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Research Article

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A Spectroscopic Study of Interactions Amoxicillin Sodium With Iodine in Acetonitrile and Formation of Complexes.

Abdul Aziz Ramadan[1*], Hasna Mandil[2], Jenan Sabouni[3]

Department of Chemistry, Faculty of Science, University of Aleppo, Syria.

*[1]*E-mail: dramadan@scs-net.org* or *dramadan1946@gmail.com;*

[2] *E-mail: promandil955@gmail.com*

[3]*E-mail: jenansabouni@gmail.com*

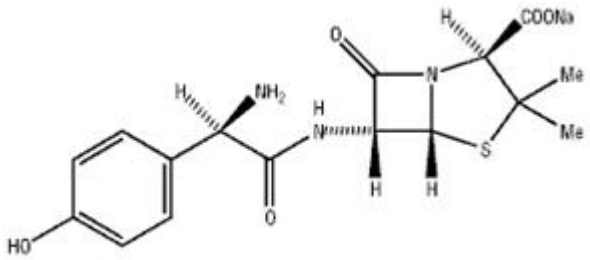
Abstract

A spectroscopic study of interactions amoxicillin sodium (Amo) with iodine in acetonitrile medium and formation of complexes was studied. The method is based on two charge transfer complex's the drug with iodine. The first complex ($AmoI^+.I^-$) is formed when the concentration of iodine is much lower than the concentration of amoxicillin and therefore a peak of this complex occurs at wavelengths 206 and 246 nm and the absorbance's were proportional to the concentration of iodine at range 1.00 to 10.00 µM in present of Amo $5.0x10^{-5}$ M. The second complex ($AmoI^+.I_3^-$) is formed occurs at wavelengths 290 and 360 nm when the drug concentration is two or more times less than iodine concentration. Under these optimized experimental conditions, Beer's law is obeyed in the concentration ranges 0.387-15.495 µg.mL^{-1} for amoxicillin sodium. The method was validated for linearity, precision and accuracy, repeatability, sensitivity and robustness. The method was successfully applied for determination of Amo in pure and pharmaceutical formulations samples with relative standard deviations did not exceed 2.9% for the concentrations of Amo (0.387 µg.mL^{-1}). This is simple, accurate and sensitive spectrophotometric method gives good results for the determination of Amo in bulk and different dosage forms.

Keywords: Amoxicillin sodium, Spectrophotometric method, Charge transfer complexes, Iodine.

Introduction

Amoxicillin sodium (Amo), chemically known as 6-(p-hydroxy-alpha-amino phenyl acetamido) penicillanic acid. Its molecular weight is 387.386 g/mol and its molecular formula is $C_{16}H_{18}N_3NaO_5S$. It is a white powder and has a water solubility of 958 $mg.mL^{-1}$, see Scheme 1[1].



Scheme 1: Chemical structure of amoxicillin sodium

Previous research that identified amoxicillin in its pure form and in pharmaceutical preparations was identified according to different methods including spectrophotometry [2-7], high-performance liquid chromatography [8], IR, NMR and mass spectra [9], capillary electrophoresis [10]. Amoxicillin has also been identified spectrally in conjunction with other compounds [11-16].

In the present work, a spectrophotometric method for the determination of amoxicillin sodium with iodine has been studied. The method is based on charge transfer complexation reaction of the drug with iodine in acetonitrile. And formation two complex's, the first complex $AmoI^+.I^-$ and the second complex $AmoI^+.I_3^-$ were formed.

Experimental

Reagents

Amoxicillin sodium (99.89%) was purchased from Lu Kang Drugs and Reagents Company (Shandong, China). All reagents (as iodine, acetonitrile and others) were of analytical grade from Merck.

Instruments and apparatus

Spectrophotometric measurements were made in T90+UV-VIS with 1.0 cm quartz cells. The diluter pipette model DIP-1 (Shimadzu), having 100 µL sample syringe and five continuously adjustable pipettes covering a volume range from 20 to 5000 µL (model Piptman P, GILSON). SARTORIUS TE64 (0.01 mg) electronic balance was used for weighing.

A stock standard solution of iodine ($1x10^{-2}$ $mol.L^{-1}$)

Dissolving 63.58 mg of iodine with acetonitrile into volumetric flask (25 mL) and diluting to mark by acetonitrile.

A stock standard solution of amoxicillin sodium ($1x10^{-3}$ $mol.L^{-1}$)

An accurately weighed 3.878 mg standard sample of amoxicillin sodium was dissolved in 0.5 mL methanol, transferred into a 10 mL standard flask and diluted to the mark with acetonitrile and mixed well.

Working solutions

The stock solutions were further diluted to obtain working solutions daily just before use in the ranges of Amo: 1.00, 2.00, 4.00, 6.00, 8.00, 10.00, 15.00, 20.00, 25.00, 30.00, 35.00, and 40.00 $\mu mol.L^{-1}$ (0.387, 0.775, 1.550, 2.324, 3.099, 3.874, 5.811, 7.748, 9.685, 11.622, 13.559, and 15.495 $\mu g.mL^{-1}$) by dilution of the volumes:

0.010, 0.020, 0.040, 0.060, 0.080, 0.100, 0.150, 0.200, 0.250, 0.300, 0.350, and 0.400 mL from stock standard solutions of amoxicillin sodium $1x10^{-3}$ mol.L^{-1} into 10 mL volumetric flask, then added 0.100 mL from stock standard solution of iodine ($1x10^{-2}$ mol.L^{-1}) and diluted to 10 mL with acetonitrile.

Samples

Commercial formulations (as vial) were used for the determination of Amo. The pharmaceutical formulations were subjected to the analytical procedures:

(1) ***AMOXYDINE*** vial, **ASIA PHARMACEUTICAL INDUSTRIES**, Aleppo - SYRIA, each vial contains: 1000 mg of amoxicillin sodium.

(2) ***AMOXYDINE*** vial, ***ASIA PHARMACEUTICAL INDUSTRIES***, Aleppo - SYRIA, each vial contains: 500 mg of amoxicillin sodium.

(3) ***Panamoxy*** vial, **ELSAAD PHARMACEUTICAL**, Aleppo - SYRIA, each vial contains: 1000 mg of amoxicillin sodium.

Stock solutions of pharmaceutical formulations

Contents of 5 vials of each studied pharmaceutical formulations were weighted accurately and mixed well. An amount of the powder equivalent to the weight 10.00 mg of samples were solved in 0.5 mL methanol, transferred into a 10 mL standard flask and diluted to the mark with acetonitrile and mixed well; this solution contents $2.581x10^{-3}$ mol.L^{-1} (1.00 mg.mL^{-1}) of Amo for all studied pharmaceutical formulations.

Working solutions of pharmaceuticals

These solutions were prepared daily by diluting 100 µL (0.100 mL) from stock solutions of pharmaceutical formulations into 10 mL volumetric flask, then added 0.100 mL from stock standard solution of iodine ($1x10^{-2}$ mol.L^{-1}) and diluted to 10 mL with acetonitrile; these solutions content 10.00 µg.mL^{-1} of Amo.

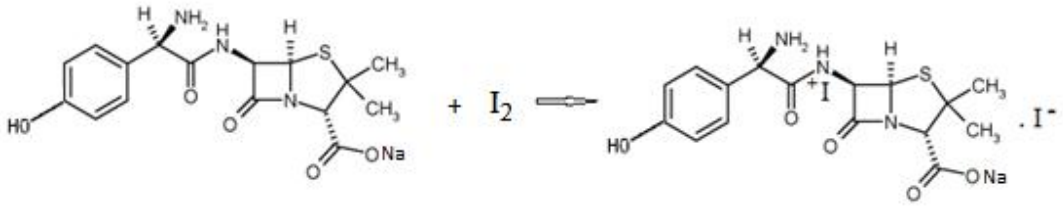
Results and Discussion

Analytical procedure

A spectrophotometric method for the determination of amoxicillin sodium with iodine has been studied. The method is based on two charge transfer complex's the drug with iodine. The first complex ($AmoI^+.I^-$) is formed when the concentration of iodine is much lower than the concentration of Amo and therefore a peak of this complex occurs at wavelengths 206 and 246 nm and the absorbance's were proportional to the concentration of iodine at range 1.00 µM (0.25381 µg.mL^{-1}) to 10.00 µM (2.5381 µg.mL^{-1}) in present of drug 50.0 µM. The second complex ($AmoI^+.I_3^-$) is formed occurs at wavelengths at 290 and 360 nm when the drug concentration is two or more times less than iodine concentration.

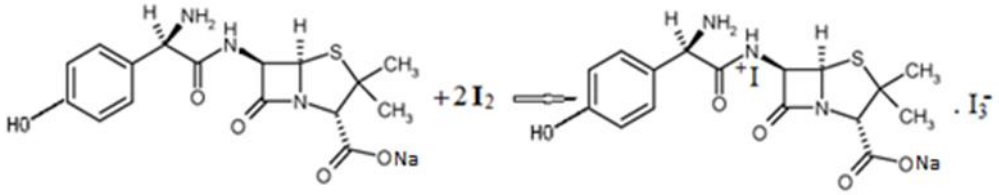
Spectrophotometric results

UV-Vis spectra of iodine, Amo, $AmoI^+.I^-$ and $AmoI^+.I_3^-$ solutions in acetonitrile were studied. The iodine solutions absorb at λ_{max} 465 nm, 360 nm and 290 nm (ε=830, 275 and 550 L. mol^{-1}. cm^{-1}, respectively). The Amo solutions do not absorb in range 280-700 nm, but absorb at λ_{max} 276 nm and 225 nm (ε=1440 and 9720 L. mol^{-1}. cm^{-1}, respectively). The $AmoI^+.I^-$ complex was formed when $C_{I2} < C_{Amo}$ according to reaction (1) and absorb at $\lambda_{max,1}$ 246 nm and $\lambda_{max,2}$ 206 nm (ε=28800 and 31000 L. mol^{-1}. cm^{-1}, respectively). The $AmoI^+.I_3^-$ complex was formed when $C_{I2} > 2C_{Amo}$ according to reaction (2) and absorb at $\lambda_{max,1}$ 290 nm and $\lambda_{max,2}$ 360 nm ($\varepsilon_{max,1}$=46000 and $\varepsilon_{max,2}$=23600 L. mol^{-1}. cm^{-1}), see Figure 1.



(1)

Amoxicillin sodium (Amo) $AmoI^{+}.I^{-}$



(2)

Amo $AmoI^{+}.I_3^{-}$

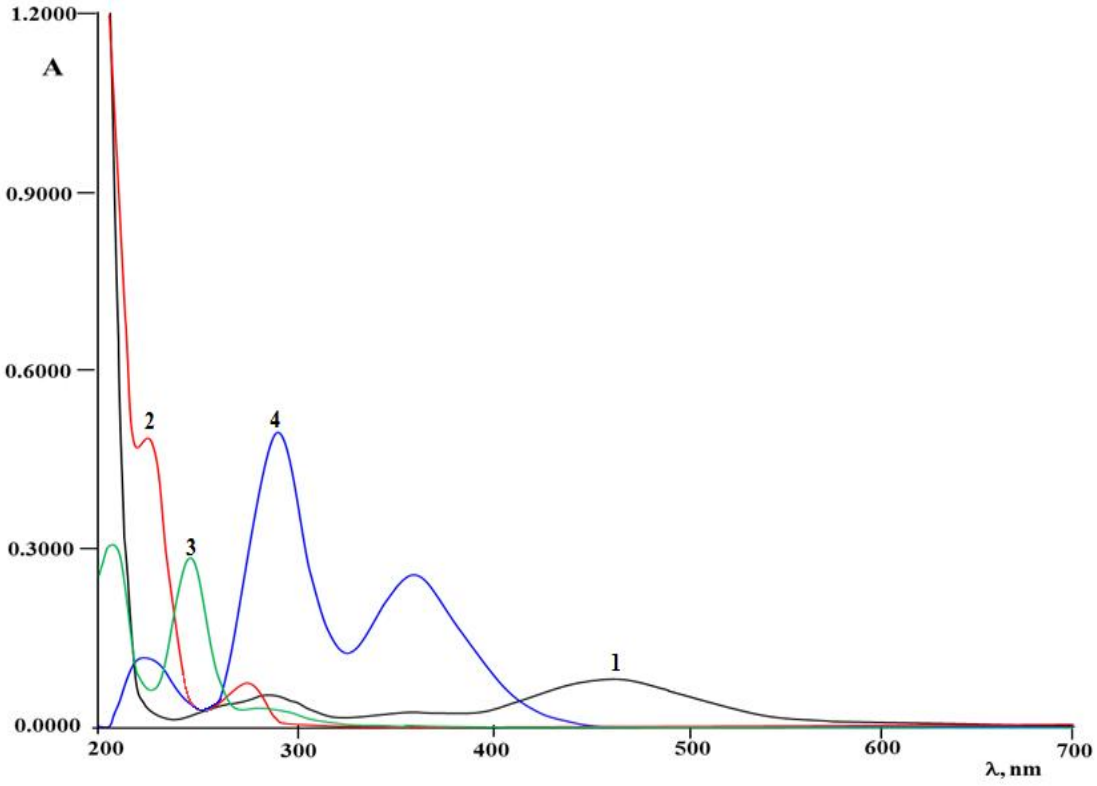
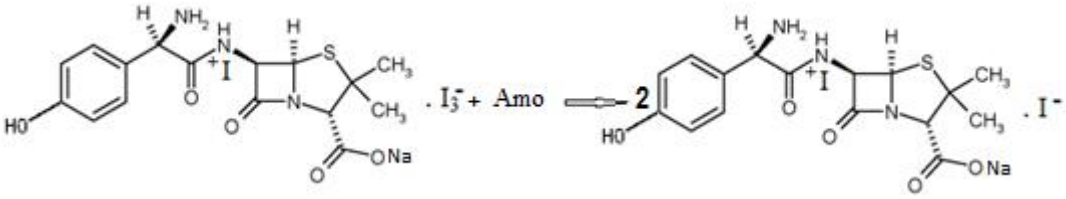


Fig. 1: UV-Vis spectra in acetonitrile of: 1- iodine $1.0x10^{-4}$ $mol.L^{-1}$; 2- amoxicillin sodium (Amo) $5.0x10^{-5}$ $mol.L^{-1}$; 3- Amo $5.0x10^{-5}$ $mol.L^{-1}$ with I_2 $1.0x10^{-5}$ $mol.L^{-1}$; 4- Amo $1.0x10^{-5}$ $mol.L^{-1}$ with I_2 $1.0x10^{-4}$ $mol.L^{-1}$ { blank for (1-2) acetonitrile, (3) Amo $5.0x10^{-5}$ $mol.L^{-1}$and (4) I_2 $1.0x10^{-4}$ $mol.L^{-1}$, ℓ =1.0 cm}.

Studying of reaction amoxicillin sodium with iodine:

The effect of time on the interaction between amoxicillin sodium and iodine in acetonitrile where the concentration of Amo is greater than twice the concentration of iodine (C_{Amo} >$2C_{I2}$) was also studied. The second complex ($AmoI^+.I_3^-$) formed significantly more than the first complex ($AmoI^+.I^-$) according to equation (2); although the iodine concentration is less than half the concentration of amoxicillin sodium and this is because the formation constant of the second complex ($AmoI^+.I_3^-$) is greater than the formation constant of the first complex ($AmoI^+.I^-$). After 160 min all the second complex converted to the first complex according to reaction (3), see Figure 2.

We also notice from Figure 2 (when C_{Amo}=$2.8C_{I2}$, C_{Amo}=$1.4x10^{-5}$ mol.L^{-1} and C_{I2}=$5x10^{-6}$ mol.L^{-1}) that, the peak of the second complex at the wavelength of 360 nm after 160 min has completely disappeared and the peak at the wavelength of 246 nm which indicates the first complex has become fixed (the concentration of first complex, $AmoI^+.I^-$, became $5x10^{-6}$ mol.L^{-1} and amoxicillin sodium $9x10^{-6}$ mol.L^{-1} remained as excess).



(3)

$AmoI^+.I_3^-$ $AmoI^+.I^-$

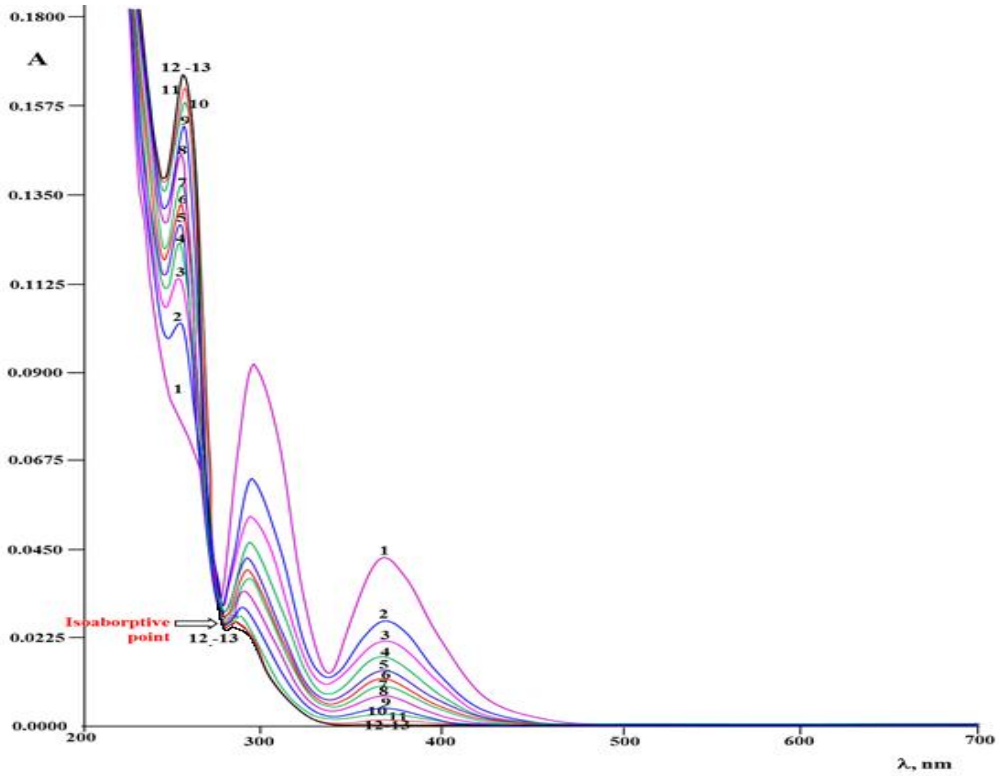


Fig. 2: The effect of time on UV-Vis spectra in acetonitrile of iodine $5.0x10^{-6}$ mol.L^{-1} with $1.4x10^{-5}$ mol.L^{-1} amoxicillin sodium (Amo): 1– 10, 2 – 20, 3 – 30, 4 – 40, 5 – 50, 6- 60, 7- 70, 8- 80, 9- 100, 10- 120, 11- 140, 12- 160 and 13- 180 min, respectively (blank is acetonitrile, ℓ =1.0 cm).

Calibration curve for determination of iodine

Calibration curve for determination of iodine in present Amo when $C_{I2} < C_{Amo}$ (Where only the first complex $AmoI^+.I^-$ is formed). It was found that, the linearity over concentration of iodine at range 1.00 µM (0.25381 µg.mL^{-1}) to 10.00 µM (2.5381 µg.mL^{-1}) in presence of 5.0×10^{-5} mol.L^{-1} of Amo in acetonitrile.

Regression equations at $^{a}_{max,1}$ 246 nm and $^{a}_{max,2}$ 206 nm were as the follows: $y_1=0.02880x+0.00045$ ($R^2=0.9996$) and $y_2=0.03100x+0.00102$ ($R^2=0.9987$) respectively in acetonitrile, see Figure 3 and Table 1.

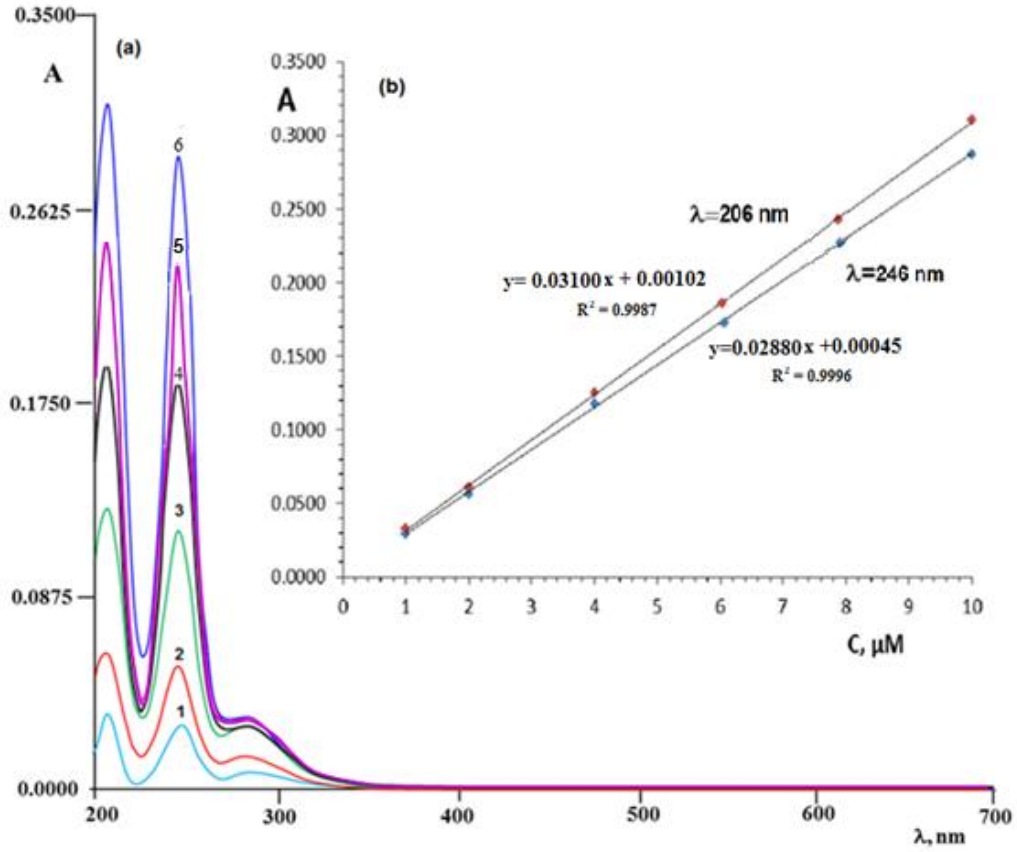


Fig 3: Spectra and calibration curve of $AmoI^+.I^-$ complex in presence of 5.0×10^{-5} M of Amo; where concentration of iodine as the follows: 1- 1.00, 2- 2.00, 3- 4.00, 4- 6.00, 5- 8.00 and 5- 10.00 µM (ℓ = 1.0 cm , blank is 5.0×10^{-5} M of Amo in acetonitrile).

Table 1. The parameters established for spectrophotometric determination of iodine by complex formation with amoxicillin sodium by formation of $AmoI^+.I^-$ complex in acetonitrile.

Parameters	Operating values
$\lambda_{max,1}$ of $AmoI^+.I^-$ complex, nm	246
$\lambda_{max,2}$ of $AmoI^+.I^-$ complex, nm	206
Beer's Law Limit, for iodineby µM	1.00 - 10.00
Molar absorptivity of $AmoI^+.I^-$ complex (ε_1), $L.mol^{-1}.cm^{-1}$	28800
Molar absorptivity of $AmoI^+.I^-$ complex (ε_2), $L.mol^{-1}.cm^{-1}$	31000
Regression equation for $AmoI^+.I^-$ at $\lambda_{max,1}$ =246 nm:	
Slope	0.02880
Intercept	0.00045
Correlation coefficient (R^2)	0.9996
Regression equation for $AmoI^+.I^-$ at $\lambda_{max,2}$ =206 nm:	
Slope	0.03100
Intercept	0.00102
Correlation coefficient (R^2)	0.9987
C_{Amo}: C_{I_2}, M	≥5:1
Stability	200 h
Temperature of solution	25±5°C

n=5, t=2.776.

Analytical results determination of iodine

Spectrophotometric determination of iodine through complex formation with $5.0x10^{-5}$ M of Amo in acetonitrile within optimal conditions using calibration curve was applied. The results of determined concentration of iodine was rectilinear over the range of 1.00 – 10.00 µM (0.25381 - 2.5381 $\mu g.mL^{-1}$), with relative standard deviation (RSD) not more than 3.5% at $\lambda_{max,1}$=246 nm, see Table 2.

Table 2: Spectrophotometric determination of iodine through complex formation with 5.0×10^{-5} M of Amo within optimal conditions using calibration curve in acetonitrile.

X_i, (Taken) µmol.L^{-1}	[a], nm	* $\bar{x}$ ±SD, µmol.L^{-1} (Found)	$\bar{x} \pm \frac{t.SD}{\sqrt{n}}$ µmol.L^{-1}	RSD%
1.00	206	1.04±0.050	1.04±0.062	4.8
	246	1.01±0.035	1.01±0.044	3.5
2.00	206	1.99±0.094	1.99±0.116	4.7
	246	2.00±0.066	2.00±0.082	3.3
4.00	206	4.02±0.177	4.02±0.220	4.4
	246	4.01±0.124	4.01±0.154	3.1
6.00	206	6.00±0.246	6.00±0.305	4.1
	246	5.99±0.174	5.99±0.216	2.9
8.00	206	7.99±0.296	7.99±0.367	3.7
	246	8.01±0.200	8.01±0.249	2.5
10.00	206	10.06±0.362	10.06±0.450	3.6
	246	10.00±0.230	10.00±0.286	2.3

***** n=5, t= 2.776.

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Calibration curve for determination of amoxicillin sodium (Amo)

The calibration curve of amoxicillin sodium in pure form through complexation with iodine showed excellent linearity over concentration range of 0.387-15.495 μg.mL^{-1} in presence of 1.0×10^{-4} mol.L^{-1} of I_2 in acetonitrile. Regression equations at $\lambda_{max,1}$ 290 nm and $\lambda_{max,2}$ 360 nm were as the follows: y=0.1219x+0.0069 (R^2= 0.9991) and y=0.0622x+0.0009 (R^2= 0.9993), respectively. Figure 4 showed the spectra of complex $AmoI^+.I_3^-$ and calibration curve for determination of amoxicillin sodium according to optimal conditions at $\lambda_{max,1}$ and $\lambda_{max,2}$ (in present of 1.0×10^{-4} M of I_2) where Amo: 0.387-15.495 μg.mL^{-1} (blank is I_2 solution in acetonitrile 1.0×10^{-4} M; ℓ = 1.0 cm).

The spectra characteristics of the method such as the molar absorptivity (ε), Beer's law, regression equation at λ_{max} (y=a.x+b); where y=absorbance, a=slope, x=concentration of amoxicillin sodium by μg.mL^{-1}, b=intercept, the correlation coefficient, limit of detection (LOD) and limit of quantification (LOQ) and the optimum conditions for spectrophotometric determination of Amo through $AmoI^+.I_3^-$ complex is summarized in Table 3.

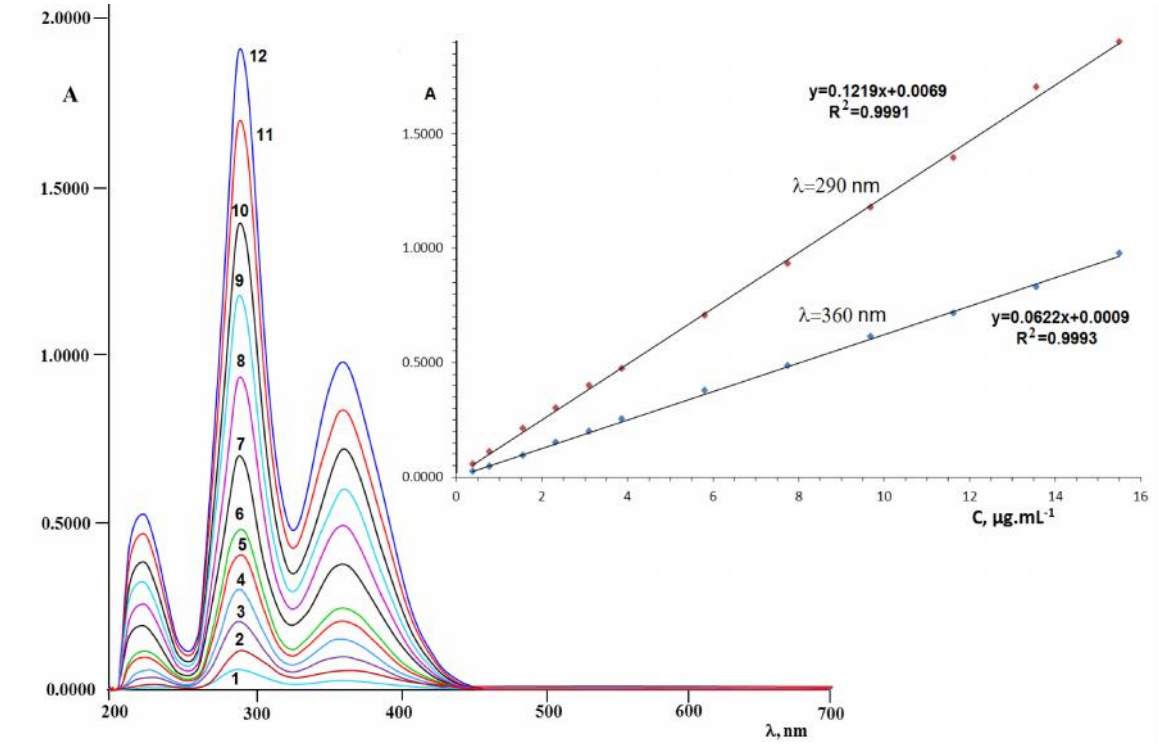

Fig.3: Spectra and calibration curve of $AmoI^+.I_3^-$ complex in presence of 1.0×10^{-4} M of I_2; where concentration of Amo as the follows: 1- 0.387, 2- 0.775 , 3- 1.550 , 4- 2.324, 5- 3.099, 6- 3.874, 7- 5.811, 8- 7.748, 9- 9.685, 10- 11.622, 11- 13.559 and 12- 15.495 μg.mL^{-1}. (ℓ = 1.0 cm , blank is 1.0×10^{-4} mol.L^{-1} of I_2 in acetonitrile).

Table 3. The parameters established for spectrophotometric determination of amoxicillin sodium by complex formation with I_2 in acetonitrile.

Parameters	Operating values
$\lambda_{max,1}$ of $AmoI^+.I_3^-$ complex, nm	290
$\lambda_{max,2}$ of $AmoI^+.I_3^-$ complex, nm	360
Beer's Law Limit, for Amo by µM	1.00 - 40.00
Beer's Law Limit, for Amo by $\mu g.mL^{-1}$	0.387 - 15.495
Molar absorptivity of $AmoI^+.I_3^-$ complex (ε_1) $L.mol^{-1}.cm^{-1}$	46000
Molar absorptivity of $AmoI^+.I_3^-$ complex (ε_2) $L.mol^{-1}.cm^{-1}$	23600
Regression equation for $AmoI^+.I_3^-$ at $\lambda_{max,1}$ =290 nm:	
Slope	0.1219
Intercept	0.0069
Correlation coefficient (R^2)	0.9991
Regression equation for $AmoI^+.I_3^-$ at $\lambda_{max,2}$ =360 nm:	
Slope	0.0622
Intercept	0.0009
Correlation coefficient (R^2)	0.9993
C_{Amo}: C_{I2}, M	≤1:2.5
Reaction time	5 min
Stability	24 h
Temperature of solution	25±5°C

n=5, t=2.776.

Analytical results determination of amoxicillin sodium

Spectrophotometric determination of amoxicillin sodium through complexation with I_2 in acetonitrile within optimal conditions using calibration curve was applied. The results, summarized in Table 4, showed that the determined concentration of amoxicillin sodium was rectilinear over the range of 0.387-15.495 $\mu g.mL^{-1}$ (1.00 – 40.00 µM), with relative standard deviation (RSD) not more than 2.9%. The results obtained from the developed method have been compared with the official HPLC method [8] and good agreement was observed between them.

Table 4: Spectrophotometric determination of amoxicillin sodium through complex formation with 1.0×10^{-4} M of I_2 within optimal conditions using calibration curve in acetonitrile.

X_i, (Taken)		[a], nm	* $\bar{x}$ ±SD, µg.mL^{-1} (Found)	$\bar{x} \pm \frac{t.SD}{\sqrt{n}}$ µg.mL^{-1}	RSD %	* $\bar{x}$, µg.mL^{-1} HPLC [8]
µmol.L^{-1}	µg.mL^{-1}					
1.000	0.387	290	0.392±0.011	0.392±0.014	2.9	0.390
		360	0.389±0.011	0.389±0.014	2.9	
2.000	0.775	290	0.779±0.023	0.779±0.028	2.9	0.777
		360	0.776±0.022	0.776±0.027	2.8	
4.000	1.550	290	1.602±0.045	1.602±0.056	2.8	1.572
		360	1.546±0.042	1.546±0.052	2.7	
6.000	2.324	290	2.341±0.063	2.341±0.078	2.7	2.340
		360	2.332±0.061	2.332±0.075	2.6	
8.000	3.099	290	3.107±0.078	3.107±0.096	2.5	3.105
		360	3.103±0.074	3.103±0.092	2.4	
10.000	3.874	290	3.873±0.093	3.873±0.115	2.4	3.874
		360	3.875±0.089	3.875±0.111	2.3	
15.000	5.811	290	5.702±0.137	5.702±0.170	2.4	5.800
		360	5.810±0.134	5.810±0.166	2.3	
20.000	7.748	290	7.625±0.175	7.625±0.218	2.3	7.752
		360	7.795±0.171	7.795±0.213	2.2	
25.000	9.685	290	9.654±0.222	9.654±0.276	2.3	9.664
		360	9.542±0.210	9.542±0.261	2.2	
30.000	11.622	290	11.421±0.251	11.421±0.312	2.2	11.521
		360	11.504±0.242	11.504±0.300	2.1	
35.000	13.559	290	13.772±0.303	13.772±0.376	2.2	13.587
		360	13.502±0.270	13.502±0.335	2.0	
40.000	15.495	290	15.493±0.372	15.493±0.462	2.4	15.503
		360	15.511±0.279	15.511±0.347	1.8	

***** n=5, t= 2.776.

Applications

The developed spectrophotometric method was applied to determine amoxicillin sodium in some Syrian pharmaceutical preparations through complex formation by I_2 in acetonitrile according to the optimal conditions. The amount (m) of Amo in one vial was calculated from the following relationship: m=h. m', where: m' is the amount of Amo in vial calculated according to the regression equation of calibration curve, h conversion factors are equal to 50 and 100 for all pharmaceuticals content 500 and 1000 mg/vial, respectively. The results of quantitative analysis for amoxicillin sodium in pharmaceutical preparations were summarized in Table 5. The proposed method was simple, direct and successfully applied to the determination of amoxicillin sodium in pharmaceutical forms. Average recovery ranged between 99.8 to 99.9%. The results obtained by this method agree well with the contents stated on the vials and were validated by HPLC method [8].

Table 5: Determination of amoxicillin sodium in some Syrian pharmaceutical preparations using spectrophotometric method through complex formation with 1.0×10^{-4} M of I_2 within optimal conditions using calibration curve in acetonitrile, $\lambda_{max,2}$ 360 nm.

Dosage form	Label Claim of Amo, mg/vial	*Mean ±SD Amo, mg/vial	RSD %	Assay %	*Mean ±SD Amo, mg/vial by HPLC [8]	* Assay %, by HPLC [8]
AMOXYDINE vial, **ASIA PHARMACEUTICAL INDUSTRIES**	1000	998±21	2.1	99.8	999±19	99.9
	500	499±11	2.3	99.8	499±10	99.8
Panamoxy vial, **ELSAAD PHARMACEUTICAL**	1000	999±21	2.1	99.9	999±18	99.9

***** n=5.

Method validation

The developed method for simultaneous estimation of amoxicillin sodium has been validated in accordance with the International Conference on Harmonization guidelines (ICH) [17].

Linearity

Several aliquots of standard stock solution of amoxicillin sodium were taken in different 10 mL volumetric flask and diluted up to the mark with acetonitrile such that their final concentrations were 0.387- 15.495 µg.mL^{-1} for amoxicillin sodium. Absorbance was plotted against the corresponding concentrations to obtain the calibration graph, see Figure 3 and Table 4. Linearity equations obtained at $\lambda_{max,1}$ =290 nm and at $\lambda_{max,2}$ =360 nm were y=0.1219x+0.0069 (R^2= 0.9991) and y=0.0622x+0.0009 (R^2= 0.9993), respectively.

Precision and Accuracy

The precision and accuracy of proposed method was checked by recovery study by addition of standard drug solution to pre-analyzed sample solution at three different concentration levels (80%, 100% and 120%) within the range of linearity for amoxicillin sodium. The basic concentration level of sample solution selected for spiking of the amoxicillin sodium standard solution was 5.811 µg.mL^{-1}. The proposed method was validated statistically and through recovery studies, and was successfully applied for the determination of amoxicillin sodium in pure and dosage forms with percent recoveries ranged from 98.9% to 102.1%, see Table 6.

Table 6: Results of recovery studies at $\lambda_{max,2}$ 360 nm (n=5).

Level	Recovery %
80%	98.9
100%	100.9
120%	102.1

Repeatability

The repeatability was evaluated by performing 10 repeat measurements for 5.811 µg.mL^{-1} of amoxicillin sodium using the studied spectrophotometric method under the optimum conditions. The found amount of amoxicillin sodium ($\bar{x}$ ± SD) 5.710 ± 0.12 µg.mL^{-1} at $\lambda_{max,1}$ and 5.800 ± 0.11 at $\lambda_{max,2}$. The percentage recovery was found to be 98.26 ± 2.1 with RSD of 0.022 at $\lambda_{max,1}$ and 99.81 ± 1.9 with RSD of 0.019 at $\lambda_{max,2}$. These values indicate that the proposed method has high repeatability for amoxicillin sodium analysis.

Sensitivity (LOD and LOQ)

The sensitivity of the method was evaluated by determining the LOD and LOQ at $\lambda_{max,2}$ 360 nm. The values of LOD and LOQ for amoxicillin sodium are 0.042 and 0.127 µg.mL^{-1}, respectively.

Robustness

The robustness of the method adopted is demonstrated by the constancy of the absorbance with the deliberated minor change in the experimental parameters such as the change in the concentration of excipients (the vials do not contain excipients), temperature (25±5^{o}C), stability (23-25 h) and reaction time (5±1 min), see Table 7 which indicates the robustness of the proposed method. The absorbance was measured and assay was calculated for five times.

Table 7: Robustness of the proposed spectrophotometric method, at $\lambda_{max,2}$ 360 nm

Experimental parameter variation	Average recovery (%)*	
	C_{Amo}	
	1.550 **µg/ml**	11.622 **µg/ml**
Temperature		
20^{o}C	99.4	99.7
30^{o}C	100.1	101.1
Stability		
23 h	100.0	100.1
25 h	100.2	100.3
Reaction time		
4.0 min	99.7	99.9
6.0 min	100.0	100.1

* n=5.

Conclusion

A spectroscopic study of the interaction of amoxicillin sodium (Amo) with iodine in acetonitrile medium and formation of complexes was studied. The method is based on two charge transfer complex's the Amo with iodine. The first complex ($AmoI^{+}.I^{-}$) is formed when the concentration of iodine is much lower than the concentration of amoxicillin and therefore a peak of this complex occurs at wavelengths 206 and 246 nm and the absorbance's were proportional to the concentration of iodine at range 1.00 to 10.00 µM in present of drug 5.0x10^{-5} M. The second complex ($AmoI^{+}.I_3^{-}$) is formed occurs at wavelengths at 290 and 360 nm when the drug concentration is two or more times less than iodine concentration. Under these optimized experimental conditions, Beer's law is obeyed in the concentration ranges 0.387-15.495 µg.mL^{-1} for amoxicillin sodium. The values of LOD and LOQ for amoxicillin sodium were 0.042 and

0.127 μg.mL^{-1}, respectively. The method was validated for linearity, precision and accuracy, repeatability, sensitivity and robustness. The method was successfully applied for determination of Amo in pure and pharmaceutical formulations samples with low RSD value. This is simple, accurate and sensitive spectrophotometric method gives good results for the determination of Amo in pure and different dosage forms.

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