INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES

(p-ISSN: 2348-5213: e-ISSN: 2348-5221)

www.ijcrcps.com

DOI: 10.22192/ijcrcps Coden: IJCROO(USA) Volume 7, Issue 8 - 2020

Research Article

DOI: http://dx.doi.org/10.22192/ijcrcps.2020.07.08.001

Spectrophotometric Study of Interactions Penicillin G Benzathine 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.

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

2 E-mail: promandil955@gmail.com

3E-mail: jenansabouni@gmail.com

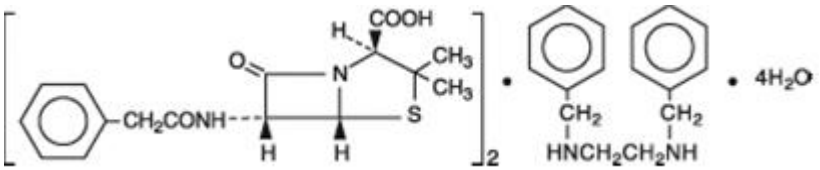
Abstract

Spectrophotometric study of interactions penicillin G benzathine (BPG) with iodine in acetonitrile and formation of complexes was applied. The method is based on two charge transfer complex's the BPG with iodine. The first complex ($BPGI^+.I^-$) is formed when the concentration of iodine is much lower than the concentration of BPG at wavelength 246 nm and the absorbance's were proportional to the concentration of iodine at range 1.00 to 10.00 $\mu mol.L^{-1}$ (0.25381-2.5381 $\mu g.mL^{-1}$) in present of BPG $2.0x10^{-5}$ $mol.L^{-1}$. The second complex ($BPGI^+.I_3^-$) is formed occurs at wavelengths 290 and 360 nm when the iodine concentration is two or more times than BPG concentration ($C_{I_2} \geq 2C_{BPG}$). Under these optimized experimental conditions, Beer's law is obeyed in the concentration ranges 0.90913-27.2739 $\mu g.mL^{-1}$ for BPG (without $4H_2O$). The method was validated for linearity, precision and accuracy, repeatability, sensitivity and robustness. The method was successfully applied for determination of BPG in pure and pharmaceutical formulations samples with relative standard deviations did not exceed 2.4% for the concentration of BPG is 1.00 $\mu mol.L^{-1}$ (0.90913 $\mu g.mL^{-1}$). This is simple, accurate and sensitive spectrophotometric method gives good results for the determination of BPG in bulk and different dosage forms.

Keywords: penicillin G benzathine (BPG), Spectrophotometric method, Charge transfer complexes, Iodine.

Introduction

Penicillin G benzathine (Bicillin L-A) is prepared by the reaction of dibenzylethylene diamine with two molecules of penicillin G. It is chemically designated as (2*S*, 5*R*, 6*R*)-3,3-Dimethyl-7-oxo-6- (2-phenylacetamido) – 4 - thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid compound with *N*,*N'* -dibenzylethylenediamine (2:1), tetrahydrate. It occurs as a white, crystalline powder and is very slightly soluble in water and sparingly soluble in alcohol. Its molecular weight is 981.19 g/mol and its molecular formula is $(C_{16}H_{18}N_2O_4S)_2$. $C_{16}H_{20}N_2.4H_2O$ (909.13 g/mol without $4H_2O$). Its chemical structure is as follows:



Penicillin G benzathine (BPG.$4H_2O$)

Prescribing penicillin G benzathine (BPG) in the absence of a proven or strongly suspected bacterial infection or a prophylactic indication is unlikely to provide benefit to the patient and increases the risk of a development of drug-resistant bacteria. Penicillin should be used with caution in individuals with histories of significant allergies and/or asthma. Care should be taken to avoid intravenous or intra-arterial administration, or injection into or near major peripheral nerves or blood vessels, since such injection may produce neurovascular damage. Prolonged use of antibiotics may promote the overgrowth of nonsusceptible organisms, including fungi [1, 2].

A new method for determining benzathine penicillin G using “zero-crossing” second derivative spectrophotometry is described. Calibration graphs were linear up to $4.40x10^{-5}$ M for benzathine penicillin G. The method was applied for determining benzathine penicillin G synthetic mixtures with good results [2, 3].

In chemistry, triiodide usually refers to the triiodide ion, I_3^-. This anion, one of the polyhalogen ions, is composed of three iodine atoms. It is formed by combining non-aqueous and aqueous solutions of iodide salts and iodine [4-11].

The spectrophotometric determination of some penicillin's, such as ampicillin and amoxicillin by iodine was used [12, 13]. The method is based on two charge transfer complex’s the drugs with iodine. The first complex's ($AmpI^+.I^-$ and $AmoI^+.I^-$) are formed when the concentration of iodine is much lower than the concentration of drugs and therefore a peak of this complex occurs at wavelength 246 nm. 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's ($AmpI^+.I_3^-$ and $AmoI^+.I_3^-$) are formed occurs at wavelengths 290 and 360 nm when the concentration of drugs 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 and 0.743-14.856 $\mu g.mL^{-1}$ for amoxicillin sodium and ampicillin sodium, respectively [12, 13].

In the present work, a spectrophotometric method for the determination of penicillin G benzathine (BPG) 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 $BPGI^+.I^-$ and the second complex $BPGI^+.I_3^-$ were formed.

Experimental

Reagents

Penicillin G benzathine (the purity is 98.42%, it is presented by ASIA pharmaceutical industries, Aleppo - Syria) was purchased from Jiangxi (CHINA). All reagents as Iodine (the purity is 99.8%), 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 5 to 5000 µL (model Piptman P, GILSON and JENCONS-SEALPETTE). 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).

A stock standard solution of penicillin G benzathine ($1x10^{-3}$ $mol.L^{-1}$)

1×10^{-3} $mol.L^{-1}$ of pure BPG (98.42%) was prepared in acetonitrile. This solution was prepared by good mixing 23.093 mg of BPG (or 24.924 mg from BPG.$4H_2O$) in 2.5 mL methanol using ultrasonic for 15 min and diluted up to mark into volumetric flask (25 mL) with acetonitrile.

Working solutions

The stock solutions were further diluted to obtain working solutions daily just before use in the ranges of BPG: 1.00, 2.00, 3.00, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 15.00, 18.00, 21.00, 24.00, 28.00 and 30.00 $\mu mol.L^{-1}$ (0.9812, 1.9624, 2.9436, 3.9248, 4.9059, 5.8871, 7.8495, 9.8119, 11.7743, 14.7178, 17.6614, 20.6050, 23.5486, 27.4733 and 29.4357 $\mu g.mL^{-1}$) by dilution of the volumes: 10, 20, 30, 40, 50, 60, 80, 100, 120, 150, 180, 210, 240, 280 and 300 µL from stock standard solutions of BPG $1x10^{-3}$ $mol.L^{-1}$ into 10 mL volumetric flask, then added 100 µL 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 BPG. The pharmaceutical formulations were subjected to the analytical procedures:

(1) ***Benzathine Penicillin*** vial, **ASIA pharmaceutical industries**, Aleppo - Syria, each vial contains: 1200000 IU Eq. to 900 mg of BPG without H_2O or 971.34 mg with H_2O (EXP. DATE 09/2021).

(2) ***Benzathine Penicillin*** vial, **ASIA pharmaceutical industries**, Aleppo - Syria, each vial contains: 600000 IU Eq. to 450 mg of BPG without H_2O or 485.67 mg with H_2O (EXP. DATE 09/2021).

Stock solutions of pharmaceutical formulations

Three vials of each studied pharmaceutical formulation were weighed accurately and mixed well; each vial weight ≥494 or 987 mg of BPG.$4H_2O$ (98.42%). An amount of the powder equivalent to the weight of 49.85 mg was solved in 2.5 mL methanol using ultrasonic for 15 min, acetonitrile was added a 25 mL flask. This solution contains the following: 2.000 $mmol.L^{-1}$ (1.9625 $mg.mL^{-1}$) of BPG.$4H_2O$.

Working solutions of pharmaceuticals

Five solutions were prepared daily by diluting 110 µL from each stock solution of pharmaceutical formulations (which is contents: 450 or 900 mg/vial of BPG), then 0.100 mL from stock standard solution of iodine was added and adjusted the volume up to 10 mL with acetonitrile; these solutions contain 20.000 $\mu g.mL^{-1}$ or 22.000 $\mu mol.L^{-1}$ of BPG (without H_2O); test solutions.

Results and Discussion

Analytical procedure

A spectrophotometric method for the determination of penicillin G benzathine with iodine has been studied. The method is based on the formation of a complex between iodine with penicillin G benzathine. The formed complex's (BPG with iodine) were measured at λ_{max} 246 nm (for first complex $BPGI^{+}.I^{-}$) or at λ_{max} 290 and 360 nm (for second complex $BPGI^{+}.I_3^{-}$) against the reagent blank prepared in the same manner.

Spectrophotometric results

UV-Vis spectra of BPG, iodine, first complex ($BPGI^{+}.I^{-}$) and second complex ($BPGI^{+}.I_3^{-}$) solutions in acetonitrile were obtained. BPG solutions do not absorb in the range 270-700 nm. Iodine solutions have absorption at λ_{max} 465 nm, 360 nm and 290 nm (ε=830, 275 and 550 L. mol^{1}. cm^{-1}, respectively). First complex ($BPGI^{+}.I$) solutions have maximum absorption at λ_{max} 246 nm (ε was 28800 L. $mol^{-1}.cm^{-1}$). Second complex ($BPGI^{+}.I_3^{-}$) solutions have maximum absorption at λ_{max} 290 and 360 nm (ε was 46000 and 23600 L. $mol^{-1}.cm^{-1}$, respectively), see figure 1.

The effect of time and temperature

The effect of time and temperature on the first and second complex's in acetonitrile were studied within the ranges 5-105 min and 15-30^{o}C. It was found that the formed complex's weren't affected by mentioned temperature. The first complex ($BPGI^{+}.I^{-}$) is clearly affected by time, so when the iodine concentration and the BPG concentration are equal, the first complex and the second complex get, which indicates that the formation of the first complex is weak. When the BPG concentration increases to 1.8 of the iodine concentration, the first complex is formed by a large percentage and becomes 100% after 105 minutes, see figure 2. Whereas, the second complex ($BPGI^{+}.I_3^{-}$) is formed directly when the $C_{I_2} \geq 2C_{BPG}$ and is not affected by time.

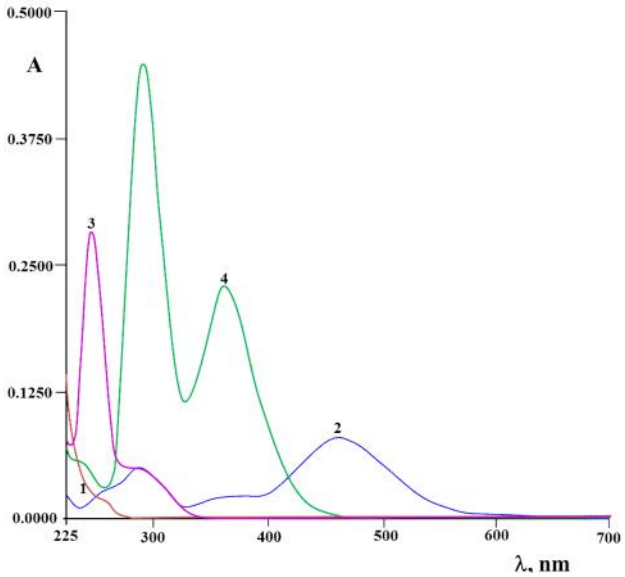


Fig. 1: UV-Vis spectra in acetonitrile of: 1- $2x10^{-5}$ $mol.L^{-1}$ of BPG; 2- $1.0x10^{-4}$ $mol.L^{-1}$ of iodine; 3- $1.0x10^{-5}$ $mol.L^{-1}$ of iodine with $2.0x10^{-5}$ $mol.L^{-1}$ of BPG (first complex $BPGI^{+}.I^{-}$) and 4- $1.0x10^{-5}$ $mol.L^{-1}$ of BPG with $1.0x10^{-4}$ $mol.L^{-1}$ of iodine (second complex $BPGI^{+}.I_3^{-}$). {blank for (1-2) acetonitrile, (3) BPG $2.0x10^{-5}$ $mol.L^{-1}$ and (4) I_2 $1.0x10^{-4}$ $mol.L^{-1}$, ℓ =1.0 cm}.

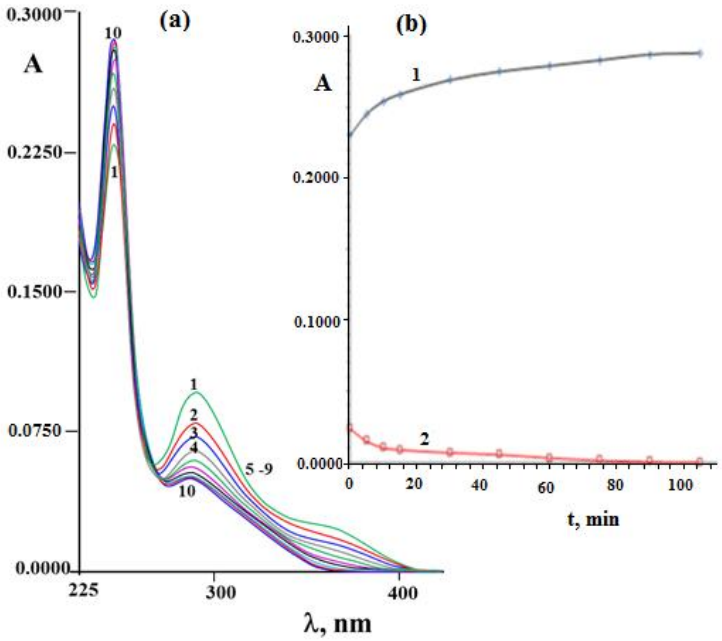


Fig. 2. The effect of time on the first complex ($BPGI^+.I^-$) in acetonitrile (C_{BPG}=1.8x10^{-5} and C_{I_2}=1x10^{-5} mol.L^{-1}): a- the spectra from time 1- 0.0, 2- 5, 3- 10, 4- 15, 5- 30, 6- 45, 7- 60, 8- 75, 9- 90 and 10- 105 min. b- The first and the second complex changes with time 1- First Complex ($BPGI^+.I^-$) at λ_{max} 246 nm , 2- Second Complex ($BPGI^+.I_3^-$) at λ_{max} 360 nm.

Stoichiometric relationship

The molar ratio method

The composition of the first complex ($BPGI^+.I^-$) was determined by the molar ratio method of continuous variation [14]. The stoichiometry of $BPGI^+.I^-$ complex was studied by molar ratio method according to the following equation: A_{max}= f ($[I_2]/[BPG]$) at λ_{max} 246 nm in acetonitrile. It confirmed that the binding ratio of $BPGI^+.I^-$ complex is equal to (1:1); where the concentration of BPG was constant (10 µmol.L^{-1}) and the concentrations of I_2 changed from 1 to 20 µmol.L^{-1}, see figure 3. The formation constant of the first complex $BPGI^+.I^-$ is 3.8x10^6 in acetonitrile.

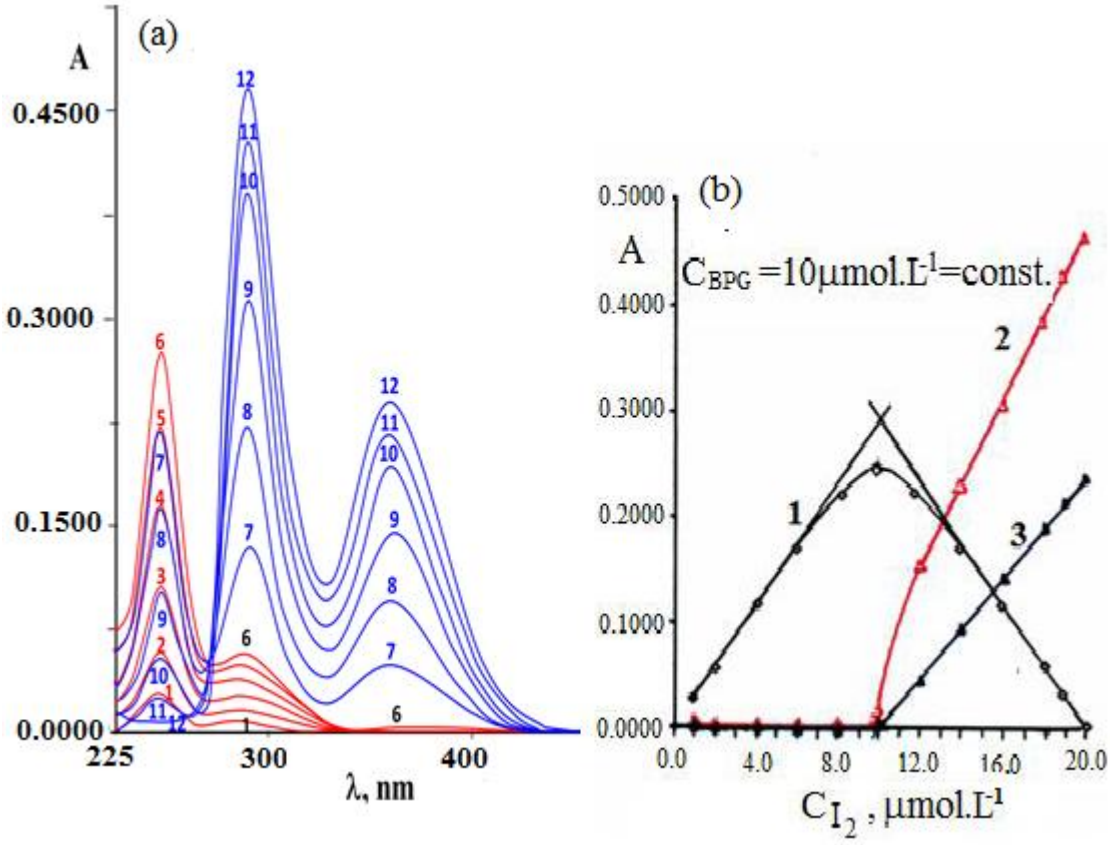


Fig. 3: Molar ratio method to calculate binding ratio of $BPGI^+.I^-$ complex at λ_{max} 246 nm in acetonitrile: (a) the spectra of BPG (10 µmol.L^{-1}) with concentrations of iodine are as follows: 1- 1.0, 2- 2.0, 3- 4.0, 4- 6.0, 5- 8.0, 6- 10.0, 7- 12.0, 8- 14.0, 9- 16.0, 10- 18.0, 11- 19.0 and 12- 20.0 µmol.L^{-1}, (b): 1- $BPGI^+.I^-$ at λ_{max} 246 nm, 2- at λ_{max} 290 nm and 3- at λ_{max} 360 nm (blank is acetonitrile, ℓ =1 cm).

Composition of the second complex ($BPGI^+.I_3^-$) was determined by the molar ratio method of continuous variation [14]. The stoichiometry of $BPGI^+.I_3^-$ complex was studied by molar ratio method according to following equation: A_{max}= f ([BPG]/[I_2]) at λ_{max} 290 and 360 nm in acetonitrile. It confirmed that the binding ratio of $BPGI^+.I_3^-$ complex is equal to (1:2); where the concentration of iodine was constant (20 µmol.L^{-1}) and the concentrations of BPG changed from 2 to 16 µmol.L^{-1}, see figure 4. It was found that the peak at the wavelength of 290 nm cannot be adopted to match the tail of the peak for the first complex, while the second peak at 360 nm is good. The formation constant of second complex ($BPGI^+.I_3^-$) is $7.1x10^7$ in acetonitrile.

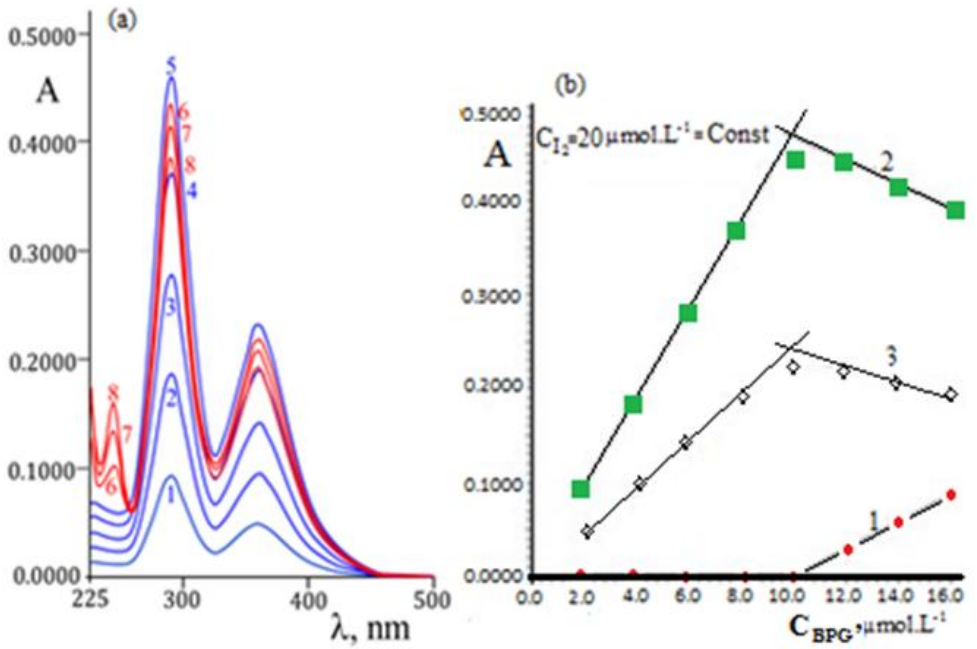
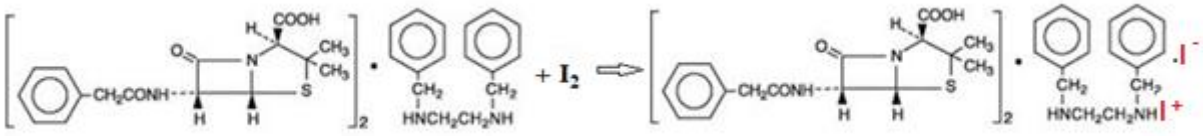


Fig. 4: Molar ratio method to calculate binding ratio of $BPGI^+.I_3^-$ complex at λ_{max} 290 nm (2), 360nm (3) and first complex ($BPGI^+.I^-$) λ_{max} 246 nm (1) in acetonitrile ($[I_2]$= 20 µmol.L^{-1}) with concentrations of BPG are as follows: 1- 2.0, 2- 4.0, 3- 6.0, 4- 8.0, 5- 10.0, 6- 12.0, 7- 14.0 and 8- 16.0 µmol.L^{-1} (blank is acetonitrile, ℓ =1 cm).

Mechanism of Reaction:

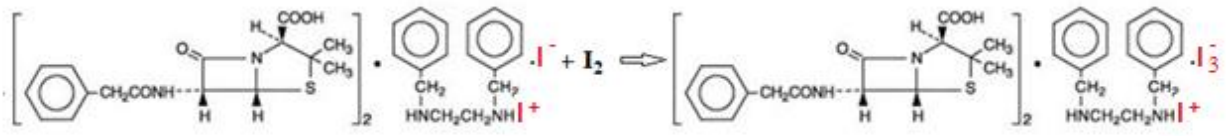
We studied the mechanism of the reaction and found that the drug interacts with iodine when its concentration is more than the concentration of iodine, and it gives the first complex according to the reaction (i), and when the concentration of the drug becomes less than twice the concentration of iodine, the second complex occurs as in reaction (ii).

i- First complex ($BPGI^+.I^-$):



BPG The first complex ($BPGI^+.I^-$)

ii-Second complex ($BPGI^+.I_3^-$):



The first complex ($BPGI^+.I^-$) The second complex ($BPGI^+.I_3^-$)

Calibration curve for determination of iodine

Calibration curve for determination of iodine in present BPG when $C_{I_2} < C_{BPG}$ (Where only the first complex $BPGI^+.I^-$ 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 2.0×10^{-5} mol.L^{-1} of BPG in acetonitrile. Regression equation at λ_{max} 246 nm was as the follows: y=0.028780x+0.00183 (R^2=0.9982) in acetonitrile, see Table 1 and 2.

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

Parameters	Operating values*
λ_{max} , nm	246
Beer's Law Limit, for iodine by µmol.L^{-1}	1.00 - 10.00
Beer's Law Limit, for iodine by µg.mL^{-1}	0.25381– 2.53810
Regression equation for $BPGI^+.I^-$ at λ_{max} =246 nm:	
Slope	0.02878
Intercept	0.00183
Correlation coefficient (R^2)	0.9982
C_{BPG}: C_{I_2}, mol.L^{-1}	≥2:1
Stability	200 h
Temperature of solution	25±5^{o}C

* n=5, t=2.776.

Table 2: Spectrophotometric determination of iodine through complex formation with 2.0×10^{-5} M of BPG at within optimal conditions using calibration curve in acetonitrile.

X_i, (Taken)		*$\bar{x}$ ±SD, µmol.L^{-1} (Found)	$\bar{x} \pm \frac{t.SD}{\sqrt{n}}$, µmol.L^{-1}	RSD%
µg.mL^{-1}	µmol.L^{-1}			
0.25381	1.00	0.98±0.047	0.98±0.058	4.8
0.50762	2.00	2.00±0.092	2.00±0.114	4.6
1.01524	4.00	4.04±0.170	4.04±0.211	4.2
1.52286	6.00	5.99±0.234	5.99±0.290	3.9
2.03048	8.00	8.01±0.288	8.01±0.358	3.6
2.53810	10.00	10.01±0.350	10.01±0.435	3.5

*n=5, t= 2.776.

Calibration curve for determination of penicillin G benzathine (BPG)

The calibration curve of BPG in pure form through complexation with iodine showed excellent linearity over concentration range of 1.00-30.00 µmol.L^{-1} (0.90913-27.2739 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_1=0.04613x+0.00061 (R^2=0.9986) and y_2=0.02362x+0.00005 (R^2= 0.9996), respectively. Figure 5 showed the spectra of complex $BPGI^+.I_3^-$ and calibration curve for determination of BPG according to optimal conditions at $\lambda_{max,1}$ and $\lambda_{max,2}$ in present of 1.0×10^{-4} M of I_2 where 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 BPG by µmol.L^{-1}, b=intercept, the correlation coefficient, limit of detection (LOD) and limit of quantification (LOQ) and the optimum conditions for spectrophotometric determination of BPG through $BPGI^{+}.I_3^{-}$ complex is summarized in Table 3.

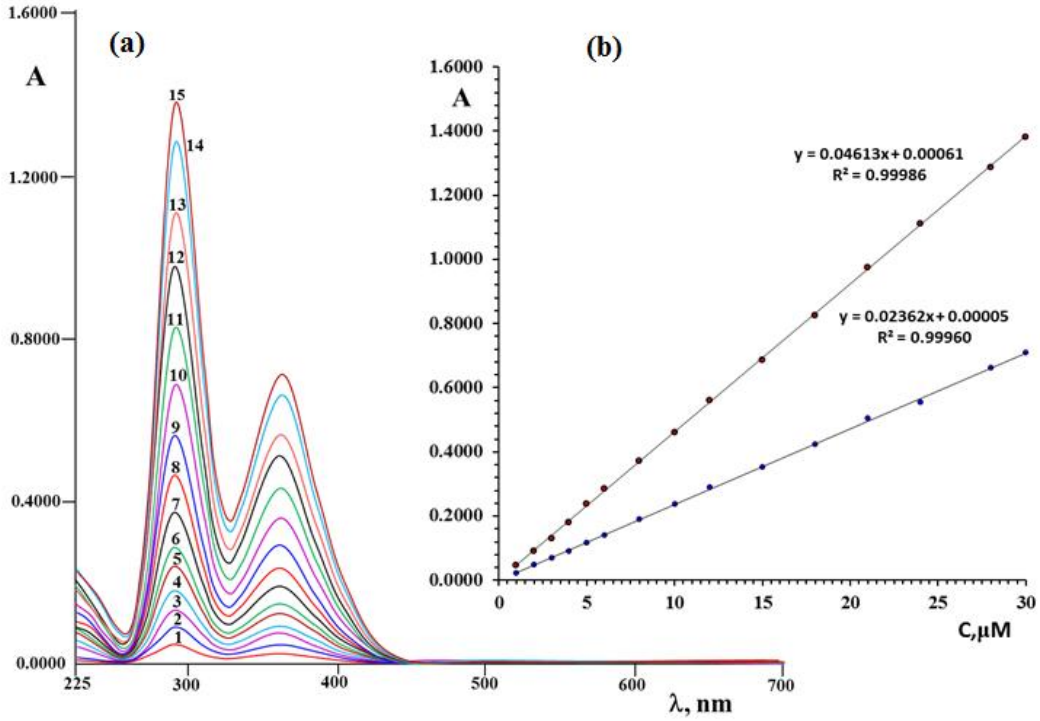


Fig. 5: Spectra and calibration curve of $BPGI^{+}.I_3^{-}$ complex in presence of 1.0×10^{-4} M of I_2; where concentration of BPG as the follows: 1- 1.00, 2- 2.00, 3- 3.00 , 4- 4.00, 5- 5.00, 6- 6.00, 7- 8.00, 8- 10.00, 9- 12.00, 10- 15.00, 11- 18.00, 12- 21.00, 13- 24.00, 14- 28.00 and 15- 30.00 µmol.L^{-1}. (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 BPG by complex formation with I_2 in acetonitrile.

Parameters	Operating values
$\lambda_{max,1}$ of $BPGI^+.I_3^-$ complex, nm	290
$\lambda_{max,2}$ of $BPGI^+.I_3^-$ complex, nm	360
Beer's Law Limit, for BPG by $\mu mol.L^{-1}$	1.00 - 30.00
Beer's Law Limit, for BPG by $\mu g.mL^{-1}$	0.90913 – 27.2739
Molar absorptivity of $BPGI^+.I_3^-$ complex (ε_1), $L.mol^{-1}.cm^{-1}$	46000
Molar absorptivity of $BPGI^+.I_3^-$ complex (ε_2), $L.mol^{-1}.cm^{-1}$	23600
Regression equation for $BPGI^+.I_3^-$ at $\lambda_{max,1}$ =290 nm:	
Slope	0.04613
Intercept	0.00061
Correlation coefficient (R^2)	0.99986
Regression equation for $BPGI^+.I_3^-$ at $\lambda_{max,2}$ =360 nm:	
Slope	0.02362
Intercept	0.00005
Correlation coefficient (R^2)	0.99960
C_{BPG}: C_{I_2}, $mol.L^{-1}$	≤1:3.3
LOD, $\mu mol.L^{-1}$	0.080
LOQ, $\mu mol.L^{-1}$	0.242
Reaction time	5 min
Stability	24 h
Temperature of solution	25±5°C

n=5, t=2.776.

Analytical results determination of penicillin G benzathine (BPG)

Spectrophotometric determination of BPG 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 BPG was rectilinear over the range of 1.00 – 30.00 $\mu mol.L^{-1}$ (0.90913-27.2739 $\mu g.mL^{-1}$), with relative standard deviation (RSD) not more than 2.4%.

Table 4: Spectrophotometric determination of BPG through complex formation with 1.0×10^{-4} mol.L^{-1} of I_2 within optimal conditions using calibration curve in acetonitrile.

X_i, (Taken drug)			λ, nm	*$\bar{x}$ ±SD, µmol.L^{-1} (Found)	$\bar{x}\pm\frac{t.SD}{\sqrt{n}}$, µmol.L^{-1}	RSD%
BPG, µmol.L^{-1}	BPG, µg.mL^{-1}	BPG[illegible] µg.mL^{-1}				
1.00	0.9091	0.9812	290	1.01±0.024	1.01±0.030	2.4
			360	0.998±0.025	0.998±0.031	2.5
2.00	1.8183	1.9624	290	2.01±0.048	2.01±0.060	2.4
			360	1.97±0.049	1.97±0.061	2.5
3.00	2.7274	2.9436	290	2.96±0.071	2.96±0.088	2.4
			360	2.97±0.071	2.96±0.088	2.4
4.00	3.6365	3.9248	290	3.95±0.091	3.95±0.113	2.3
			360	3.94±0.095	3.94±0.117	2.4
5.00	4.5457	4.9059	290	5.01±0.110	5.01±0.137	2.2
			360	5.03±0.116	5.03±0.144	2.3
6.00	5.4548	5.8871	290	5.92±0.130	5.92±0.162	2.2
			360	6.05.±0.139	6.05.±0.173	2.3
8.00	7.2730	7.8495	290	8.06±0.177	8.06±0.220	2.2
			360	8.04±0.177	8.04±0.220	2.2
10.00	9.0913	9.8119	290	10.02±0.210	10.02±0.261	2.1
			360	10.04±0.221	10.04±0.274	2.2
12.00	10.9096	11.7743	290	12.02±0.252	12.02±0.093	2.1
			360	12.04±0.265	12.04±0.330	2.2
15.00	13.6370	14.7178	290	15.00±0.300	15.00±0.372	2.0
			360	14.97±0.314	14.97±0.391	2.1
18.00	16.3643	17.6614	290	17.93±0.359	17.93±0.445	2.0
			360	17.96±0.377	17.96±0.468	2.1
21.00	19.0917	20.6050	290	21.98±0.440	21.98±0.546	2.0
			360	21.96±0.439	21.96±0.545	2.0
24.00	22.7283	23.5486	290	23.95±0.455	23.95±0.565	1.9
			360	24.04±0.481	24.04±0.597	2.0
28.00	25.4556	27.4733	290	28.07±0.533	28.07±0.662	1.9
			360	27.96±0.531	27.96±0.660	1.9
30.00	27.2739	29.4357	290	30.02±0.540	30.02±0.671	1.8
			360	30.01±0.540	30.01±0.671	1.8

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

Applications

The developed spectrophotometric method was applied to determine penicillin G benzathine (BPG) in some Syrian pharmaceutical preparations through complex formation by I_2 in acetonitrile according to the optimal conditions. The amount (m) of BPG in one vial was calculated from the following relationship: m=h. m', where: m' is the amount of BPG in vial calculated according to the regression equation of calibration curve, h conversion factors are equal

to 22.5 and 45.0 for all pharmaceuticals content 450 and 900 mg/vial of BPG, respectively. The results of quantitative analysis for BPG in pharmaceutical preparations were summarized in Table 5. The proposed method was simple, direct and successfully applied to the determination of penicillin G benzathine in pharmaceuticals. Average recovery ranged between 100.4 to 100.7%. The results obtained by this method agree well with the contents stated on the vials.

Table 5: Determination of penicillin G benzathine (BPG) 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 BPG, mg/vial	*Mean ±SD BPG, mg/vial	RSD%	Assay%
Benzathine Penicillin vial, ASIA PHARMACEUTICAL INDUSTRIES	900	904±17	1.9	100.4
Benzathine Penicillin vial, ASIA PHARMACEUTICAL INDUSTRIES	450	453±9.1	2.0	100.7

***** n=5.

Method validation

The developed method for simultaneous estimation of penicillin G benzathine has been validated in accordance with the International Conference on Harmonization guidelines (ICH) [15].

Linearity

BPG were taken in different 10 mL volumetric flask and diluted up to the mark with acetonitrile such that their final concentrations were 0.90913-27.2739 μg.mL^{-1} for BPG. The calibration graph, see figure 5 and Table 4. Linearity equations obtained at $\lambda_{max,1}$ =290 nm and at $\lambda_{max,2}$ =360 nm were y_1=0.04613x+0.00183 (R^2= 0.99986) and y_2=0.02363x+0.00005 (R^2= 0.99960), 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 BPG. The basic concentration level of sample solution selected for spiking of the BPG standard solution was 8.00 μmol.L^{-1}. The proposed method was validated statistically and through recovery studies, and was successfully applied for the determination of BPG in pure and dosage forms with percent recoveries ranged from 99.9% to 101.0%, see Table 6.

Table 6: Results of recovery studies at λ_{max} 360 nm (n=5).

Level	Recovery%
80%	100.4
100%	99.9
120%	101.0

Repeatability

The repeatability was evaluated by performing 10 repeat measurements for 6.00 µmol.L^{-1} of BPG using the studied spectrophotometric method under the optimum conditions. The found amount of BPG ($\bar{x}$ ± SD) 6.03 ± 0.13 µmol.L^{-1} at $\lambda_{max,1}$ and 6.02 ± 0.14 µmol.L^{-1} at $\lambda_{max,2}$. The percentage recovery was found to be 100.5 ± 2.2 with RSD of 0.022 at $\lambda_{max,1}$ and 100.3 ± 2.2 with RSD of 0.022 at $\lambda_{max,2}$. These values indicate that the proposed method has high repeatability for BPG analysis.

Sensitivity (LOD and LOQ)

The sensitivity of the method was evaluated by determining the LOD and LOQ. The values of LOD and LOQ for BPG are 0.080 and 0.242 µmol.L^{-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), reaction time (5±1 min) and λ_{max} (360±1 nm), 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 λ_{max} 360 nm.

Experimental parameter variation	Average recovery (%)*	
	C_{BPG}	
	4.00 **µmol.L^{-1}**	21.00 **µmol.L^{-1}**
Temperature		
20^{o}C	99.8	99.9
30^{o}C	101.0	101.3
Stability		
23 h	100.1	100.1
25 h	100.4	100.2
Reaction time		
4.0 min	100.2	100.4
6.0 min	100.3	100.4
λ_{max}, nm		
359	99.7	99.9
361	100.1	100.2

* n=5.

Conclusion

Spectrophotometric study of penicillin G benzathine (BPG) with iodine in acetonitrile and formation of complexes was applied. The method is based on two charge transfer complex's the drug with iodine. The first complex ($BPGI^+.I^-$) is formed when the concentration of iodine is much lower than the concentration of BPG at wavelength 246 nm and the absorbance's were proportional to the concentration of iodine at range 1.00 to 10.00 µmol.L^{-1} (0.25381-2.5381 µg.mL^{-1}) in present of BPG 2.0x10^{-5} mol.L^{-1}. The second complex ($BPGI^+.I_3^-$) is formed occurs at wavelengths 290 and 360 nm when the iodine concentration is two or more times than drug concentration ($C_{I_2} \geq 2C_{BPG}$). Under these optimized experimental conditions, Beer's law is obeyed in the concentration ranges 0.90913-27.2739 µg.mL^{-1} for BPG. The method was validated for linearity, precision and accuracy, repeatability, sensitivity and robustness. The method was successfully applied for determination of BPG in pure and pharmaceutical formulations samples with relative standard deviations did not exceed 2.4% for the concentration of BPG is 1.00 µmol.L^{-1} (0.90913 µg.mL^{-1}). This is simple, accurate and sensitive spectrophotometric method gives good results for the determination of BPG in bulk and different dosage forms.

References

1. Schanzer H, Gribetz I, Jacobson JH, 1979. " Accidental intra-arterial injection of penicillin G". JAMA. 242(12):1289-1290.
2. Franz Kreuzig, 1982. "Penicillin-G Benzathine". Analytical Profiles of Drug Substances.11:463-482.
3. Murillo Pulgarin J, Alañón Molina A, Rodríguez Flores J, 1992. "Simultaneous determination of procaine penicillin G and benzathine penicillin G by second derivative spectrophotometry". J Analytical Letters. 25(7):1275-1288.
4. Ramadan AA, Sabagh G, 1994. "The effect of non-aqueous solvents on potentiometric titration of Cu(II) and Pb(II) by iodide and determination of pollution by copper and lead using ion selective electrodes". Res J Aleppo University. 17:93-112.
5. Edrees G MSc. Thesis, 2005. "High frequency titration of halogens in non-aqueous media". Aleppo University, Aleppo, Syria.
6. Ramadan AA, Agasyan PK, Petrov SI, 1974. "Spectrophotometric and potentiometric study of formation I_3^- complex in mixture solvents". Gen Chem. 44:983-992.
7. Ramadan AA, Agasyan PK, Petrov SI, 1974. "Spectrophotometric determination of formation constant I_3^- complex in some organic solvents". Gen Chem. 44:2299-2307.
8. Ramadan AA, Agasyan PK, Petrov SI, 1973. "Potentiometric titration of iodine by a thiosulphate solution in non-aqueous media and the formation of I_3^- complex ion". Zh Anal Khim. 28:2396-2401.
9. Ramadan AA, Agasyan PK, Petrov SI, 1974. "Effect of mixed solvents on determination of iodine by sodium thiosulphate solution with potentiometric detection of an end point". Zh Anal Khim. 29:544-550.
10. Ramadan AA, Ashur S, 1989. "Spectophotometric determination of iodine and iodide, bromide and chloride by iodine in non-aqueous solvents and formation of polyhalogen complexes". Res J Aleppo Univ. 11:57-76.
11. Ramadan AA, Ashur S, 1995. "Spectophotometric and potentiometric study of I_2:Y^- (Y^-=I^- or Br^- or Cl^-) complexes in non-aqueous solvents". Res J Aleppo Univ. 20:143-163.
12. Ramadan AA, Mandil H, Sabouni J, 2019. "Spectrophotometric study of the interaction of ampicillin sodium with iodine in acetonitrile and formation of charge transfer complexes". Int J Curr. Res Chem Pharm Sci, 6(7):11-20.
13. Ramadan AA, Mandil H, Sabouni J, 2020. "A Spectroscopic Study of Interactions Amoxicillin Sodium With Iodine in Acetonitrile and Formation of Complexes". Int J Curr. Res Chem Pharm Sci, 7(5): 14-27.

14. Sawyer DT, Heineman WR, Beebe JM, 1984. "Chemistry Experiments for Instrumental Methods". Wiley, New York. P.198– 200.
15. ICH: Proceedings of the International Conference on Harmonization of Technical Requirement of Registration of Pharmaceuticals for Human Use (ICH Harmonized Tripartite Guidelines), 2005.

Access this Article in Online

Website: www.ijcrcps.com

Subject: Chemistry

Quick Response Code

DOI: 10.22192/ijcrcps.2020.07.08.001

How to cite this article:
Abdul Aziz Ramadan, Hasna Mandil, Jenan Sabouni. (2020). Spectrophotometric Study of Interactions Penicillin G Benzathine With Iodine in Acetonitrile and Formation of Complexes. Int. J. Curr. Res. Chem. Pharm. Sci. 7(8): 1-15.
DOI: http://dx.doi.org/10.22192/ijcrcps.2020.07.08.001

