Journal homepage www.jzs.uos.edu.krd
Journal of Zankoy Sulaimani
Part-A- (Pure and Applied Sciences)

Batch and Flow injection Spectrophotometric Methods For Determination of Folic acid in Pharmaceutical Preparations using Cerium(IV) ammonium sulphate(CAS) as an oxidant.

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Article info

Original: 29 May 2017
Revised: 7 May 2018
Accepted: 11 June 2018
Published online: 20 June 2018

Key Words:
Spectrophotometric
Folic acid
Flow-injection,
Methylene blue
Cerium (IV)
ammonium sulphate

Abstract

A simple, rapid and sensitive batch and flow injection spectrophotometric methods have been developed for the determination of folic acid (FA) in pure form and in its pharmaceutical preparations. The proposed methods involve the addition of a measured excess of cerium (IV) ammonium sulphate (CAS) in acidic medium followed by determination of unreacted CAS by reacting with a fixed amount of methylene blue (MB) and measuring the absorbance at 665 nm. The optimum reaction conditions and other analytical parameters have been evaluated. Linearity was observed from 1-10 and 4.0-20.0 µg/ml folic acid by batch and flow injection procedures, respectively. Statistical analysis of the results and comparison with results by the British Pharmacopoeia method are also reported.

Introduction

Folic acid (FA) is a member of the vitamin-B group(vitamin B9) in some term called folate[1], and chemically named as N-[4-[[(2-amino-1,4-dihydro-4-oxo-6-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid Fig. (1) [4]. The molecular formula is $C_{19}H_{19}N_7O_6$ and molecular weight 441.40 gm/mole. It is reduced in the body to tetrahydrofolate, which is a coenzyme for various metabolic processes including the synthesis of purine and pyrimidine nucleotides, and hence in the synthesis of DNA. FA plays a major role in the synthesis of red blood cells, in the formation of RNA and DNA, in the development of tissues and the brain of the fetus and the growth of a baby [3]. There are various methods described for the determination of folic acid are such as high-performance liquid chromatography using different detectors [4-6], spectrophotometry [7-10] ,Derivative spectrophotometry [11] ,chemiluminescence [12].

However, some of these procedures suffer from one or another disadvantage such as narrow range of determination; require heating or extraction, long time for the reaction to complete, and also instability of the coloured product produced. The use of CAS as an analytical reagent offered adequate sensitivity and accuracy, also the simplicity and low cost of the analytical method. The use of CAS for the determination of FA has not been reported yet. Therefore, the present study was undertaken to evaluate CAS as an analytical reagent for the spectrophotometric determination of FA. Flow injection (FI) system is adequate procedure to use in routine analysis in pharmaceutical laboratories due to their simplicity, high analytical frequency and capacity to reduce reagent consumption when compared with batch procedure.

The purpose of the present investigation was to develop, two simple, rapid and sensitive batch and FI methods using spectrophotometric detection were described for the determination of folic acid . The method was based on the oxidation of folic acid by a known excess of cerium(IV)ammonium sulphate (CAS) in acidic medium followed by reaction of the excess oxidant with methylene blue (MB) to bleach its blue colour. The proposed methods have been successfully applied to the determination of FA in different brands of tablets.

MATERIAL preparations and Instrumentation:

Materials

All chemicals were of analytical reagents grade.

Folic acid stock standard solution.

A solution of 1000 $\mu g.ml^{-1}$ was prepared by dissolving 0.1000 g of FA (provided by the Company for Drug Industries and Medical Applications SDI, Samarra, Iraq) in 10 ml of 0.1 M sodium hydroxide and then the volume was made up to 100 ml in a volumetric flask with the same solvent and kept in the dark at 5^0C in plastic container. This solution is stable for one week. Working standard solutions were prepared by suitable dilution of the stock standard solution.

Cerium (IV) ammonium sulphate (100µg/ml)

Prepared by dissolving 0.1g of CAS (BDH) (Ce $(NH_4)_4$ $(SO_4)_4.2H_2O$) in a least amount of 0.5M H_2SO_4 and diluted to 1000 ml with distilled water. Working standard solutions were prepared by dilution with distilled water.

Methylene blue (100µg/ml)

Prepared by dissolving 0.1g of MB (E. Merck)(3,7 bis(Dimethyl amino)-phenothiazin-5-ium chloride)($C_{16}H_{18}ClN_3S$) in water and diluted to 1000 ml with distilled water. Working standard solutions were prepared by dilution with distilled water.

Sulphuric acid (1M)

A 1.0 M of H_2SO_4 (SDFCL) was prepared by diluting 5.4 ml of concentrated acid (Sp.gr. 1.8, 98%) to 100 ml with distilled water. More dilute solutions were prepared by dilution with distilled water.

Solutions of interferences.

A stock standard solution of each interfering species (sodium chloride, glucose, sucrose, fructose, lactose, galactose, and starch) was prepared by dissolving 0.1 g of the compound in distilled water then the volume is completed to 100 ml in calibrated flask. Other solutions were prepared by serial dilutions of the stock solution.

Preparation of tablet solutions. (Each one tablet contains 5 mg folic acid).

An accurately weighed amount of powder that was contain 0.01 g folic acid (0.191 g powder brand A, 0.209g powder brand B, 0.2256g powder brand C, and 0.2444g powder brand D) is mixed with about 30 ml of 0.1 M NaOH, and stirred to increase solubility. The insoluble mass is filtered off on a Whatman No.41 filter-paper, washed with NaOH and the filtrate plus washings are diluted to 100 ml with (0.1 M) NaOH in a volumetric flask.

Instrumentation

1- Spectral and absorbance measurements for batch and FI methode were carried out by a Bio-Tek Instrument UV-Vis spectrophotometer (model J643002, Vermont, USA), using 1.0 cm quartz cell. . Spectral and absorbance measurements were carried out by an A&ELAB Instrument UV-Vis spectrophotometer (model AE-S60-2U, P.R.C.), using 1.0 cm quartz cell. Flow cell with 30µl and 10mm path length quartz was used.Multi-channel peristaltic pump (Watson-Marlow 5012, USA) used for propelling merged streams. A 6-way injection valve with various sample loops was used.

2- HPLC made by a shimadzu(Japan),Column name (Intert sustain) Size (4.6mm) , Chromspher C8 is suitable.

Procedures

Recommended batch procedure.

A series of 25 ml volumetric flasks an increasing volume of FA solution (100 µg/ml) transferred to cover the range of the calibration graph (1.0 – 10.0 µg/ ml). Then 0.4 ml of H_2SO_4 (1 M) and 2.5 ml of CAS (20 µg/ml) were added. The solutions were lifted for 10 min at room temperature (25˚C), finally adding 1.5 ml of MB (10 µg/ml) then dilution to the mark with distilled water. The absorbance was measured after 5 min at 665 nm versus the reagent blank, prepared in the same manner but containing no drug.

Recommended FI procedure.

A volume of 100 µl of the prepared sample solution of (FA) was loaded into the sample loop by means of a syringe. The sample was injected into a 0.02 M H_2SO_4 carrier stream pumped at a rate of 0.80 ml/min. The CAS solution (30 µg/ml) was added to the carrier stream at a rate of 0.80 ml/min in a confluence manner downstream to ensure rapid and adequate mixing. After that, the MB solution (15µg/ml) was added to a stream containing the unreacted CAS at a rate of 0.80 ml/min. After injection, the valve was returned to the load position when the maximum change in absorbance value had been reached. The absorbance was monitored at 665 nm in a quartz flow cell, at which the maximum absorption occurred and connected to a recorder, at 0.5 mV and with a chart speed of 25 cm/h. FI system is shown in Fig. (5).

Result and Discussions

CAS has been used as an analytical reagent for many organic compounds .In the present work, it was found that CAS can oxidize FA in an acidic medium. In addition, it reacts immediately with MB also in an acidic medium to bleach out its blue colour. After the oxidation of the FA by CAS, the excess CAS will reacted with the MB as shown in the following scheme.

FA+ CAS (excess) → FA (oxidation product) +CAS (unreacted)
CAS (unreacted) + MB → Oxidation product of MB + Unreacted MB (indicator bleaching)
↓
(Measured spectrophotometrically at λmax=665 nm)

The absorption spectrum of the MB at 665 nm is shown in Fig. (2). Therefore, the different parameters affecting the oxidation reaction, and hence the subsequent determination of these drugs were optimized.

Optimization

Batch method

Effect of methylene blue amount.

The effect of amount of MB dye concentration on the intensity of colour was carried out in the range of 0.5 - 2 mL. As shown in Fig (3) it was found that the optimum concentration to maximum absorbance was obtained with 1.5 mL 10 $\mu g.ml^{-1}$ of methylene blue dye in final volume of 25 ml.

Effect of oxidant amount.

Cerium ammonium sulfate(CAS) was found to be a useful oxidizing agent, also the effect of different volumes (0.5 to 4.5) ml of 20 $\mu g.ml^{-1}$ of CAS on the colour of the dye was studied. As shown in Fig. (4) the results indicated that 2.5 ml of CAS solution was enough to obtain a maximum bleaching of the colour of methylene blue dye therefor it was recommended in the subsequent experiments.

Nature and amount of acid

The reactions were tested in HCl, H_2SO_4, HNO_3, and CH_3COOH solutions.Indicated that the reaction is suitable in sulphuric acid medium. The variation in H_2SO_4 amount from 0.1 to 1.0 ml has been studied. The maximum absorbance was achieved when the H_2SO_4 was 0.4 ml of 1 M, which was used in all subsequent experiments. As shown in Table (1).

Effect of temperature.

The effect of temperature on the colour intensity of methylene blue colour was studied. In practice a maximum absorbance was obtained when the colour was developed at room temperature Table (2), decrease in colour intensity and stability was observed at low or high temperature, therefor room temperature is recommended for subsequent experiments.

Order of addition.

To obtain optimum results the order of addition of components should be studied. The results in Table (3) indicated that the order of addition of reagents should be followed as given by the procedure, otherwise, a loss in colour intensity and stability are observed.

Effect of time on oxidation.

It was observed that if methylene blue was added immediately to the solution containing FA and CAS in acidic medium the resulted solution is bleached rapidly and the absorbance is very low. This can be explained by the fact that the drug oxidation by CAS is a time developing reaction and thus the influence of the reaction time was studied. In this respect, solutions containing 3.0 ml of 100µg/ml FA, 0.4 ml of 1M H_2SO_4 and 2.5 ml of (20µg/ml) CAS have been let to react at darkness in different times before adding the indicator and measuring the absorbance at 665nm. It was observed that the absorbance of these solutions increases with in time 10 minutes and then it becomes constant was shown in table (4).

Stability of colour.

The colour intensity was stable for 24 hours as shown in table (5).Hence at room temperature reaction time 10 min were selected between CAS and folic acid and directly bleaching MB were selected.

Optimization of FI method

Optimization of chemical parameters

Batch method for the determination of FA was adopted as a basis to develop FI procedure. The manifold used for the determination of folic acid is shown in Fig. (5). The parameters of flow in the determination of FA were optimized. According to the results of preliminary spectrophotometric studies concerning the effect of acidic medium on the absorbance of the product, sulphuric acid was used for the FI method.

Effect of H_2SO_4 concentration

The effect of the concentration of sulphuric acid was studied in the range 0.01-0.1 M with fixed FA concentration of (10µg/ml). A gradual increase in the analytical signal was observed with increasing the H_2SO_4 concentration. However, the stability of the baseline and consequently the reproducibility of the results were significantly reduced at higher H_2SO_4 concentrations. Therefore, 0.02 M $H_2SO_4$was used as a carrier as it gives a reasonable sensitivity and baseline stability Fig. (6).

The effect of CAS concentration

The influence of the CAS concentration on the absorbance was studied at constant concentrations of FA. Fixed volumes (100 µl) of FA was used and injected into the H_2SO_4 stream. The effect of changing the concentration of CAS in the range (10-45) µg/ml on the absorption peak height was shown in Fig. (7).The figure shows that, a maximum analytical signal was achieved when the CAS concentration reached to 30 µg/ml , and was chosen for further use.

Effect of MB concentration

The effect of a different concentration of MB in the range of (5-30) µg/ml at constant concentrations of FA (10 µg/ ml) on the absorption peak height is shown in Fig. (8). The figure shows that maximum analytical signals were achieved when the MB concentration reached to 15µg/ml, and was chosen for further use.

Optimization of physical parameters

Effect of flow rate

Various flow rates (0.5, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.4 and 3.6) ml/min for propelled solutions for the system were studied. As it is shown in Fig. (9) the maximum absorbance value was achieved when the final solution passed through the detector with a flow rate of 2.4 ml/min for the system.

Effect of coil length

In order to investigate the effect of this factor, Three coils with different lengths (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100) cm were examined for the system. As shown in Fig. (10) , the optimum values of coils lengths for the mixing coil and for both first and second reaction coils were found to be (20, 60 and 20 cm) respectively.

Effect of sample volume

After injection the sample zone moves downstream through the manifold toward the detector, so the influence of different sample volumes (25, 50, 75, 100, 125 and 150) µl. as shown in Fig. (11), the absorbance increases with increasing the volume of the sample introduced in to the flow system up to 75 µl for CAS system after which the absorbance slightly decreases as a result of sample dilution.

Quantitation

Calibration curve:

Under the optimum conditions for batch and FI systems, two calibration curves were constructed. A straight line was achieved by plotting absorbance against concentration of standard folic acid solution. As illustrated in Fig. (12), for batch method Beer's law was obeyed in the concentration ranges (1.0-10) µg/ml of standard folic acid using MB as oxidizing reagents. while for FI method, Beer's law was obeyed in the ranges (4.0-20.0)µg/ml of standard folic acid using MB as oxidizing reagents Fig. (13).

Analytical characteristics

Analytical characteristics such as regression equation, linear range, relative standard deviation, relative error, molar absorptivity and Sandell's sensitivity values of each method were determined under the optimized conditions as shown in Table (6).The limits of detection (LOD) and quantitation (LOQ) were calculated according to the International Union of Pure and Applied Chemistry (IUPAC) definition18 using the formula: LOD = 3S/b and LOQ = 10S/b

Where: S is the standard deviation of blank absorbance values and b is the slope of the calibration plot.The high values of molar absorptivity and low values of Sandell's sensitivity and LOD indicate the high sensitivity of the proposed methods.

Precision and accuracy:

Under the optimum conditions and using the recommended procedures, the precision and accuracy of the proposed batch and FI methods were checked depending on the values of relative standard deviation percent (RSD %), and relative error percentage (E_{rel} %) respectively. These values were calculated by doing five replicates for three concentration levels of synthetic sample solutions (1,5and 10 µg/ml) and (4,12 and 20 µg/ml) prepared by using standard folic acid solution using CAS reagent for batch and FI methods respectively. The precision and accuracy of data for the method are shown in Table (7).

Interference studies

In order to assess the possible analytical applications of the proposed analytical method described to the assay of commercial FA formulations, the effect of some excipients frequently present in the pharmaceutical preparations were investigated by carrying out the determination of FA in the presence of different excipients. Experimental results in Table (8) showed that sodium chloride, glucose, sucrose, fructose, lactose, galactose, and starch had no effect on the determination of FA with concentration (5 µg/ ml) and (10 µg/ ml) of batch and FI methods respectively .The tolerant limits of the studied species were taken to be the amount that caused a relative error percent ≤ ±5.0% of the absorbance measurement of the standard Folic acid solution.

Application to analysis of tablets

The proposed batch and FIA spectrophotometric methods were successfully applied to the determination of FA in different brands of tablets. The results were summarized in Table (9). In comparison of the batch and the FIA procedure with standard method (HPLC) from Awamedica Company for drugs in Erbil-Iraqi Kurdistan regin which depending on British pharmacopeia, applied for analysis of the samples, and there was a reasonable agreement between them.

Conclusion

In the current research study the followings are concluded, two simple methods have been developed to determine Folic Acid in pharmaceutical preparations. The developed procedures based on addition of a measured excess of CAS in acidic medium followed by determination of unreacted CAS by reacting with a fixed amount of methylene blue and measuring the absorbance at 665 nm. The proposed methods need neither temperature nor pH control and nor long time for the reaction to complete. The methods were successfully applied in different brands of tablets. Calibration curve the obeyed Beer's law were obtained of batch and FI methods of folic acid reaction using cerium(IV) ammonium sulfate reagents individually in the concentration ranges of (1.0-10.0 µg/ml,4.0-20.0 µg/ml) with a detection limit, molar absorptivity and correlation coefficient (r) 0.03µg/ml ,0.4 µg/ml ,$4.7x10^4$ l/mol.cm, $0.8960x10^4$ l/mol.cm, and (0.9980)(0.9989) for batch and FI methods respectively.

The results of tables (6) shown the sensitivities for both methods are nearly the same, the relative error percentage of folic acid from synthetic samples for the recommended batch and FI methods together were found to range from (-5.0% to +5.0%), indicating the suitability of the methods for the determination of folic acid in synthetic samples of pharmaceutical products at a concentration level of traces, also the proposed methods can be used for routine analysis of folic acid in pharmaceuticals and in quality control, since there was no serious interference from the common excipients that might be found in commercial products. Both reagents show quite good selectivity and sensitivity.

Table (1): Effect of different types of acid and the volume of the acid for CAS oxidizing reagent.

No.	Acid types (1 M)		H_2SO_4 amount (ml)	Absorbance/ FA (5 µg / ml)
1	Acetic acid	0.496	0.2	0.445
2	Hydrochloric acid	0.511	**0.4**	**0.531**
3	**Sulpheric acid**	**0.579**	0.6	0.382
4	Nitric acid	0.527	0.8	0.285
			1	0.281

Table (2): Effect of temperature and time.

Temperature C°	10	20	30	40	50	60
5	0.401	0.399	0.403	0.401	0.398	0.397
10	0.411	0.412	0.410	0.410	0.413	0.409
(RT)	**0.556**	**0.554**	**0.555**	**0.556**	**0.553**	**0.554**
50	0.433	0.431	0.432	0.437	0.433	0.430

Table (3): Effect of order of reactants addition.

Reaction component	Order number	Absorbance
S+A+OX+DYE	I	**0.571**
S+OX+A+DYE	II	0.503
S+OX+DYE+A	III	0.445
S+A+DYE+OX	IV	0.391

S = FA (5µg/ ml), A = H_2SO_4, OX = CAS, DYE = MB

Table (4): Effect of time on oxidation and bleaching the color of MB.

Addition of oxidant(min)	5	10	20	30	40	50	60
5	0.513	0.513	0.514	0.510	0.511	0.510	0.510
10	0.576	0.575	0.576	0.577	0.574	0.575	0.573
15	0.544	0.542	0.541	0.543	0.542	0.540	0.539
20	0.438	0.435	0.437	0.433	0.435	0.432	0.431

Table(5): Effect of stability colour intensity

Time (minute)	Absorbance
Immediately	0.541
5	0.543
10	0.543
15	0.542
20	0.543
25	0.542
30	0.542
35	0.541
40	0.541
45	0.542
50	0.541
55	0.543
60	0.541
70	0.544
80	0.543
90	0.541
100	0.542
120	0.542
24h	0.521

Table (6): Analytical characteristics of the proposed methods.

Parameter	Batch method	FI- method
Beer's law range (µg / ml)	1.0-10.0	4.0-20.0
Detection limits (µg / ml)	0.03	0.1
Quantitation limit (µg/ml)	0.1	0.4
Molar absorptivity (l /mole.cm)	4.753×10^4	0.8960×10^4
Sandell's sensitivity (µg /cm2)	0.009	0.04
Regression equation (Y = a + bC)*		
Intercept (a)	0.0073	0.0169
Slope (b)	0.1077	0.0203
Determination coefficient (R^2)	0.9961	0.9982

Table (7): precision and Accuracy data for the proposed batch and FI spectrophotometric determination of folic acid using CAS as oxidizing reagent.

Method	Folic acid Conc. (µg/ml)	Found(µg/ml)	SD	RSD%	E_{rel}%*
Batch method	1.0	0.99	0.0054	4.9	-1
	5.0	4.89	0.0065	1.1	-2.2
	10.0	10.42	0.0519	4.3	4.2
FI- method	4.0	4.20	0.0015	1.4	5
	12.0	11.87	0.0025	0.89	-1.02
	20.0	20.1	0.0021	0.25	0.50

Table (8): Foreign species influence on the batch and FI spectrophotometric determination of folic acid using CAS oxidizing reagent.

	Batch method		FI method	
Excipients	Maximum Allowable Conc.(µg/ml)	Error %	Maximum Allowable Conc.(µg/ml)	Error %
Lactose	20	4.3	60	-0.6
Fructose	40	-2.8	80	-1
Glucose	20	-3.6	80	-1.3
Starch	16	3.3	100	1.5
Sucrose	25	4.4	100	2.4
Sodium chloride	10	1.6	80	-0.3

Table (9) : Application of the proposed method for the determination of folic acid in different brands of tablets.

Method	Pharmaceutical products	Composition	Content (mg) declared	Found (mg)				Error %*
				standared method(HPLC)	Batch method	Error%*	FI method	
Using (CAS) oxidant	Folic awa	Folic acid	5	5.69	5.49	-3.51	5.44	-4.38
	Actavis Folic acid-	Folic acid	5	4.80	4.77	-0.62	4.60	-4.01
	Julphar Folicum-	Folic acid	5	4.38	4.59	4.79	4.25	-2.96
	Femitol	Folic acid	5	5.01	4.83	-3.53	4.83	-3.53

***Average of five determinations.**

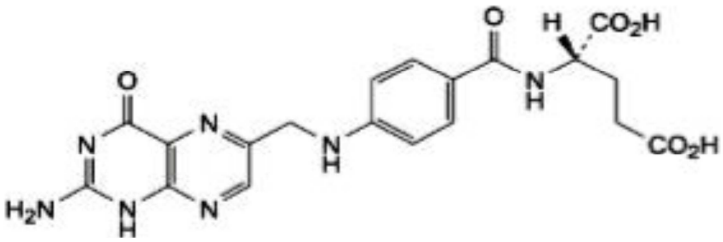

Figure (1): Structure of Folic acid

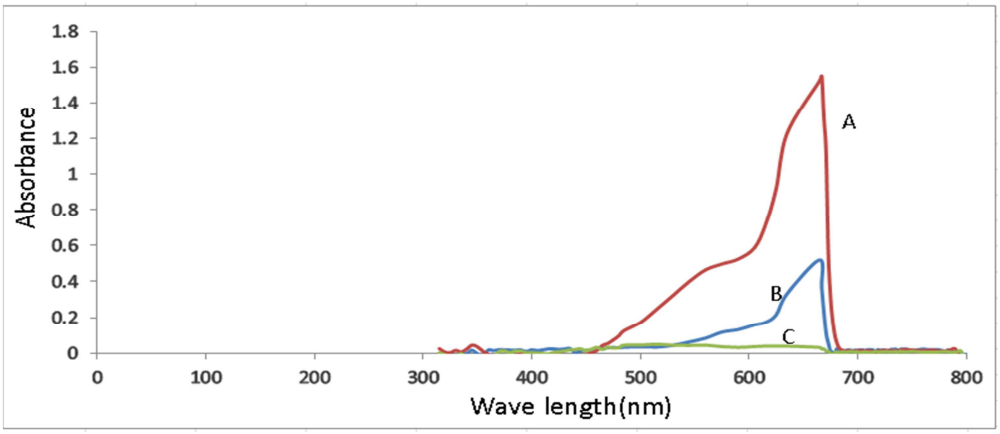

Figure (2): (A) Absorption spectra of 1.5 ml of (10.0 µg mL-1) methylene blue, (B) Absorption spectra of 5.0 µg/ml folic acid against reagent blank, (C) reagent blank against distilled water.

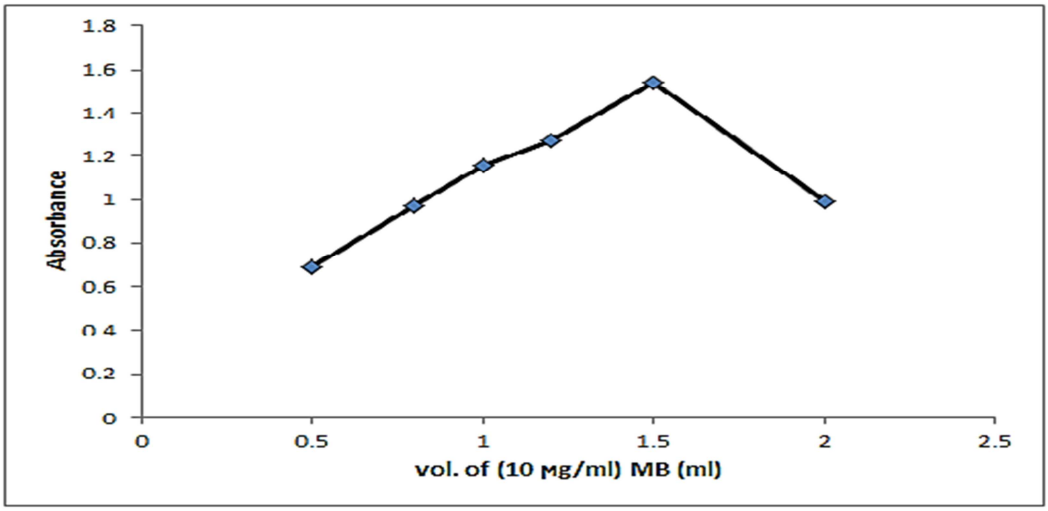

Figure (3): Effect of MB amount on the colour intensity.

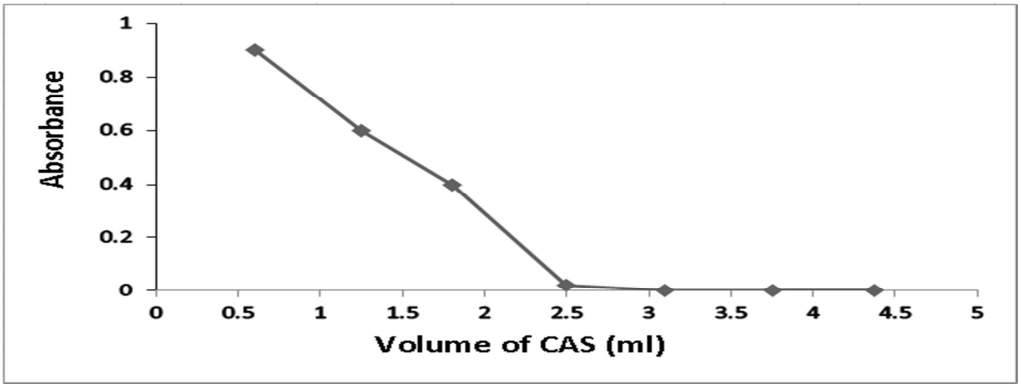

Figure (4): Effect of CAS amount on bleaching the colour of MB dye.

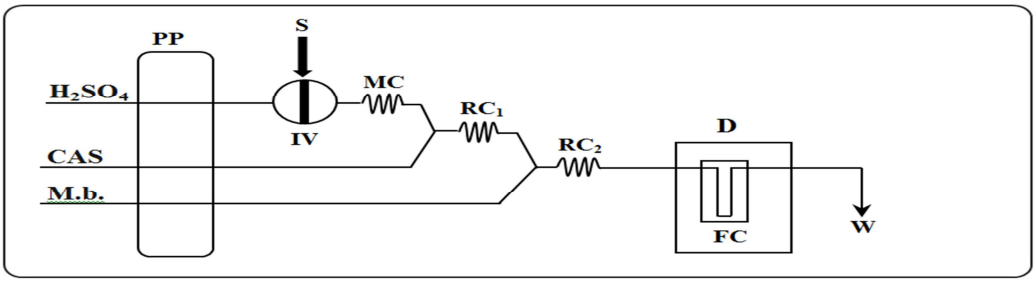

Figure (5): FIA manifold used for determination of folic acid using CAS as oxidizing reagent: (PP) peristaltic pump, (S) sample injected, (MC) mixing coil, (RC_1) 1st reaction coil, (RC_2) 2nd reaction coil, (IV) injection valve, (D) detector, (FC) flow cell, (W) waste.

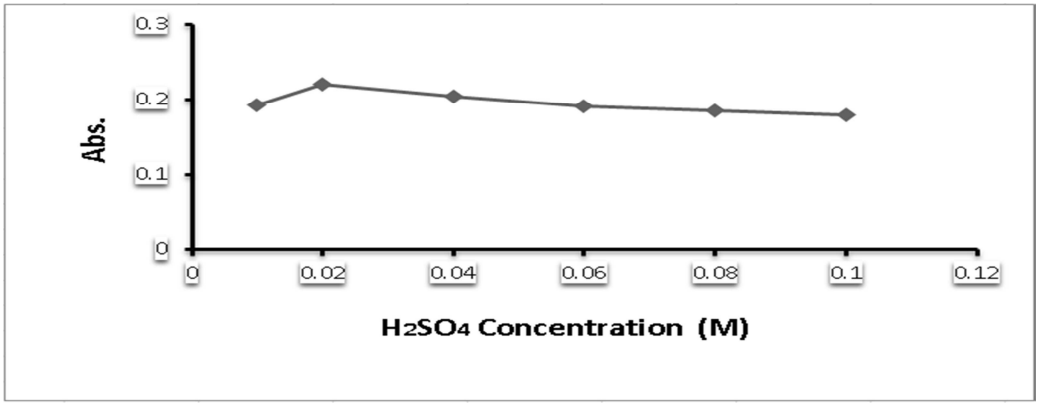

Figure (6): Effect of H_2SO_4 concentration on absorbance, using CAS as oxidizing reagent.

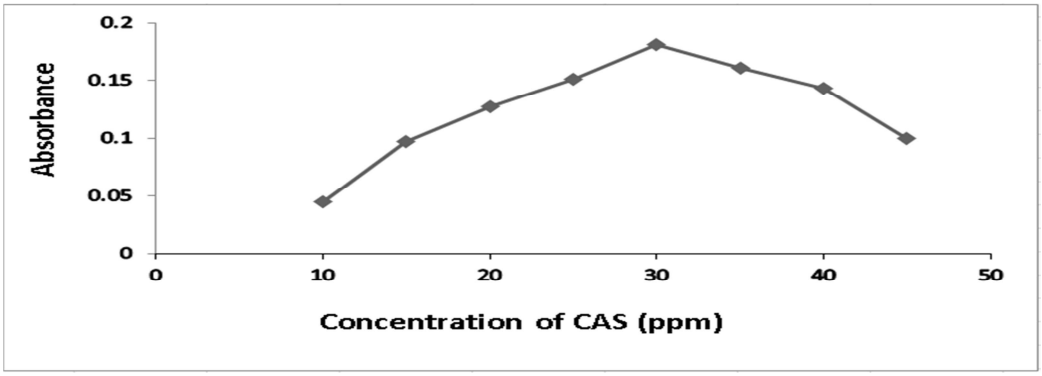

Figure (7): Effect of CAS concentration on absorbance, using CAS as oxidizing reagent.

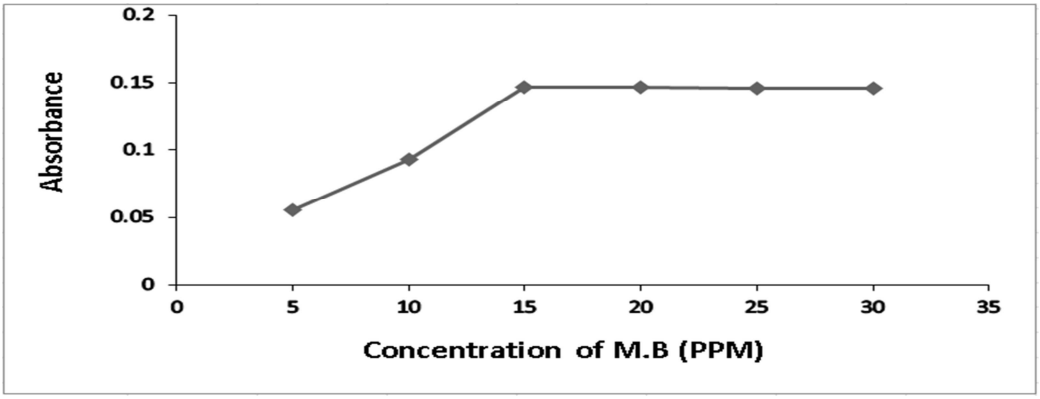

Figure (8): Effect of M.B concentration on absorbance, using CAS as oxidizing reagent

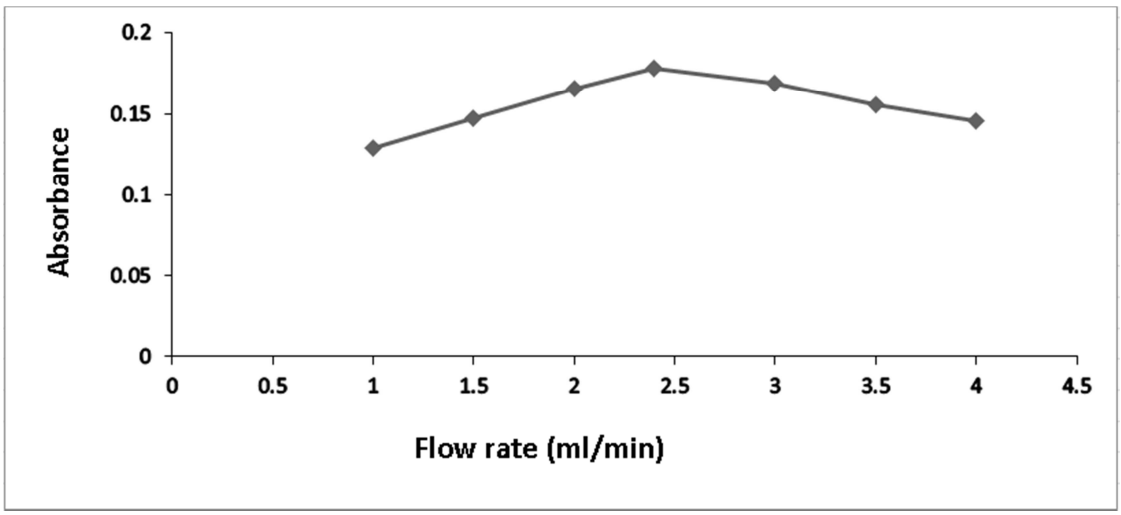

Figure (9): Effect of flow rate on absorbance, using CAS as oxidizing reagent

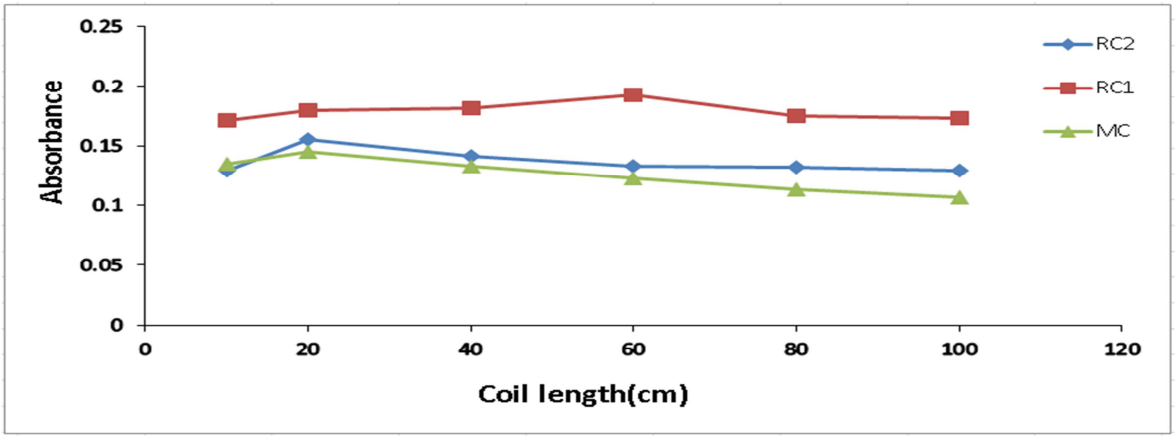

Figure (10): Effect of coil length on absorbance, using CAS as oxidizing reagent.

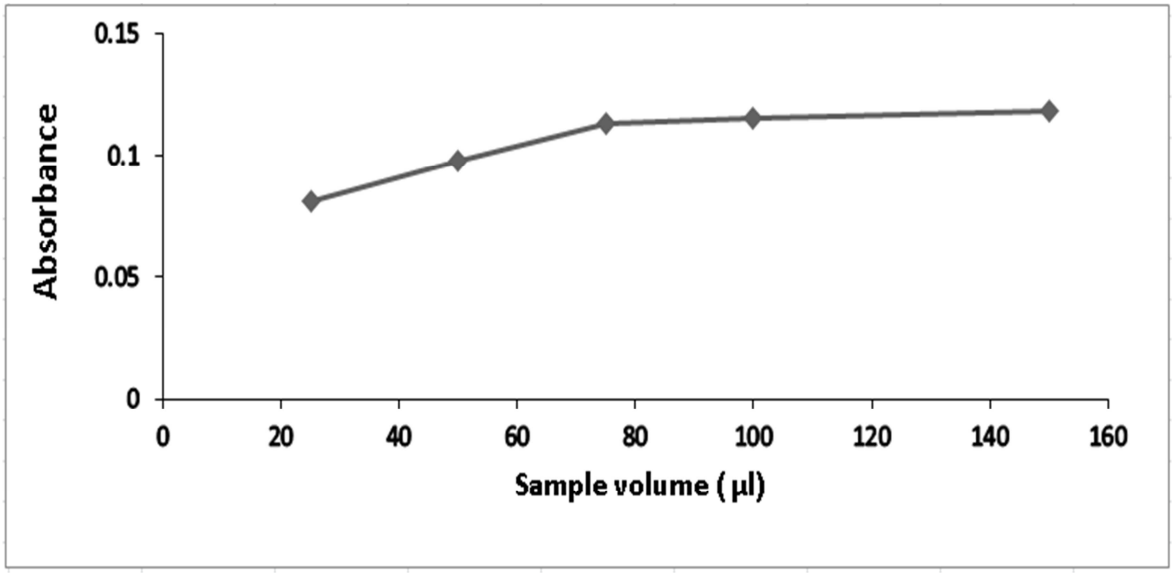

Figure (11): Effect of sample volume on absorbance, using CAS as oxidizing reagent.

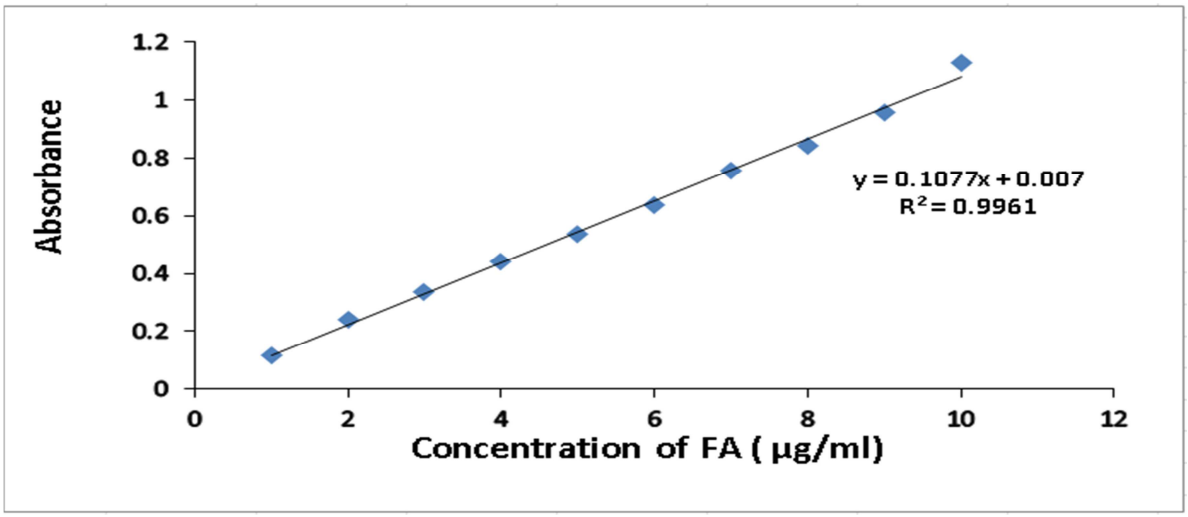

Figure (12): Calibration curve for spectrophotometric determination of folic acid using CAS as a oxidizing reagent.

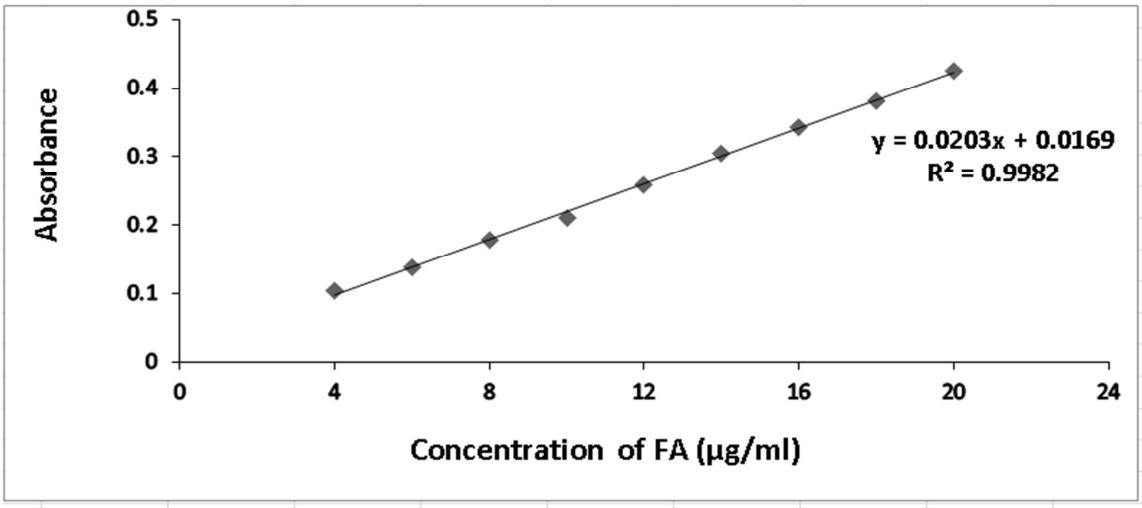

Figure (13): Calibration curve for FI spectrophotometric determination of Folic acid using CAS as oxidizing reagent.

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