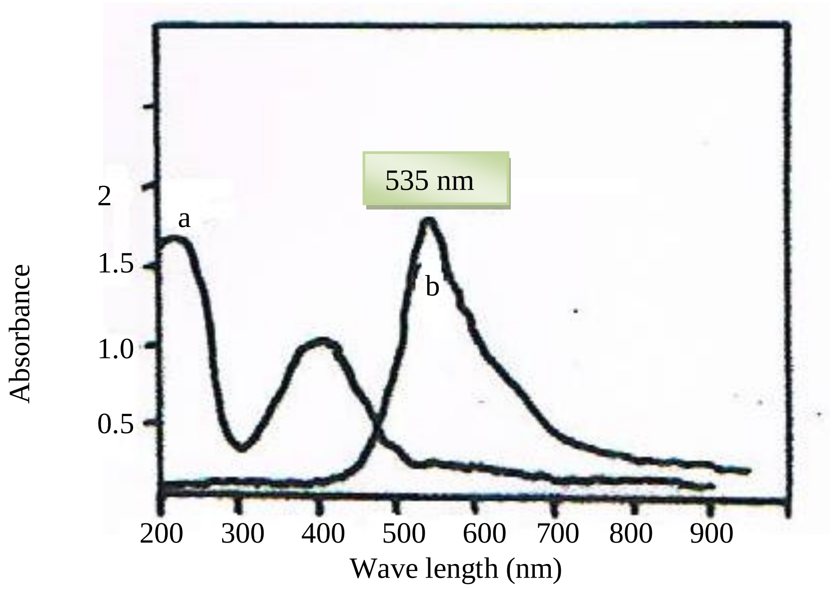


Figure 3- UV-Vis spectrophotometric of reddish-orange species by folic acid -Ce(IV)-H_3O^+. a: for folic acid , b: for colour species.

The same colour product of the oxidized species formed by F.A- Ce(IV)-H_3O^+ system was measured using a homemade Ayah 3Sx3-3D-solar cell FI photometer at three different light emitted diode (LED) [blue 470nm, green 525 nm, and red 635 nm]. A maximum response was measured in mV when using green light emitted diode (525 nm) as a source as shown in figure-4.

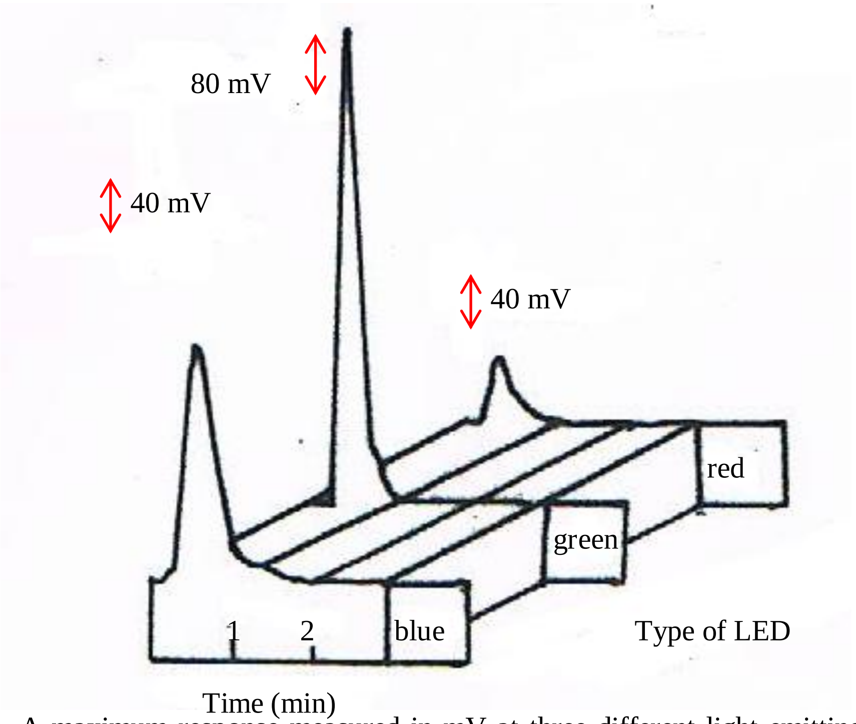


Figure 4- A maximum response measured in mV at three different light emitting diode at folic acid 8 mmol.L^{-1}, 150 μL .

3-2- Optimum conditions for F.A- Ce(IV)-H_3O^+ system using Ayah 3Sx3-3D-solar cell FI photometer

3-2-1- Chemical variable :

- Effect of Ce(IV) concentration :

A series of Ce(IV) sulphate solutions (in 0.5 mol.L^{-1} H_2SO_4) were prepared ranging (0.1-1) mmol.L^{-1} establish the optimum concentration that can be used. The study was conducted that 0.5 mmol.L^{-1} of Ce(IV) sulphate was necessary to obtained the maximum colour intensity of the product figure-5, in addition to gave a regular and sharp maxima with suitable peak height comparing with lower concentration (< 0.5 mmol.L^{-1}).

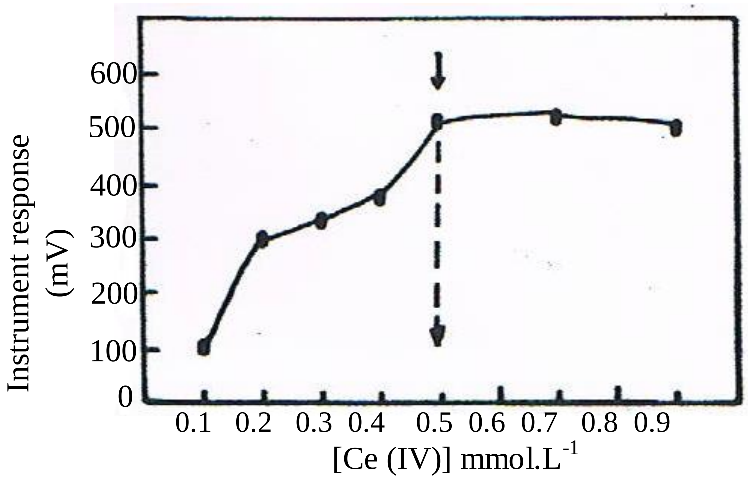


Figure 5- Variation of Ce(IV) sulphate concentration on instrument response using F.A. (8 mmol.L^{-1}), 150 μl

- Variation of sulphuric acid concentration :

A series of solutions were prepared by different concentrations of sulphuric acid (0.1-1) mol.L^{-1} with optimum concentration of Ce(IV) sulphate (0.5 mmol.L^{-1}), sample volume 150μl. The results obtained shown in figure-6. It was noticed that the increase of the sulphuric acid concentration up to 0.5 mmol.L^{-1} gave a regular and sharp response while an increase above 500 mmol.L^{-1} will decrease in the response height, it might be attributed to the probability of dissociation of coloured species. Therefore 0.5 mol.L^{-1} was chosen as the optimum concentration.

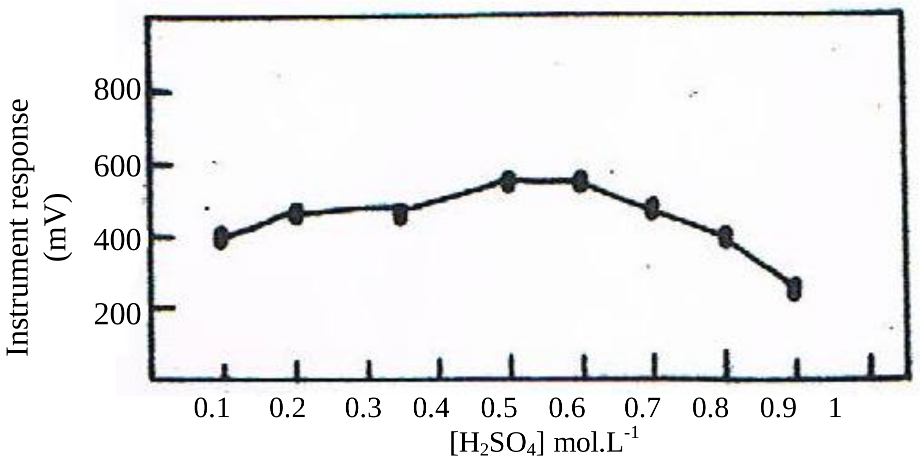


Figure 6- Variation of sulphuric acid concentration on Instrument response using F.A (8 mmol.L^{-1}).

3-2-2- Physical variable :

- Effect of flow rate :

A set of experiments were carried out for the optimization of preferred flow rate that extent from 0.5 to 2 ml/min for both line figure-2, using Ce(IV) sulphate (0.5mmol.L^{-1}) in 0.5 mol.L^{-1} H_2SO_4 with 150 µl sample volume and open injection value as a purge time. The results are tabulated in table-1. It was noticed that at low flow rate there is an increase in peak base width (Δt_B) as shown in figure-7A this might be due to dispersion and dilution. While at higher speed >1 ml.min-1 slightly increase in the response and height. Therefore 1 ml.min-1 as a flow rate was chosen as optimum to obtain regular response, narrower Δt_B and minimized the consumption in the reactants solutions, as shown in figure-7B.

Table 1- Variation of flow rate on the instrument response (mV) using folic acid (8mmol.L^{-1}).

Pump speed	Flow rate for both line (ml/min^{-1})	Response n=3 $\bar{y}_i$ (mV)	Peak base width Δt_B (sec)	t (sec)
10	0.5	220	60	45
15	0.7	350	40	36
20	0.9	480	30	32
25	1	520	20	25
30	1.3	525	18	20
35	1.5	430	18	18
40	2	380	16	12

t= the arrival time of sample segment to the flow measurement cell.

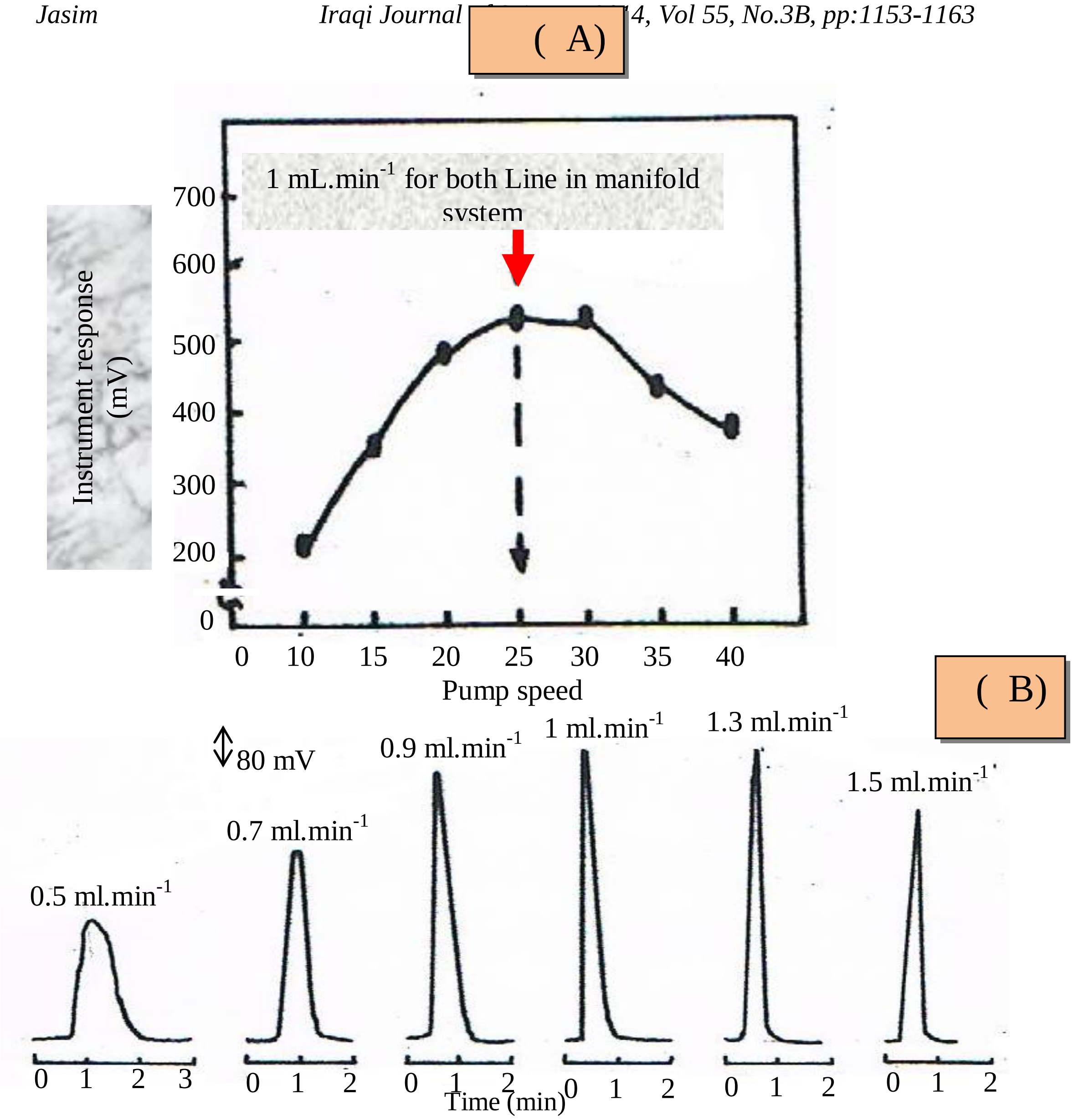


Figure 7- Variation of flow rate on:
A: Instrument response in mV
B: response profile using 8 mmol.L^{-1} of folic acid.

- Effect of sample volume :

Using the optimum flow rate 1ml.min^{-1}. Variable sample volume (50, 100, 150, 200) µl were injected using open valve mode i.e allowance for continuous purge of sample from the sample loop in the injection valve. The data obtained were plotted as shown in figure-8. showing that the optimum sample volume is 100µL. Regular clear response were obtained and increase of response height. Using larger volume i.e > 100 µl it gave an increase in the Δt_B of the response which was probably attributed to the continuous longer duration of sample segment in front of detector as shown in figure-8B.

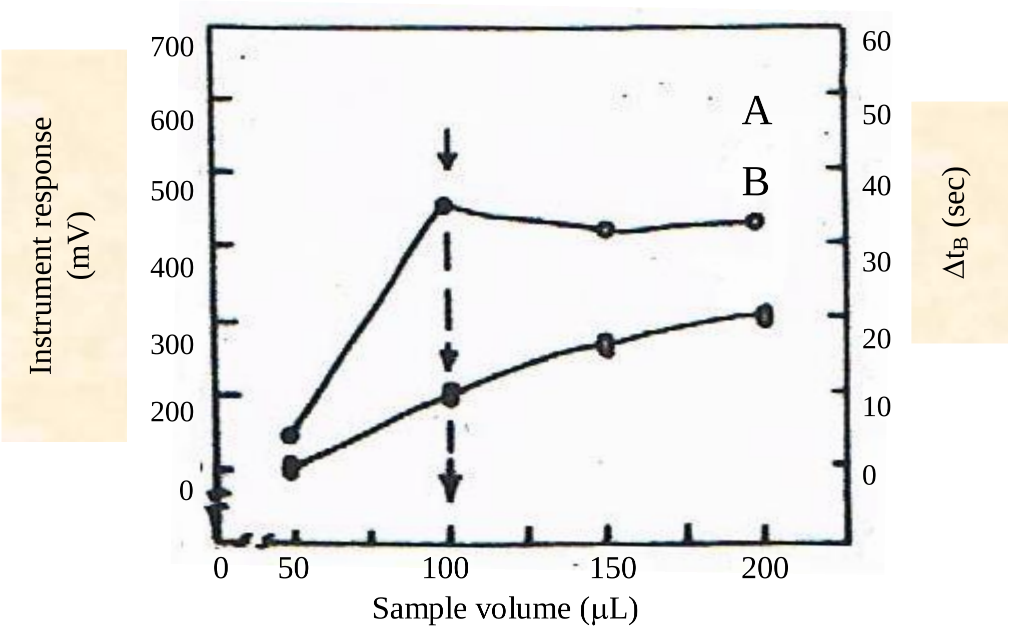


Figure 8- Variation of injected sample volume on: A: Instrument response, B: base width [Δt_B] using optimum conditions

3-3- Calibration graph for folic acid- Ce(IV)-H_3O^+ system and detection limit :

Fixing all the achieved parameters whether it is physical or chemicals. A series of solutions for folic acid 0.005-25 mmol.L^{-1} were prepared, a calibration graph for the variation of instrument responses with F.A. concentration for 0.005-20 mmol.L^{-1} shown in figure-9. Above 20 mmol.L^{-1} the value of correlation coefficient (r) will decreased, which might be attributed to the un oxidized folic acid. The obtained results were tabulated in table-2.

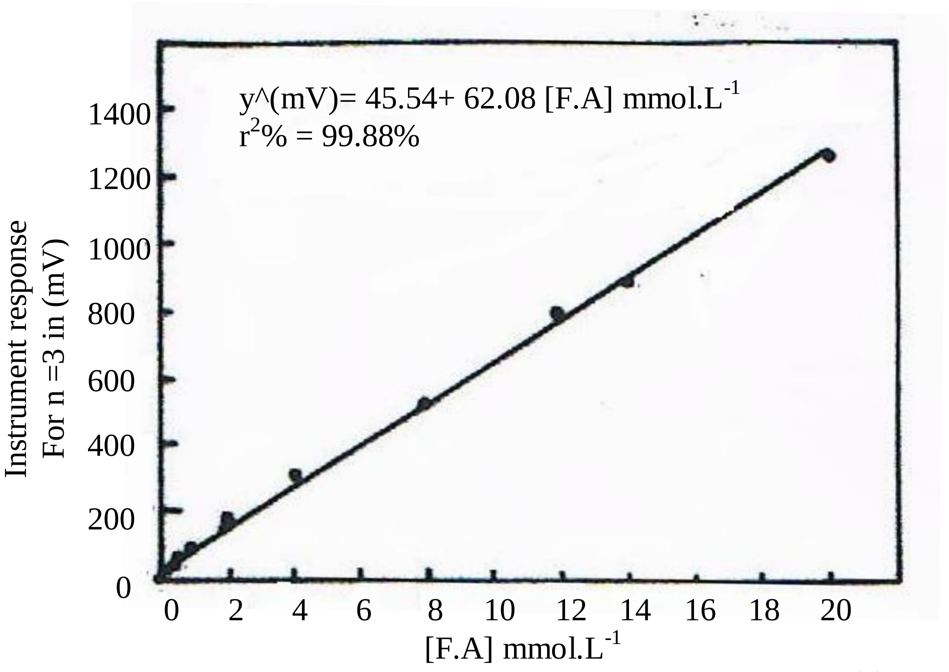


Figure 9- Variation in an instrument response versus concentration of folic acid

Table 2- summary of calibration graph results for the determination of folic acid using Ce(IV) as oxidizing agent

Measured [folic acid] mmol.L^{-1}	Range of calibration graph n=11	$\hat{y}_i$ (mV)= a+b [folic acid] mmol.L^{-1}	r, r^2, r^2%
0.005-25	0.005-20	45.54+62.08 [F.A] mmol.L^{-1}	0.9994, 0.9988 99.88%

Limit of detection for folic acid was conducted from gradual dilution for the minimum concentration in the linear calibration graph which equal to 0.5 µmol.L^{-1} at 100 µl sample volume.

- Repeatability:

The repeatability was studied for the determination of folic acid via measurements of oxidized folic acid at concentration (8, 12) mmol.L^{-1} of six successive injected sample measurements as shown in figure-10. The results obtained are tabulated in table-3.

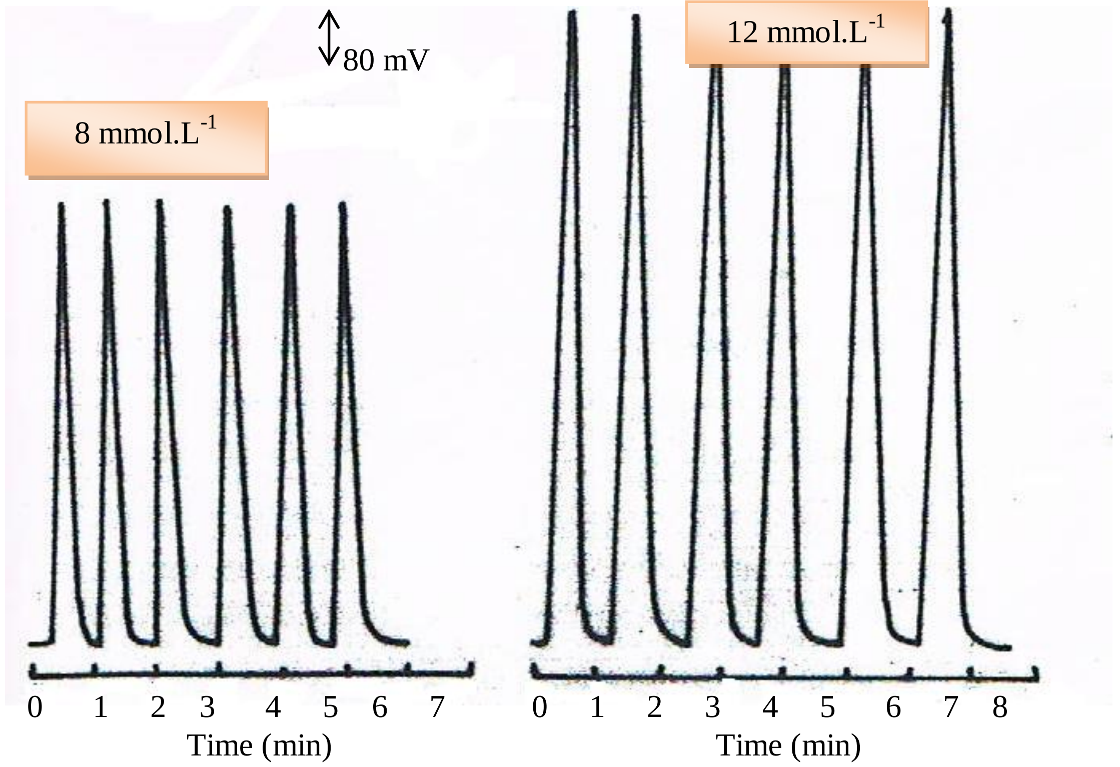


Figure 10- successive repeatability measurements of folic acid (8, 12) mmol.L^{-1} using Ayah 3Sx3-3D-solar cell FI photometer, 100µl sample volume .

Table 3- Repeatability of folic acid

[folic acid] mmol.L^{-1}	$\bar{y}_i$ (mV) n=6	σ_{n-1}	RSD%	Confidence interval of the mean $\bar{y}_i \pm t * \frac{\sigma_{n-1}}{\sqrt{n}}$
8	560	1.49	0.27	560 ± 7.30
12	800	3.29	0.41	800 ± 8.17

t*= $t_{\frac{0.05}{2}, n-1}$ = 4.303

3-4- Analysis of pharmaceutical preparation

The proposed method achieved in this work was used for the analysis of folic acid in pharmaceutical preparation and was compared with the UV-VIS spectrophotometry method via the measurement at λ_{max}= 535nm, linear calibration curve was obtained for the concentration range of 0.01-1.5 mmol.L^{-1} as shown in table-4.

Thirteen tablets were weight, crushed and grinded. A 2.5 mmol.L^{-1} solutions were prepared by weighting a mount equivalent to 110.35mg active ingredient for each pharmaceutical preparation. The powder was dissolved in deionized water followed by filtration to remove any undissolved residue affecting on the response. The filtrate was completed to 100ml.

1.5ml was drown to each of five 25ml volumetric flasks followed by the addition of gradual volumes of standard folic acid (0, 1, 2, 3, 4) ml of 2.5 mmol.L^{-1} to obtain (0.15, 0.25, 0.35, 0.45 and 0.55) mmol.L^{-1}. The measurement were conducted by proposed method and classical method (UV-VIS spectrophotometry). The results were mathematically treated by standard addition method. The results were tabulated in table-5 at confidence interval 95% [15]. Paired t-test was used as shown in table-6.

The obtained results indication clearly that there was no significant difference between newly developed method (Ayah 3Sx3-3D-solar cell FI photometer) with classical method at 95% confidence interval as the calculated t value is less than tabulated t-value.

Table 4- Summary of calibration graph results for determination of folic acid using UV-VIS spectrophotometry

Measured [folic acid] mmol.L^{-1}	Range of calibration graph mmol.L^{-1}	$\hat{y}_i$ (mV) a+b [F.A.] mmol.L^{-1}	r, r^2, r^2%
0.005-15	0.01-1.5	0.207+1.179 [F.A] mmol.L^{-1}	0.9876, 0.9754, 97.54%

Table 5- Results for the determination of folic acid at different drugs using F.A- Ce(IV)- H_3O^+ system via standard addition method

Name, content manufactures and country	Confidence interval of average weight $\bar{w}_i \pm t \frac{\sigma_{n-1}}{\sqrt{n}}$	Sample weight (g)	Theoretical content for active ingredient at 95% (mg)	Practical content for active ingredient at 95% (mg)	Recovery %
Folic acid 5mg SDI Iraq	0.1235 ± 0.0045	2.7256	5 ± 0.182	4.58 ± 0.98	91.6%
Folic acid 5 mg Actavis UK	0.1432 ± 0.0056	3.1604	5 ± 0.196	5.13 ± 1.02	102.6%

Table 6- Paired t-test for comparison between proposed method with classical method for the determination of folic acid

Sample no.	Practical content (mg)		X_d	$\bar{x}_d$	σ_{n-1}	Paried t-test $\frac{\bar{x}_d\sqrt{n}}{\sigma_{n-1}}$	t_{tab} at 95% for n-1 n=2
	Proposed method	Classical method					
1	4.58	4.39	0.19	0.22	0.042	7.41 << 12.7	
2	5.13	4.88	0.25				

4- Conclusion:

The proposed method is simple, rapid and inexpensive with a high sensitivity for the determination of folic acid which based on it is oxidation to coloured species by Ce(IV) sulphate. This method can be used for the analysis of folic acid in pharmaceutical preparation and pretreatment of the sample is not necessary because the preparation of the pharmaceutical formulation and reagent is done simply by dissolving in water, in this manners and since they were found not to interfere with the determination folic acid, therefore this system is particularly useful for the implementation of routine analysis.

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