Tikrit Journal of Pharmaceutical Sciences

Journal Homepage: http://tjo-ps.com

Determination of Amoxicillin in Pharmaceutical preparations by spectrophotometric and flow Injection – activated chemiluminescence methods

Abdul Majeed K.Ahmed[1], Shahla J. Shakkor[2]

1- Dep. of basic science college of Nursing, Kirkuk university, Kirkuk Iraq.
2- Dep. of chemistry ,college of Science, Kirkuk university, Kirkuk Iraq.

DOI: http://dx.doi.org/10.25130/tjops.14.1.07

Article info.

Article history:
-**Received**: 5 / 9 / 2018
-**Accepted**: 31 / 1 / 2019
-**Available online**: 20 / 6 / 2019

Keywords:
(Amox.), (FIA-Cl), amoxicillin, FIA-CL,

***Corresponding author:**

Email :
shalajamal.sj2@gmail.com

Mobile : 009647701005503

Contact To Journal

E-mail: tjops@tu.edu.iq

009647835274189

009647835274189

Abstract

This investigation involves development of a simple pectrophotometric method and a flow injection – activated chemiluminescence (FIA-Cl) for the determination of amoxicillin (Amox.) in pharmaceutical formulations. Spectrophotometric method was based on the oxidation of the (Amox.) with alkaline potassium permanganate, the reaction was followed spectrometrically by measuring the absorbance of (Amox.) at 600 nm. The reaction time of oxidation at (20 min) method is adopted for determining the drug concentration. The calibration graph was linear in the range of (5-50)µg.ml-1 with a correlation coefficient of (0.9995), detection limit of (4.76) µg.ml-1, molar absorption coefficient is 2557.8 L/mol.cm and a relative standard deviation RSD% of (1.84%). The method of FIA-CL was based on the activation of luminol – cobalt – H2O2 chemiluminescence by (Amox.). The linearity is (8-32) µg.ml-1 with detection limit of (6.2)µg.ml-1, and correlation coefficient was (0.9998) n=6 and the relative standard deviation was(1.48%). The two methods were applied successfully to determine the content of (Amox.) in pharmaceutical preparations with a recovery of 98.9% .

تقدير الاموكسيسيلين في المستحضرات الصيدلانية باستخدام الطريقة الطيفية وطريقة الحقن الجرياني المقترن بالبريق الكيميائي المنشط

عبد المجيد خورشيد احمد شهله جمال شكور

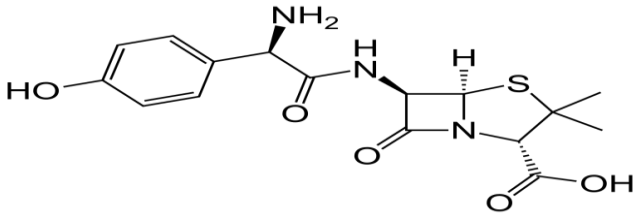
الخلاصة

تضمنت هذه الدراسة تطوير طريقة طيفية بسيطة وطريقة الحقن الجرياني المقترن بالبريق الكيميائي المحفز لتقدير الاموكسيسيلين في المستحضرات الصيدلانية, استندت الطريقة الطيفية على أكسدة الاموكسيسيلين مع برمنكنات البوتاسيوم القاعدية (KMnO4) ومتابعة التفاعل طيفيا" من خلال قياس التغيير بالامتصاص عند طول موجي (600) نانوميتر. وجد أن الزمن اللازم لإكمال التفاعل هو (20) دقيقة ومدى الخطية لمنحني المعايرة يتراوح بين (5-50) مايكروغرام / مل ومعامل الارتباط (0.9995), وحد الكشف مقداره (4.76) مايكروغرام / مل أما معامل الامتصاص المولاري 2557.8 لتر/مول.سم وبلغت قيمة الانحراف القياسي النسبي (RSD%) 1.84%. أما الطريقة الثانية فقد استندت على تحفيز البريق الكيميائي لنظام (لومينول – كوبلت – بيروكسيد الهيدروجين) بواسطة الاموكسيسيلين وكانت الحدود الخطية للطريقة تتراوح بين (8-32) مايكروغرام / مل وبحد كشف (6.2) مايكروغرام / مل وبمعامل ارتباط (0.9998) وانحراف قياسي نسبي مئوي 1.48% .طبقت هاتين الطريقتين بنجاح لتقدير الاموكسيسيلين في المستحضرات الصيدلانية وكان الاسترداد المئوي (98.9%) .

Introduction

amoxicillin [2] is (2S, 5R, 6R) – 6 – {[(2R) -2- amino-2- (4- hydroxyl phenyl) – acetyl] amino} – 3,3 – dimethyl – 7- oxo – 4 thia -1- azabicyclo[3,2,0] heptane -2- carboxylic acid, which have the following structures.

Amoxicillin is a moderate- spectrum bacteriolytic, β- lactam antibiotic used to treat bacterial infections caused by susceptible microorganisms and it is used in the treatment of a number of infection. [1] The chemical name for



Amoxicillin ($C_{16}H_{19}N_3O_5S$), M. wt = 365.4 gm/mole

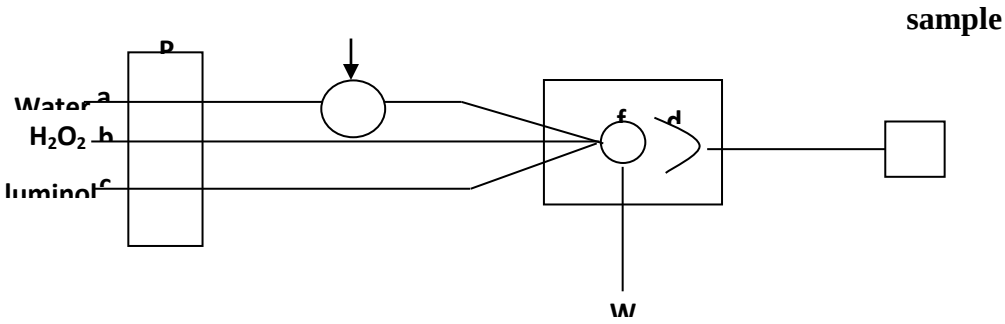
Various methods based on spectrophotometry [3-7] have been developed for determination of (Amox.) as its metabolites, sequential injection analysis [8,9], flow – injection [10- 13], FIA-Chemiluminescence [14-16], electro chemical method [17,18], chromatography [19,20]. HPLC [21-24] and polarography [25]. The present study describes a spectrophotometric method for the determination of (Amox.) in pharmaceutical preparations based on the oxidation with alkaline potassium permanganate and also includes development of FIA-CL method.

Experimental

Apparatus:

A) A spectrophotometer of type Shimadzu UV-160 model Kyoto, Tokyo, Japan was used, with quartz cells of 1 cm width.

B) FIA Chemiluminescence configuration which was outlined in figure (1) was used for the determination of (Amox.).



P- Peristaltic pump V- Injection valve F- Flow cell d- Photomultiplier

R- Recorder W- Waste solution .

Fig.(1):- Chemical reaction manifold used for the Flow-Injection Chemiluminescence determination of Amox.

Reagents

Reagents of analytical grade and distilled water were used through out the study. Solutions were prepared by appropriate dissolution as shown in table (1).

Table (1):- Preparation of some solutions

Substance	Molar Concentration (M)	Dissolved weight (gm)	Final Volume (ml)
NaOH	**1.5**	**6.000**	**100**
$KMnO_4$	**0.05 , 0.1**	**7.900 , 15.800**	**1000 , 1000**
Na_2CO_3	**0.1**	**10.599**	**1000**
$Na_2C_2O_4$	**0.1**	**13.390**	**1000**

***** Luminol solution was prepared by dissolving the required weight in a solution of (0.1 M Na_2CO_3) while $KMnO_4$ has been prepared and then standardized by (0.1M $Na_2C_2O_4$).

***** Hydrogen peroxide solution (1M) was prepared by diluting 48.85 ml of H_2O_2 (48%) in 1 L of distilled water and standardized against standard solution of 0.1 M $KMnO_4$.

***** 0.1 M of sulfuric acid was prepared by diluting 0.55 ml of concentrated acid (96%) in 100 ml of distilled water and standardized against 0.1 M Na_2CO_3. Cobalt (100 μg.ml^{-1}) was prepared by dissolving 0.4039 gm of $CoCl_2.6H_2O$ in 20 ml of 5 x10^{-3} M H_2SO_4 and diluted to 1L with distilled water. Finally, Amox. stock solution (100μg.ml^{-1}) was prepared by dissolving 0.1 gm of Amox. powder in 1L of (0.7 μg.ml^{-1}) cobalt (II) solution. Solutions of lower concentrations were prepared by appropriate dilution.

Pharmaceutical preparation

Amoxicillin (SDI Samara - Iraq). Ten capsules were taken and a certain portion of the powder was accurately weighed to give an equivalent to 500 mg of Amoxicillin and then dissolved with (25) ml of methanol. The resulting solution was washed by shaking with methanol and filtered on Whatman filter paper No.4 to remove any suspended particles. Then this solution was evaporated to dryness at 60C and the residue was redissolved in 25 ml distilled water to form a solution of 100 μg.ml^{-1}. The same method was adopted for preparation of Amoxicillin capsules, and finally dissolving in (0.7 μg. ml^{-1}) cobalt salt insolvent was carried out by ultrasonic bath.

***Procedure (1)*: Spectrophotometric determination of Amoxicillin in pharmaceutical preparation.**

General procedure for the spectrophotometric method

Initial rate method: Aliquots of 5×10^{-2} M $KMnO_4$ solution (3.0 ml) and 1.5M NaOH solution (1.5 ml) were transferred into a 25 ml volumetric flask. An accurate volume of the working solution of Amox. (3) ml was added and diluted to volume with distilled water. The contents of the mixture were shaken well and immediately transferred to the spectrophotometric cell at room temperature . The absorbance of the oxidation reaction of (Amox.) was found at 600 nm as a function of time against reagent blank. In the second procedure, the absorbance was measured at a fixed time of (20 min) and was plotted against the final concentration of Amox. and the content of the drug was calculated from either the calibration graph or regression equation.

Results and Discussion

In an alkaline medium, potassium permanganate oxidizes Amox. resulting in the formation of manganate ion[26], which showed an absorption peak at 600 nm. The various experimental parameters affecting the formation of the reaction product were optimized as follows:

Effect of the $KMnO_4$ concentration

To study the effect of the $KMnO_4$ concentration, aliquots of amoxicillin containing 10 μg .ml^{-1} were transferred into a series of 10 ml volumetric flasks, followed by addition of varying volumes of 0.05M $KMnO_4$ (0.5-5) ml and 1.5 ml of 1.5M NaOH solution. The absorbance at 600 nm was measured at a fixed time of 20 minute. It is obvious from figure (2) that the absorbance

increased with increasing volume of the $KMnO_4$ solution, and became constant at 3 ml of $KMnO_4$ so it used in subsequent experiment.

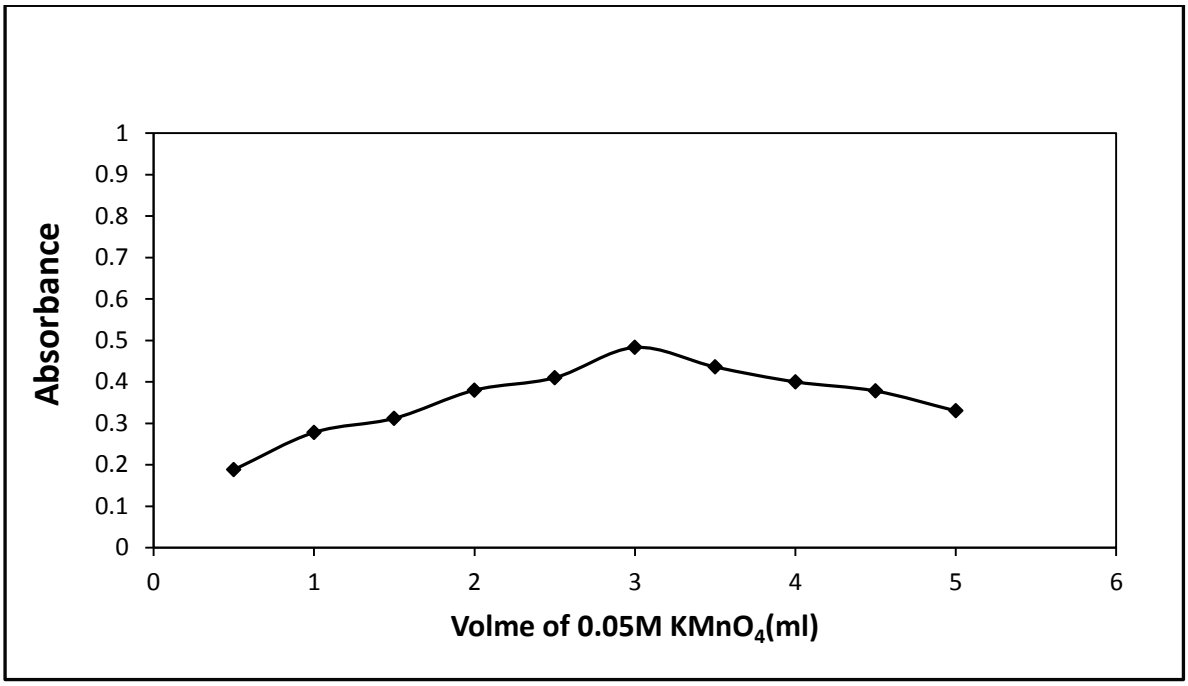


Fig. (2):- Effect of the volume of 0.05M $KMnO_4$ on the intensity of color produced for the reaction (Amox. 10µg / ml , 1.5 ml of 1.5 M NaOH).

Effect of the NaOH concentration

Influence of the NaOH concentration on the formation of MnO_4^{2-} was examined critically. Figure (3) shows that the maximum absorbance was obtained with 1.5 ml of the 1.5M NaOH, so the optimum volume of 1.5 ml was chosen.

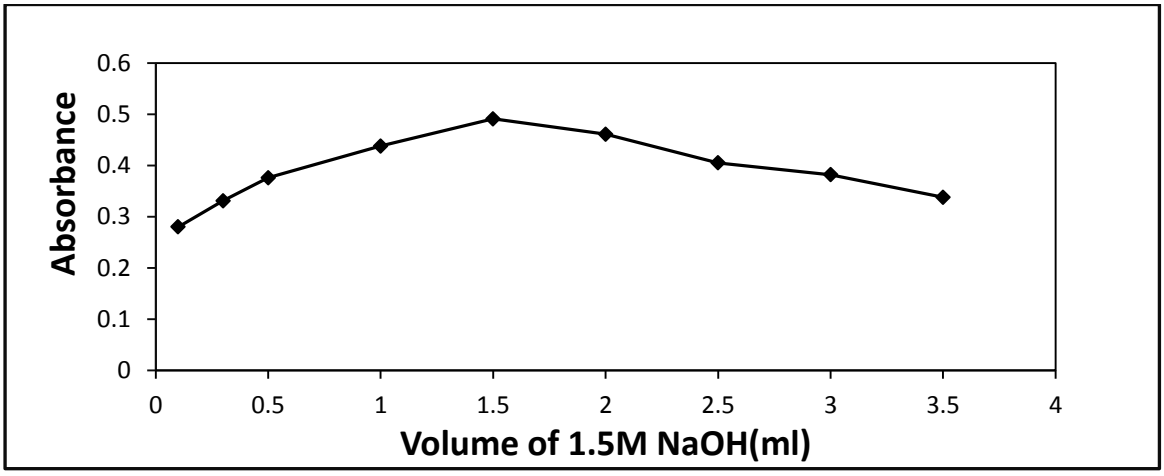


Fig.(3):- Effect of the volume of 1.5M NaOH on the intensity of color produced for the reaction (Amox. 10 µg /ml, 3.0 ml of 0.05 M $KMnO_4$).

Effect of oxidation time

It was found that the most acceptable value was obtained at a fixed time of 20 min. (Figure 4) and therefore was considered to be the most suitable time of oxidation reaction.

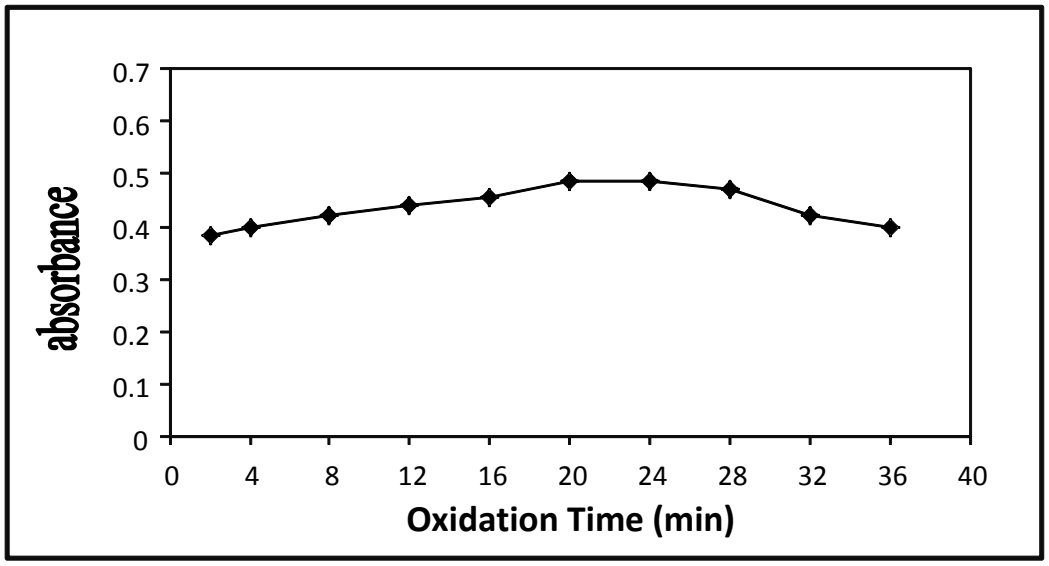


Fig.(4):- Effect of time on the oxidation of Amoxicillin by 0.05M $KMnO_4$

Final absorption spectrum of (Amox.)

A clear peak of MnO_4^{-2} at 600 nm (figure 5) was obtained after the oxidation of Amox. by 0.05M $KMnO_4$ solution in an alkaline medium of 1.5M NaOH.

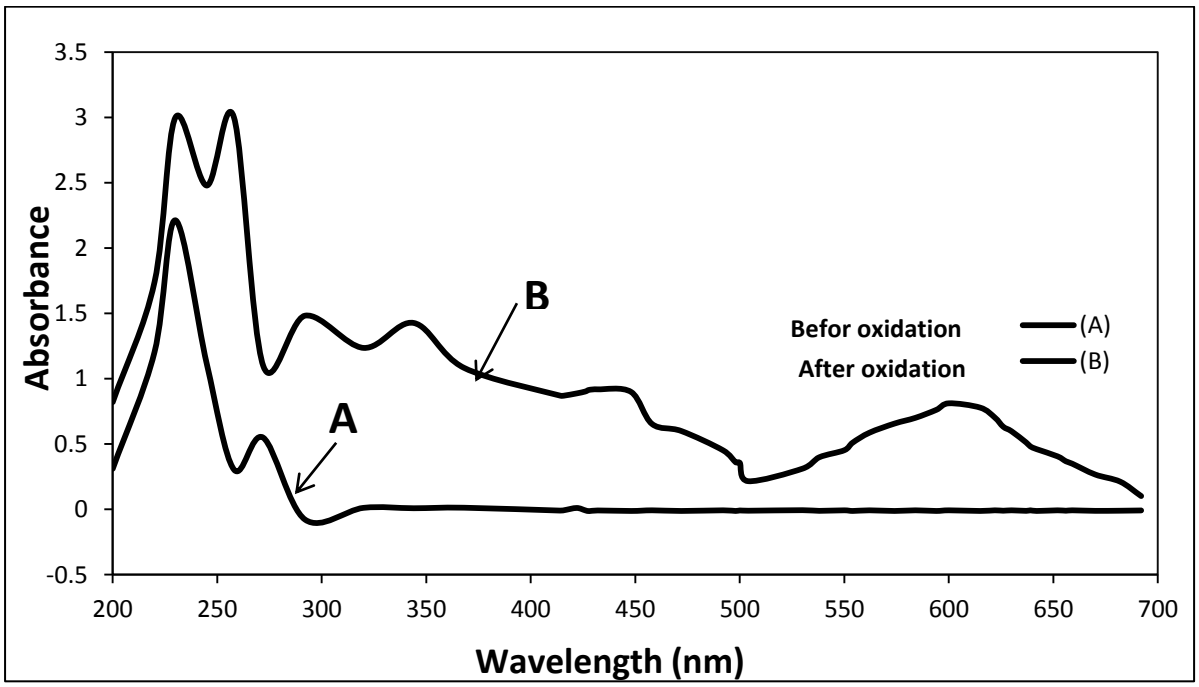
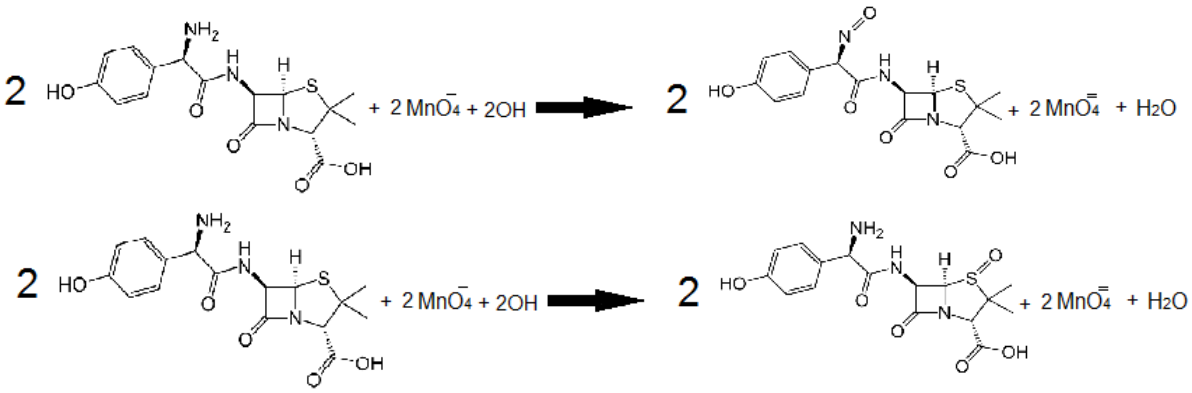


Fig.(5):- A - absorption spectrum of (Amox.) before oxidation with $KMnO_4$.
B - absorption after the oxidation of (Amox.) with $KMnO_4$.

Amoxicillin contains (NH_2) and (S) groups which has an ability to be reaction mechanism is proposed and given in the following equations [26]



Recommended analytical conditions

According to the obtained results, the optimum conditions for the determination of Amox. using a spectrophotometric method are given in table (3).

Table (3):- Optimum conditions for the determination of Amox. using the spectrophotometric method.

$KMnO_4$		NaOH		Fixed time	Wave length
Conc. M	**Vol.ml**	**Conc. M**	**Vol.ml**	**Min**	**nm**
0.05	3	1.5	1.5	20	600

Calibration graph

A linear calibration graph for amoxicillin (Fig.6) under the optimized conditions was obtained in table (3). Beer's law is obeyed over the concentration range of (5-50) $\mu g.ml^{-1}$ with correlation coefficient of 0.9995 and molar absorbance ϵ_{max} 2557.8 $l.mol^{-1}.cm^{-1}$and detection limit 4.76 $\mu g.ml^{-1}$.

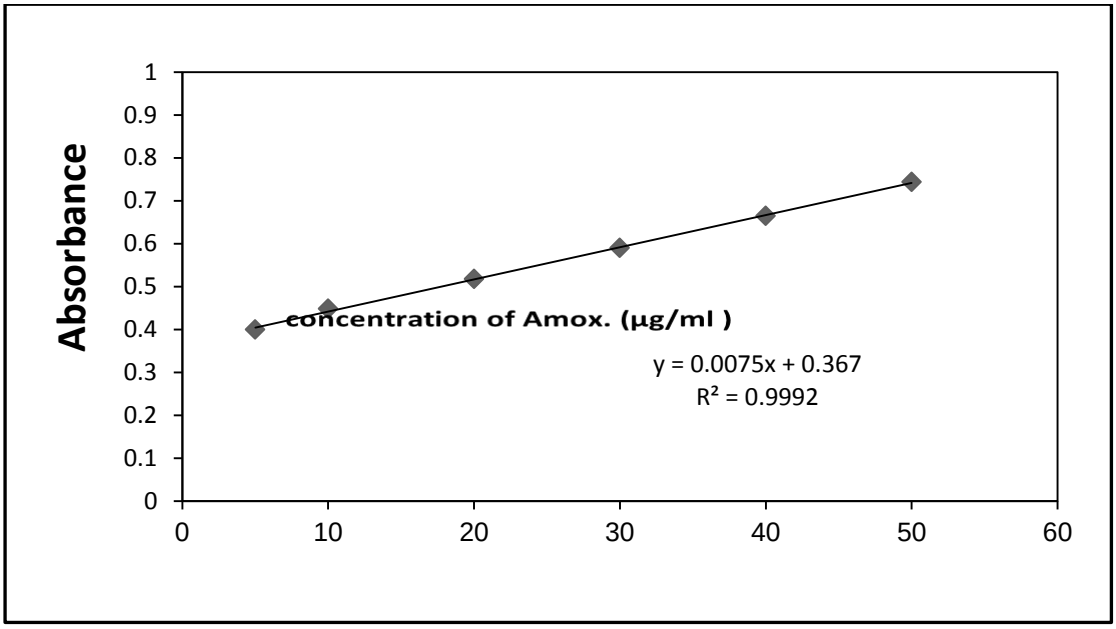


Fig.(6):- Calibration graph for the determination of Amox. by the spectrophotometric method.

The relative standard deviation for the method was ranged 1.84 -5.45 % (n = 6). Table (4) shows the accuracy and precision of the calibration graph. Table (5) shows summary of analytical data for the determination of amoxicillin using spectrophotometric method.

Table (4):- Accuracy and precision of the calibration graph for the determination of (Amox.) by spectrophotometric method.

Taken amount of Amox. µg/ml	Found amount of Amox. µg/ml	*Recovery (%)	Average recovery (%)	*RSD (%)	Detection limit
5.0	5.06	101.2	100.6	5.45	4.76
20.0	20.2	101.0		1.87	
50.0	49.85	99.7		1.84	

***Average of six determinations.**

Table(5):-Analytical data for the determination of Amox. by the spectrophotometric method.

Analytical data	Value
Linear range µg. ml^{-1}	5-50
Correlation coefficient	0.9995
Regression equation	Y= 0.0075 X + 0.367
RSD %	1.84
LOD µg. ml^{-1}	4.76
ε_{max} $L.mol^{-1}.Cm^{-1}$	2557.8

Pharmaceutical Application

The proposed method was applied for the determination of amoxicillin in capsules. The result in table (4) refer to good precision and recovery. This method was successfully compared with the British Pharmacopoeia[(2)] standard method. The results obtained are summarized in table(6).

Table (6):- Application of the proposed Spectrophotometric method for the determination of Amox. in Pharmaceutical Preparations

Sample	Recovery %		RSD %
	*Proposed method	Standard method	
Pure amoxicillin	101.20	99.83	1.84
Amoxidal	100.54	99.69	1.80

***Average of six determinations**

Procedure (2): Determination of Amoxicillin using a FIA-CL method.

General procedure for the FIA – Cl method*:*

The FIA-CL scheme is outlined in Figure (1). Various concentrations of

Amox. were prepared in Co^{2+} (0.7 $\mu g.ml^{-1}$) solution. 200 µl aliquot of each solution was injected through the sample loop into the stream of Co^{2+} solution, which then was combined with luminol and hydrogen peroxide streams in the flow cell which situated in front of the photomultiplier tube (PMT).

Results and discussion

The chemiluminescence of luminol–hydrogen peroxide–Co (II) System is very intense [27]. However, in this work, trace amounts or Amox. were found to be strongly activate chemiluminescence signal of this system.

Effect of reagents concentration

The effect of luminol concentration on the net chemiluminescence intensity was studied . Different concentrations of luminol (1×10^{-5} –8×10^{-5} M) were used to establish the best emission intensity – time profile that can be obtained. Figure (7) shows that (5×10^{-5} M) of luminol is the optimal concentration.

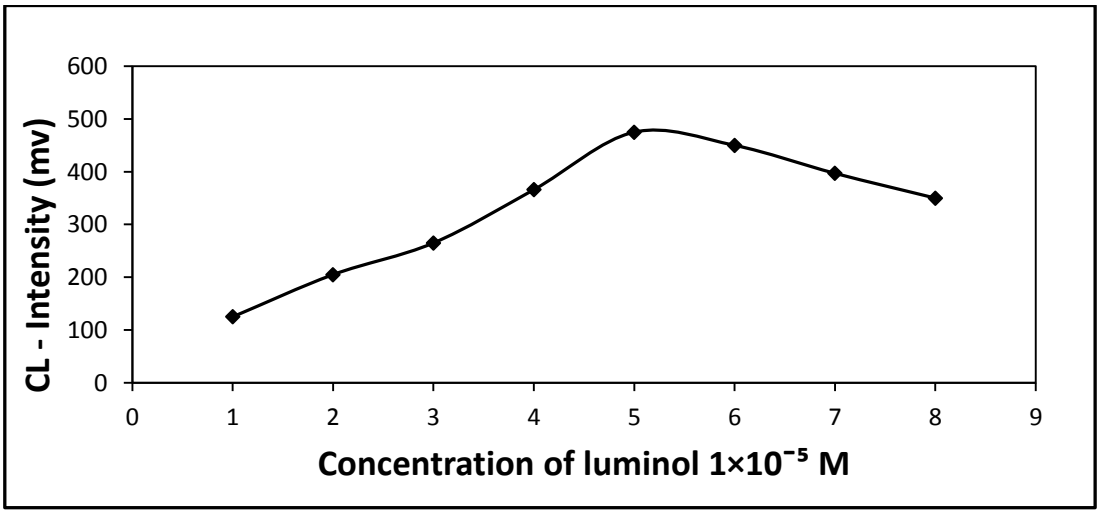


Fig. (7):- Effect of concentration of Luminol on the CL-intensity.

Effect of hydrogen peroxide concentration

The effect of H_2O_2 concentration was investigated; from the results of Figure (8), the concentration of (1×10^{-2} M) H_2O_2 was selected to be the optimum concentration.

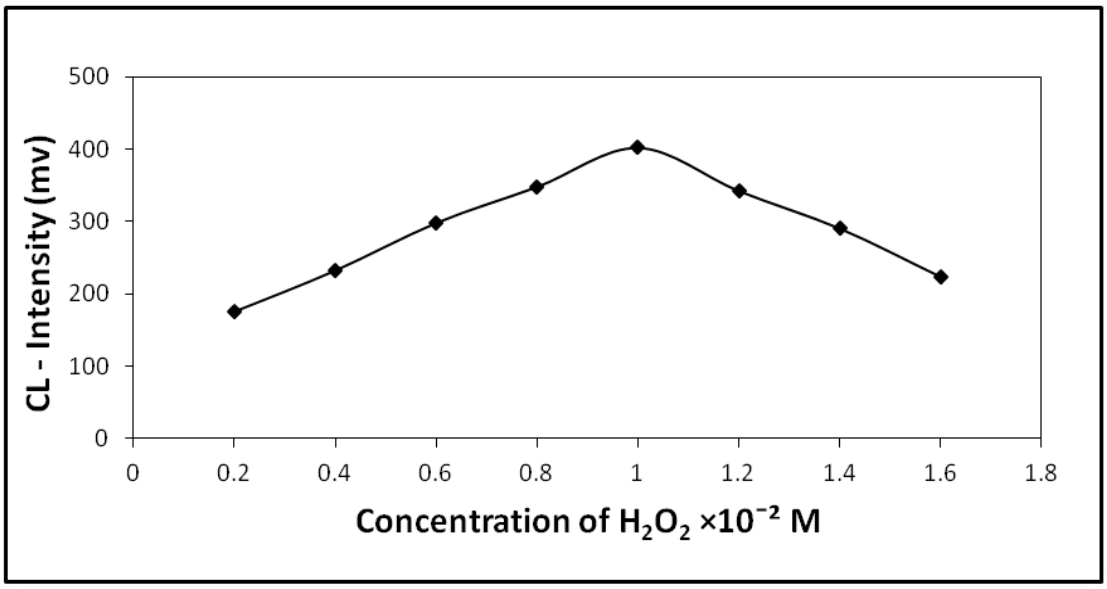


Fig.(8):- Effect of concentration of H_2O_2 on the CL-intensity

Effect of Sulphuric acid and Cobalt Concentrations

The effect of the acidity of cobalt Co^{2+} solution was also studied; three concentration of cobalt Co^{2+} (0.2, 0.5, 0.7) µg. ml^{-1} with varying concentrations of (5×10^{-3} - 1×10^{-4}) M of H_2SO_4 were investigated. The best intensity was obtained at the concentration of ($5x10^{-3}$) M of H_2SO_4. The effect of cobalt concentration was also studied, figure (9) Shows that the Concentration of Co^{+2} is (0.7 µg / ml^{-1}) gives a suitable CL-intensity

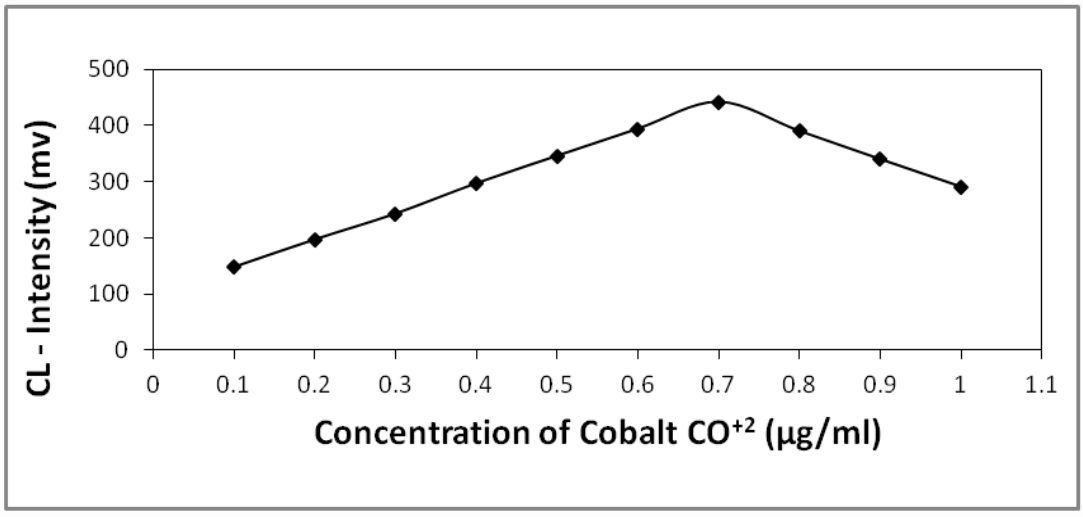


Fig.(9):- Effect of Cobalt concentration on the CL-intensity.

It is worth noting here that as the concentration of H_2SO_4 increases the CL intensity increases, this is because of increasing the catalyzed oxidation of luminol by H_2O_2 [27]. At $1x10^{-2}$ M H_2SO_4 the CL intensity decreases, this fact can be attributed to the cleavage of the formed fluorescent compound.Table (7) shows that as the concentration of H_2SO_4 increases, the

time of analysis decreases accompanied with decreasing band width.

Table (7):- Effect of sulphuric acid concentration on band width and time of analysis.

H_2SO_4 Concentration (M)	Base band width (mm)	Time of analysis (sec)
$1x10^{-4}$	30.0	80.0
$5x10^{-4}$	28.0	72.0
$1x10^{-3}$	25.0	55.0
$5x10^{-3}$	15.0	49.0
$1x10^{-2}$	9.8	44.0

Effect of flow rate

A flow rate ranged from (1-10) ml/ min was investigated. Table (8) shows that the CL intensity increased with the increase of flow rate. However, a flow rate of 5ml/min is recommended for all streams because of satisfactory CL intensity, less reagent consumption and short analysis time.

Table (8):- Effect of flow rate on the emission intensity, analysis time and peak width using 0.7 µg. ml^{-1} of Co^{+2}

Flow rate ml/min	Intensity (m v)	Analysis time (sec)	Peak width mm
1.0	223	70	26
2.0	278	45	18
3.0	489	42	12
4.0	550	38	10
5.0	670	35	8
6.0	720	32	7
7.0	743	27	6

8.0	776	25	5
9.0	820	21	4
10.0	842	15	3

Recommended Analytical Conditions

A Summary of the optimum experimental conditions for the determination of Amox. in pharmaceutical preparations are in Table (9).

Table (9): The recommended analytical conditions for the determination of Amoxicillin using FIA – Chemiluminescence system

Parameter	Recommended value
Conc. Of luminal	$5x10^{-4}$ M
Conc. Of H_2O_2	$1x10^{-2}$ M
Conc. Of Co^{+2}	0.7 µg. ml^{-1}
Conc. Of H_2SO_4	$5x10^{-3}$ M
Flow rate	5 ml/ min
Volume of Co^{+2} injected	200 µl

Calibration graph

A calibration graph of relative chemiluminescence intensity against the Amoxicillin concentration was established by applying the optimal conditions . The regression equation is (Y = 13.42X + 1.4643), and the linearity is in the range of (8-32) µg . ml^{-1} of Amox. (Fig.10)

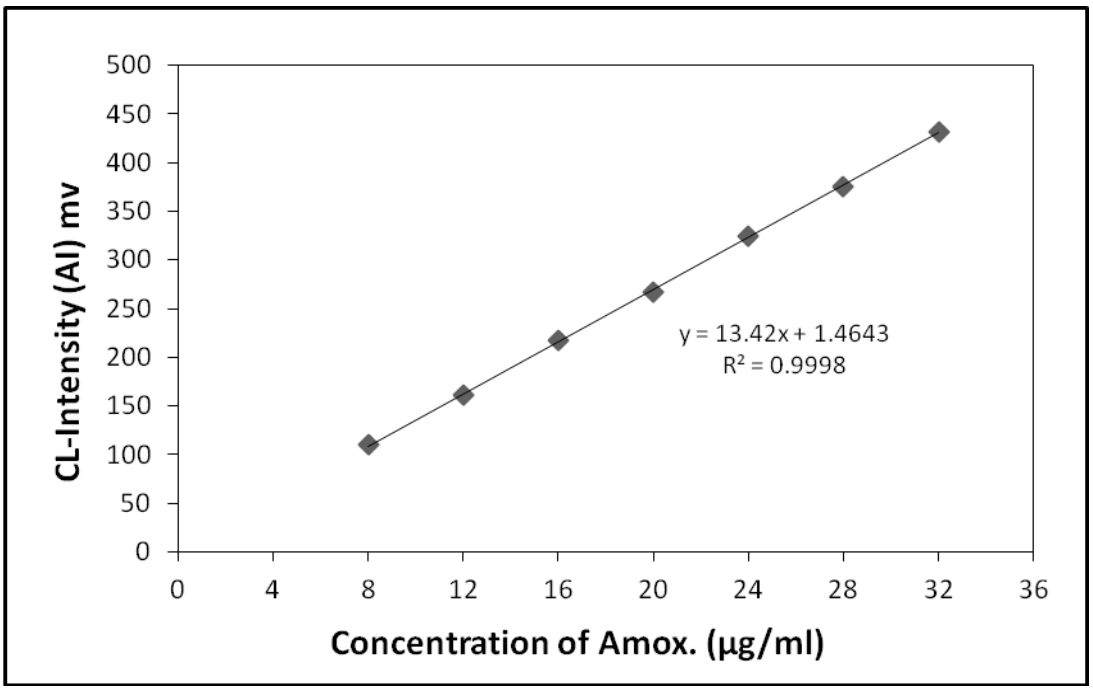


Fig.(10):- Calibration graph of relative Chemiluminescence intensity against the Amox. concentration.

The analytical data obtained from the calibration graph are summarized in table (10)

Table (10):- Analytical data for the determination of amoxicillin using FIA-CL method.

Analytical data	Value
Linear range µg. ml^{-1}	8-32
Correlation coefficient	0.9998
Regression equation	Y= 13.42 X+1.4643
RSD %	1.48
Average Recovery %	98.9
LOD µg. ml^{-1}	6.2

Interferences

Amoxicillin is usually formulated in capsules, and injections forms, therefore, the effect of some common excipient substances usually present in pharmaceutical preparation were investigated. The presence of Croscarmellose Sodium, Magnesium stearate and Iron oxides gave no significant interfering effect on the

chemiluminescence intensity of Amoxicillin.

Application of developed FIA-CL method for the determination of Amox. in pharmaceutical preparations;

Amoxidal capsule containing amoxicillin was analyzed using the developed method and the results are compared with the British pharmacopoeia[2] standard method, Table(11).

Table (11):- Application of the proposed method for the determination of Amox. in Pharmaceutical Preparations

Sample	Recovery %		*RSD %
	*Proposed method	Standard method	
Pure amoxicillin	98.9	99.3	1.48
Amoxidal	98.4	99.5	1.39

***Average of six determinations**

Comparison between the two methods

The two proposed methods were compared with other methods as shown in table (12). The value (0.45) of calculated (F test) for FlA-CL / spectrophotometric methods is much less than the value (4.95) for tabulated (F test) which indicates good agreement.

Table (12):- The statistical comparison of results for the spectrophotometric and FIA-CL method

The method	Linearity (µ g/ml)	Correlation Coefficient (r)	Recovery %	RSD %	L.O.D µg.ml	Reference
Spectrophotometri*c*	5.0-50.0	0.9992	100.6	1.84	4.76	Present method
FIA-CL	8.0-32.0	0.9998	98.9	1.48	6.2	Present method

FIA-CL	0.1-50 mg/L	-	-	0.3-0.6	0.05 mg/L	(14)
FIA-CL	0.1-10 mg/L	-	-	0.8-2.0	-	(15)
FIA-CL	0.05-10	-	-	2.3	0.02	(16)
HPLC	0.15-600	-	-	-	0.05	(23)
Spectrophotometric	0.6-24	0.9999	99.92	1.83	-	(5)
Spectrophotometric	7.5-75	–	–	1	-	(7)
Polarography	0.15-7.3	0.9986	98.0	-	-	(25)

Conclusion

This study included two main parts: The first part involved the development of a simple kinetic spectral method for the determination of amoxicillin based on oxidation of this drug with KMnO4 and spectral follow-up by measuring the change in absorption at a wavelength of 600 nanometres. In the second part the chemiluminescence was found to be activated by Amox. and this is the base of the developed new method. The proposed method offers advantages of simplicity, rapidity, high sensitivity and low reagent consumption. These two methods were successfully applied to pharmaceuticals and had percentage recovery (98.9%).

References

1. Amoxicillin (http: // www.drug. Com/ monograph/ Amoxicillin. html) The American Society of the health – system pharmacists. (2011).
2. British pharmacopoeia vol. 2, (2002).
3. G. A. Saleh, (1996), Analyst, 121, pp. (641-645)
4. T. S. Al-Ghabsha and T. V. Alsabha (2001), accepted in Raf. J. Sci.
5. F. M. AL –Esawati, (2002), M. Sc. Thesis, Mosul university.
6. Th. Rojanarata, P. Opanasopit, T. Ngawhirunpat, Ch. Saehuan, S. Wiyakrutta, V. Meevootisom, Enzyme and Microbial Technology, Vol. 46, Issues 3–4, (2010), PP. (292-296) .
7. G.G Mohamed, Cairo University, Giza, Egypt, Journal of Pharmaceutical and Biomedical Analysis, Vol. 24, Issue 4, (2001), PP. (561–567).
8. A. Pasamontes, M.P. Callao Analytica Chimica Acta, Vol. 485, Issue 2, (2003), PP.(195-204).
9. A. Pasamontes, M.P Callao, Analytica Chimica Acta, Vol. 515, Issue 1, (2004), PP.(159-165).
10. M.F. El-Shahat, N. Burham, S.M. Abdel Azeem, Journal of Hazardous Materials, Vol. 177, Issues 1–3,(2010), PP. (1054-1060).

11. K. Mervartová, M. Polášek, J. M. Calatayud, Journal of Pharmaceutical and Biomedical Analysis,Vol. 45, Issue 3, (2007), PP. (367–381).
12. Ch. Thongpoon, B. Liawruangrath, S. Liawruangrath, R. Alan Wheatley, A. Townshend, Journal of Pharmaceutical and Biomedical Analysis, Vol. 42, Issue 2, (2006), PP. (277–282).
13. F. Hasanpour, Ali A. Ensafi, T. Khayamian, Analytica Chimica Acta, Vol. 670, Issues 1–2,(2010), PP.(44-50).
14. Li Yinhuan, Lu. Jiuru, Luminescence(Journal of Biological and Chemical Luminescence) Vol. 21, Issue 4, (2006), pp. (251–255) .
15. Yuhai Tang, Hong Yao, Junmei Fu., Luminescence (Journal of Biological and Chemical Luminescence, Vol. 18, Issue 6, (2003), pp. (313–317).
16. Du Jianxiu, Li Yinhuan and Lu Jiuru, Analytical Letters**,** Volume 35, Issue 14, (2002) .
17. M Hernández, F Borrull, M Calull, Journal of Chromatography B: Biomedical Sciences and Applications, Vol. 731, Issue 2, (1999), PP. (309-315).
18. P. K. Brahman, R. A. Dar, K. S. Pitre, Sensors and Actuators B: Chemical, Vol. 176, (2013), PP. (307-314).
19. S. De Baere, P. De Backer, Analytica Chimica Acta, Vol. 586, Issues 1–2, (2007), PP. (319-325).
20. J. Haginaka, J. Wakai Journal of Chromatography B: Biomedical Sciences and Applications**,** Vol. 413, (1987), PP. (219–226).
21. Herman, J. Mascher, Christian kikuta, J. of chromatography A, vol. 812, Issues 1,2,3 (1998), pp. 221-226 .
22. G. Hoizey, D. Lamiable, C. Frances, Th. Trenque, M. Kaltenbach, J. Denis, H. Millart, Journal of Pharmaceutical and Biomedical Analysis, Vol. 30, Issue 3, (2002), PP.(661-666) .
23. N. Tavakoli, J. Varshosaz, F. Dorkoosh, M. R. Zargarzadeh Journal of Pharmaceutical and Biomedical Analysis, Vol. 43, Issue 1, (2007), PP. (325-329) .
24. B. Lugoboni, T. Gazzotti, E. Zironi, A. Barbarossa, G. Pagliuca
Journal of Chromatography B, Vol. 879, Issue 21, (2011), PP.(1980-1986).
25. S. J. lyle, Sheikheldin S. Yassin, chemistry Dept., king Fahd university of petroleum and Minerals, Dhahran 31261 (2001).
26. N. Rahman, M. Kashif, Analytical Science, Vol. 19, (2003), PP. (907).
27. Matindal: The extra pharmacopoeia, (1993), the pharmaceutical press, London 30th ed. PP. (901).