Iraqi Journal of Market Research and Consumer Protection
(2021) 13(2): 43-55
DOI: http://dx.doi.org/10.28936/jmracpc13.2.2021.(4)
Efhema et, al.

المجلة العراقية لبحوث السوق وحماية المستهلك

ULATRAVIOLET ABSORBANCE SEPECTRA FOR ANTIBIOTIC DERIVATIVES (AMINO GLYCOSIDES) USED IN MEDICAL AND PHARMACEUTICAL INDUSTRY

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Received 12/ 10/ 2020, Accepted 15/ 7/ 2021, Published 31/ 12/ 2021

ABSTRACT

Ultraviolet spectrophotometric studies for antibiotic (amino glycoside) derivatives including, *Neomycin*, *Streptomycin*, *Gentamycin* and *Kanamycin* with special reagents, which are benzoyl chloride; benzene sulfonyl chloride, toluenesulfonyl chloride and phthalic anhydride were made. *Amino glycosides* derivatives were followed through measurements of the ultraviolet absorbance (A) from which the absorptivity (ε) of the complexes was deduced and molar absorbances using Ultraviolet for products and calculate the number of reagents molecule that combine to amino glycosides.

Key words: Amino glycosides, organic reagents, ultraviolet-visible spectrometer, melting point apparatus.

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امتصاصية طيف الأشعة فوق البنفسجية لمشتقات المضادات الحيوية الأمينوكلايكوسايد المستعملة في الصناعات الطبية والصيدلانية

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Received 12/ 10/ 2020, Accepted 15/ 7/ 2021, Published 30/12/ 2021

الخلاصة

دراسة طيف الاشعة فوق البنفسجية لمشتقات المضادات الحيوية (الامينوكلايكوسايد) التي تتضمن مركبات الأمينوكلايكوسايد متعددة الصيغ الكيميائية منها *Neomycin* و*Streptomycin* و*Gentamycin* و*Kanamycin*، ونظرا لضعف امتصاصيتها بالأشعة فوق البنفسجية، تم معالجتها بكواشف لها امتصاصية واضحة مثل benzoyl chloride وbenzene sulfonyl chloride وtoluene sulfonyl chloride وphthalic anhydride للحصول على معقدات يمكن قياس امتصاصيتها باستعمال طيف الأشعة فوق البنفسجية لهذه النواتج والتي عن طريقها يتم حساب عدد جزيئات الكاشف التي محتمل ارتباطها بكل من مركبات الامينوكلايكوسايد.

الكلمات المفتاحية: الامينوكلايكوسايد، الكواشف العضوية، مطياف الاشعة فوق البنفسجية، جهاز قياس الذوبانية.

INTRODUTION

Amino glycoside are groups of antibiotics includes, *Gentamycin, Kanamycin, Neomycin* and *Streptomycin*. Some of these antibiotics are complex and consists of two (or three) major isomeric components. Streptomycin. This antibiotic effective against tuberculosis .Neomycin is analogous to streptomycin and isolated from streptomycesfaradiae **(Tsuji & Jenkens 1986)**. Also Kanamycin produced by streptomyces kanamycin antimicrobial spectrum is similar to neomycin **(Erice *et al.*, 2006; Sybil 1998)**. Gentamycin, Neomycin, Kanamycin and streptomycin are antibiotic belonging to the aminoglycoside group and they are effective against wide verity of microorganisms **(Goda & Khtar 1987; Usui & Umezawa 1987)**. Because these antibiotics are not Uv-absorbent so we need to introduce a suitable organic reagent as chromophore in pre-column derivatization high performance liquid chromatography (HPLC) to enhance solubility, separation and delectability. Its bactericidal power derives from the binding of the molecule to the protein of the bacterial subunit 30S, which disturbs protein synthesis (i.e. inhibits protein synthesis) **(Eugene *et al.*, 2011)**.

Some antibiotic have hydroxyl and amino functional groups (i.e, poly functional with two types of groups) which effected by reaction conditions **(David 2009)**. For trace analysis acid chloride the suitable reagent for quantification by HPLC. The reaction of amino sugar with acid chloride is effected by base as catalysts which enhance the reaction and neutralized the liberated acid **(Snyder *et al.*, 2009; Furniss *et al.*, 1989)**.The effects of solvents and/or catalysts play important parts in types of products. (i.e. complete or incompletes) **(Ziadan 1989)**. Problems facing amino glycoside (antibiotics) are:

The quantitative determination of antibiotics is one of the most difficult areas of pharmaceutical analysis **(Snyder *et al.*, 2009)**. Derivatization products recovery from solvent shows difficulty especially that used for re crystallization. The derivatives of amino glycoside under investigation which gives more than one type of products (ester and/ or amide) **(Ziadan 1989)** and this made difficulty to find a suitable solvent to give precise Uv-result. Most aminoglycoside derivatives have not sharp melting point **(Furniss *et al.*, 1989; DuPont & George 1986)**. Spectra in the visible and ultraviolet region arise from transitions between different energy levels of electrons in atoms or molecules. The essential fact about atomic or a molecule can occur only if the energy of the absorbed atom equals the energy difference ΔE between two energy levels in the atom or molecule.

$\Delta E = E_2 - E_1 = h\nu$, Where: ν is The frequency which is $\nu = (C/\lambda) = C\bar{\nu}$

C is speed of light. λ is Wavelength. $\bar{\nu}$ is Wave number **(Skoog *et al.*, 1998)**. Log $(I_o / I) = A = \varepsilon.C. L$, Where: A is absorbance.ε is molar absorption or molar absorptivity. C is Concentration of absorbing species. L is Sample thickness.

I_o is Intensity of incident light. I am Light intensity through sample transmitted.

Absorption of ultraviolet radiation by an organic molecule leads to electronic excitation among various energy levels within the molecule. The transition generally occur in between a bonding or lone–pair orbital and occupied non–bonding or ant bonding orbital. The ultraviolet visible electronic regions are as following: The Beer -Lambert law. States that the proportion of light absorbed by a solute in a transparent solvent is depends on the intensity of the incident light and is a proportional to the number of a absorbing molecules in the light path **(Furniss *et al.*, 1989)**.

Important useful terms concerning ultraviolet spectroscopy. The colored substances owe their color to the presence of one or more unsaturated groups responsible for electronic absorption. These groups are called chromophores. Typical examples are C=C, C=N, C=O, N=N, etc; they all absorb intensely at the short wavelength end of the spectrum but some of them (e.g. carbonyl) have less intense bands at higher wavelength owing to the participation of electrons. An auxochrome is an auxiliary group which interacts with the chromophore causing a bathochromic shift. Typical examples are amino and substituted amino groups (NH_2,NHR and NR_2), hydroxyl and alkoxy groups. In general, an auxochrome is a group that deepens color; its presence because a shift in the UV or visible absorption maximum to a longer wavelength **(Jag 2004)**.

Shift of an absorption maximum to longer wavelength .It is produced by a change of medium ($\pi \rightarrow \pi^*$)transitions shift with an increase in the polarity of the solvent) or when an auxochrome is attached to a carbon-carbon double bond. Ethylene, for example, absorbs at 175 nm in comparison to1-butene (λ_{max} = 185 nm) or isobutene (λ_{max} = 188 nm). The bathochromic shift is progressive as the number of alkyl groups' increase.

A shift of absorption maximum to shorter wavelength is known as hypsochromic shift. This may be caused by a change of medium ($n \rightarrow \pi^*$)transitions undergo hypsochromic shift with an increase in the polarity of (solvent). It is the effect leading to increased absorption intensity. For example the intensities of primary and secondary bands of phenol are increased in phenolate. It is the effect leading to decreased absorption intensity. For example, the intensities of primary and secondary bands of benzoic acid are decreased in benzoate **(Jag 2004)**. The most important factors affecting the position of ultraviolet bands.

1. Effect of steric hindrance on planarity (steric inhibition of resonance). Distortion of chromophore may lead to red or blue shifts depending upon nature of distortion.
2. Solvent shifts in polyenes (alkenes) and enones (ketone) are due to the difference in relative capabilities of solvents to sulfate the ground and excited state of molecule. Solvent effects of up to 20 nm may be observed with carbonyl compounds. Thus the ($n \longrightarrow \pi^*$) absorption of acetone occur at 279 nm in hexane; In water λ_{max} is 264.5 nm. Weak absorption band in region 280 to 290 nm, which displaced toward shorter wavelength with

increased solvent polarity Strong absorption band indicates the presence of carbonyl Group **(Skoog & Donald 1998)**. As the solvent polarity increases, blue shift is observed in the maximum absorption wavelength (λ_{max}) (hypsochromic shift) **(Jag 2004)**.

MATERIALS AND METHODS

Derivatization reagents, benzoyl chloride, benzene sulfonyl chloride and toluene sulfonyl chloride used sulfonamide respectively **(Furniss *et al.*, 1989; Vogel 1970)**. Phthalic anhydride used **(Vogel 1970; Morrison & Boyd 2010)**. The product called sulfonate **(Graham *et al.*, 2020)**.

Antibiotic derivatization, *Neomycin, Kanamycin, Gentamycin* and *Streptomycin* have OH-Groups and NH_2-groups, and according to these functional groups; they have amine and alcohol character. In general, the reaction of amine and alcohol with acid chloride (*Benzoyl chloride, BZ.Cl*) using catalysis to from amide and ester respectively in "*Schotten–Baumann*" reacts as follows:-

$$R\text{-}NH_2 + BZ.Cl \xrightarrow{NaOH} R\text{-}NH\text{-}\overset{\overset{O}{\|}}{C}\text{-}Ph + NaCl + H_2O$$

$$R\text{-}OH + BZ.Cl \xrightarrow{NaOH} R\text{-}O\text{-}\overset{\overset{O}{\|}}{C}\text{-}Ph + NaCl + H_2O$$

These are simple molecules, but for complex molecule such as *Neomycin, Kanamycin, Gentamycin* and *Streptomycin* the product is dependent on reaction conditions **(Jupille 1979; Lawerence 1979)**.

INSTRUMENTS

The main instruments used in this study were:

1. Ulatraviole-Visible. Spectrometer, double-beam UVE–Unicam, ATI UNICAM. UnicamUv series, prism software. (U.K). Uv–sample cells path length 4 cm (10 mL).
2. Melting point apparatus. Philip Harris, Shinnston-England, serial No. B/A-211.
3. Weight balance; Sartorius analytic type. A200S. Made in Germany.

PROCEDURE

Benzoylation an acid chloride is widely used as derivatizing reagent to enhance detect ability for isolation and quantification analysis. The benzoyl chloride used for separation of some carbohydrate by HPLC with pre-benzoylation **(Lehrfeld 1976)**. Some polyhydric alcohols as were separated as -P-NO2-benzene **(Schwarzenback 1977)**, -P-NO_2-benzoate used as derivative from Neomycin complex for separation by HPLC **(Ziadan 1998)**.

Amidation, acid chlorides were used also for amidation of amino groups to produce poly amidation, it is simple and rapid procedure **(Linskens & Jackson 1987)**. The reaction of acid chloride with primary and secondary amines also used **(Iwamori & Costello 1979)**. The benzoyl chloride used for analysis of amino glycoside antibiotics as benzoyl derivatives by HPLC and its application to quantitation of Neomycin inperilymph **(Harada & Iwamori 1985)**. The benzoyl chloride used for analysis pre-colomnderivatization of Neomycin complex **(Ziadan 1989)**. Detection system. The main limitation of analysis by HPLC lies in the detection system. The most commonly used detectors are ultraviolet spectrophotometers. Many compounds of interest don't contain Uv-absorbing chromophores, and therefore, cannot be detected in this way. This problem can often be solved by the preparation of suitable Uv-absorbing derivatives **(Lawrence 1979; Knox 1986)**.

Benzoyl chloride derivatization procedure **(Furniss *et al*., 1989; Vogel 1970)**.

Sodium hydroxide (NaOH):

Dissolve 1.0 g of drugs (*Gentamycin, Kanamycin, Neomycin* and *Streptomycin*) in 50 ml of H_2O in 150 mL conical flask then add 12 mL of benzoyl chloride and 30 mL of 10% NaOH solution. Stopper the flask and shake vigorously at frequent intervals until the odor of benzoyl chloride disappear (about 5 min) and crystalline product precipitates out. Collect the crystals by suction filtration and wash well with water. The crude product was re crystallized from ethanol and dried in Pyridine:

Dissolve 1.0 g of drugs in 30 mL pyridineand 15 mL of chloroform in 150 mL conical flask. Then 12 mL benzoyl chloride was added to the mixture. Stopper the flask and shake vigorously for about 10 min. Leave the reaction 24 hr at 0ºC. Dilute the reaction mixture with 75 mL $CHCl_3$ in separating funnel (500 mL), wash with 2 M sulfuric acid (H_2SO_4), distal water (H_2O), sodium hydrogen carbonate ($NaHCO_3$), then dry over anhydrous sodium sulfate, remove $CHCl_3$, dry the product. benzene sulfonyl chloride derivatization procedure **(Furniss *et al*., 1989; Vogel 1970)**.

Sodium hydroxide (NaOH):

Dissolve 1.0 g of drugs in 30 mL of 10% aqueous NaOH solution in 150 mL conical flask and then add 3.0 mL of benzene sulfonyl chloride (toluene-p- sulfonyl chloride) in 13 mL cold acetone, cork the flask securely and shake the flask frequently for duration 15-20 min. Cool the flask in running water from the tap and then pour its contents into about 150 mL water. Stir the aqueous mixture well and wash the crystal with H_2O and drain. Re crystallize the product from methylated spirit and dry on filter paper in the air.

Pyridine:

Dissolve 1.0 g of drugs in 30 mL pyridine and 15 mL of chloroform in 150 mL conical flask 12 mL of benzene sulfonyl chloride (toluene-p- sulfonyl chloride). Stopper the flask and shake vigorously for about 10 min leave the reaction over night at 0ºC. Dilute these contain with 75 mL $CHCl_3$ in separating funnel (500 mL),wash with 2 M sulfuric acid (H_2SO_4), distal water (H_2O), sodium hydrogen carbonate ($NaHCO_3$), then dry over anhydrous sodium sulfate, remove $CHCl_3$, dry the product.

Phthalic anhydride derivatization:

Heat mixture of 1.0g of drugs, 7.5 g of phthalic anhydride and 20 mL of dry pyridine on water bath for 1 hr and then allow the products to cool. Dissolve the resulting viscous mass in equal volumes of acetone (25mL), slowly, and preferably with stirring, 55 mL of concentrated HCl and with crushed ice, if oil is completely precipitate. This usually sets to hardness with 1-2 hr. If the resulting is semi-solid or pasty transfer to large flask and dry the product **(Furniss *et al*., 1989; Vogel 1970)**.

Determination of OH groups procedure (Vogel 1970):

1. a- 1 M phthalic anhydride. b- 10 mL equivalent D-glucose.
   c- 12.5 mL of reagent and 1.8 g D-glucose in round bottom flask 1hr on steam bath. D- 12.5 mL of reagent without sample as blank heated on steam bath for 1hr. e- 5 mL of distilled water to both (c, d) and heat for 5 min. cooling then titrated both blank and sample with 0.5 N NaOH using pH indicators.
2. a- 0.5 g D-glucose and 2.0 g phthalic anhydride in (15 mL pyridine+ 35.0 mL H_2O) shake the mixture then after 5 min. take 5 mL from this mixture with drops of pH and titration with 0.1 N NaOH. b- Repeat (a) at different time.
3. Theoretical treatment for experimental data. **(Smith & March 2017)**.

RESULTS AND DESCUSON

ULATAVIOLET MEASUREMENT RESULTS:

Figure (4, 5, 6, 7, 8, 9, 10, 11, 12 and 13) show the variation of absorbance with concentration in methanol for reagents (BZCl, BZ.S.Cl, T.P.S.Cl, and Ph.A). As well as calculated $\varepsilon_{max.}$ Explain the variation of absorbance with concentration in the methanol for the antibiotic-derivatives that were prepared in different medium (pyridine and aq. NaOH) also calculated ε_{max}. The compose (Table 1) show the possible number of substituted reagent on antibiotic derivatives calculated from Uv data (n_c, n_ε) .the method that are used to test the validity of the theoretical expression which applied to calculate and (Table1) to small molecule (D-glucose) represented in the (Table 2).

This table contains glucose-Ph.A derivative which was prepare in pyridine using titration methods OH-groups and occupation, (Table 1). Compare with that calculated from-data. Also glucose-BZ derivative in aq. NaOH medium by comparing penta-benzoate. Derivatives as given in literature with that calculated from Uv-data. (Figure 1, 2, 3 and 4) shows the uv-spectrum of reagents (BZCl, BZ.S.Cl, T.P.S.Cl and Ph.A), while (Figure 5, 6, 7) are the Uv-spectrum of streptomycin and strpt.-derivatives, while (Figure 8, 9, 10, 11, 12 and 13) are the uv-spectrum of (Kan. and Gen.) derivatives. The most antibiotics does not show Uv-absorption above 215 nm so we need to induced suitable chromophore (reagents) to the yield product orderto get Uv-absorption to simplify separation and quantization **(Freifelder 1982)**.

Electronic band shift depends on solvent polarity, pH and the relative orientation of neighboring chronophers **(Freifelder 1982)**. If the groups are relatively close to each other or are separated by conjugated system across which electronic band shift may be transferred. If the two chromospheres are separated by saturated chain of three or more carbon atoms each exerts it^s own effect, so that the absorption spectrum of derivative is almost the same as those of reagent **(Samuel 2010)**. the Uv-technique and their high sensitivity, Accuracy, reliability and precision make Uv-absorption analyzers suited to many process steam analysis problem **(Galen 2013)** .in solution many molecules form dimmer or higher polymers as concentration increase. Another fraction at high concentration is aggregation this lead to increase or decrease wavelength of Uv-absorption due to electronic interaction **(Freifelder 1982)**. The derivatization under takes of the two types catalytic condition, sodium hydroxide And/ or pyridine. Amine reacts so rapidly in pyridine, usually complete after refluxing for 10-15 minutes **(Furniss *et al.*, 1989).**The yield for benzoylation of hydroxyl group by pyridine is the higher than the yield by sodium hydroxide **(Paul 1976)**. Uv-absorption of antibiotic-derivatives. (Table 1) shows the antibiotic-derivatives also the reaction conditions which are used for derivatization as it found in the literature **(Furniss *et al.*, 1989)**. The physical properties recorded for the first time in this study. The antibiotic-Ph. A derivative shows the melting point (164-165ºC) and white powder in color and shape, this indicates that the derivative is proceed in the same way. The melting point for Kan-BZ, and Neo-BZ, in NaOH medium show 105, 75 and 105ºC respectively. Also the melting point of the derivative, Kan.-BZ., Gen.-BZ.S and strep.-BZ.-S in NaOH medium are 150ºC and 190ºC respectively, and gives different color and shape solid product. The Kan.-T.P.S., Gen.T.P.S.,Neo.-T.P.S and Stept.-P.T.S. derivatives in NaOH medium having 58, 95, 135 and 95ºC respectively. In general alcohol functional groups when it react with the reagents in used give low melting ester derivative **(Martin 2012)**. Some of these antibiotic-derivatives give oily products. As well some of them give liquid crystals phase properties **(Furniss *et al.*, 1989)**. Because the antibiotics have different functional groups (alcohol and amine) so we get the different derivatives product for the same reagent or the different Reagent for the same antibiotic. The

ε_{max}. for reagent and antibiotic-derivatives calculated from experimental uv-data. The possible number of substituted reagents were calculated in the two ways (n_ε and n_c) summarized in (Table 2).The compares between these values (n_ε and n_c) for each antibiotic-derivatives show relatively not much different except for Srept.-derivatives, Neo.-T.P.S.(n_ε =8.43 and n_c =2.13), Gen.-BZ.(n_c =6.8 and n_ε =1.53) and Gen.-BZ./BZ (n_c=2.3 and n_ε=6.2). This deviation is due to solubility of these an antibiotics derivatives in solvent which used for uv-measurements, some antibiotics show the dual polarity affecting solubility **(Martin 2012)**. In order to check methods validity for possible number of reagents substituted on antibiotic molecules, small molecule such as D-glucose have (5-OH groups) table(29) which is similar to subunit of antibiotics under investigation. The G-BZ.derivtive is give, n_c =5.56 which nearly equal to experimental result penta-benzoate. G-ph.A derivatives according OH-groups determination method **Vogel (1970)** give the value 5.052, occupation method of **Smith & March (2001)**.The value is 404 while uv-data calculation gives (n_c =3.7) and(n_ε =5.23). The uv-spectrum for reagents (Figure 1, 2, 3 and 4), (Figure 5) represent the uv-spectrum of streptomycin and (Figure 6) is for strept.-BZ. The similarity between uv-spectrum (Figure 1, 5, 6) specially those peaks at λ_{max}. =284, 277 and 273 nm respectively, indicate the uv-spectrum (Figure 6) is look like hyperdized spectrum of that in (Figure 1) and (Figure 5). The reagent chromospheres absorbance are at λ_{max} (287±7) for Ph.A, (280±4) for BZ.Cl and (273±2) for BZ.S.Cl and T.P.S.Cl which are reflected with antibiotic-derivatives as λ_{max} (280±4) for Kan.-T.P.S. The reagent that gives the higher absorptivity (ε) is the best for the detection limits for example phthalic acid anhydride reagent in methanol λ_{max} 274 nm (ε =1290). While in benzoyl chloride reagent At λ_{max} 271nm (ε =1000). The effect of solvent clearly appear in uv-spectrum (Figure 9) Kan.-BZ.S in $CHCl_3$,λ_{max} =274 nm(a) and (Figure 9) Kan.-BZ.S. in 5% NaOH, λ_{max}= 275nm (b), while uv-spectrum (Figure 12) show solvent effect as well as antibiotic-derivatives solubility effect according to difference in absorbance value (a) A=0.946, when the solvent is methanol and (b) A =2.406 when the solvent is 5% NaOH. Also this Uv-spectrum show shift in wavelength from λ_{max} =272 nm (a) to higher wavelength, λ_{max} 293 nm (b) which clearly appear the pH of the solvent affecting the absorption of chronopher **(Freifelder 1982)**. Temperature undergoes transition to the liquid state. These intermediate states have been called liquid crystals.

Table (1): Possible number of reagent molecules substituted on antibiotic sample to form derivative calculated according to ultraviolet.

Derivatives	Reag.	Uv-Derivatives data				Reag. Number	
Antib. -Reag.	ε_R	ε_C	C_C	A_C	C_{C-R}	n_c	n_ε
Kan.-BZ. /A	0.655	5.728	0.0583	1.850	0.706	12.12	12.13
Kan.-BZ. /B	0.655	3.600	0.1200	2.059	0.786	6.55	7.7
Kan.-BZ.S. /B	1.084	3.547	0.1440	2.222	0.513	3.56	5.78
Kan.-T.P.S./A	0.611	2.032	0.1200	2.352	0.9624	8.02	8.34
Kan.-T.P.S./B	0.611	2.365	0.0734	1.439	0.589	8.02	7.37
Neo. -BZ. /A	0.655	2.717	0.1200	1.895	0.7233	6.03	5.8
Neo. -BZ. /B1	0.655	3.0559	0.12	2.222	0.8481	7.07	6.56
Neo. -BZ. /B2	0.655	4.6093	0.12	2.4	0.916	7.6	9.89
Neo. -T.P.S./A	0.611	0.6841	0.12	2.471	1.01105	8.43	2.13
Gent. -BZ. /A	0.655	0.714	0.139	2.48	0.9466	6.8	1.53
Gent. -BZ. /B	0.655	3.6737	0.12	2.144	0.8183	6.82	7.88
Gent. -BZ.S. /B	1.084	3.8285	0.095	0.946	0.2183	2.3	6.2
Gent. -T.P.S. /A	0.611	3.2107	0.12	2.75	1.1252	9.4	10.01
Gent. -T.P.S. /B	0.611	2.0268	0.1391	2.844	1.16367	8.37	6.32
Gent. -Ph.A /B	1.048	3.7957	0.12	2.35	0.560485	4.67	5.36
Strept. -BZ. /A	0.655	4.10	0.012	2.273	0.867557	7.23	8.68
Strept. -BZ. /B	0.655	0.4137	0.1395	2.643	1.0087	6.14(3.8)	0.778
Strept. -BZ.S. /A	1.084	0.3514	0.12	0.457	0.1054	0.878(0.47)	0.489
Strept. -BZ.S. /B	1.084	2.5034	0.065	2.12	0.4889	7.5	4.08
Strept.-T.P.S. /A	0.611	1.2376	0.12	2.193	0.897	7.5 (3.8)	3.76
Strept.-T.P.S. /B	0.611	1.2256	0.119	2.18	0.892	7.5 (3.8)	3.76
Strept.-Ph.A /B	1.048	2.1965	0.12	2.302	0.54914	4.57 (2.3)	3.03
Strept. /average =(0.0578+0.0443)/2=0.05105					εC-0.05105		

Table (2): methods validity test for small molecule D- glucose.

Methods / Derivatives	Titration		Uv at λmax. =282, 275 nm, EtOH						
	OH Groups	Occupation	ε_R	ε_C	C_C	A_C	C_{C-R}	n_c	nε
G-Ph.A/B	5.052	4.04	1.048	3.7	0.09	1.374	0.33	3.7	5.23
G-BZ. /A	M.P of Penta benzoate								
	Literature	Experiment							
	179 ˚C	172 ˚C	0.655	3.65	0.07	1.022	0.39	5.56	7.8 *

*Calculated only according to a single absorbance value.

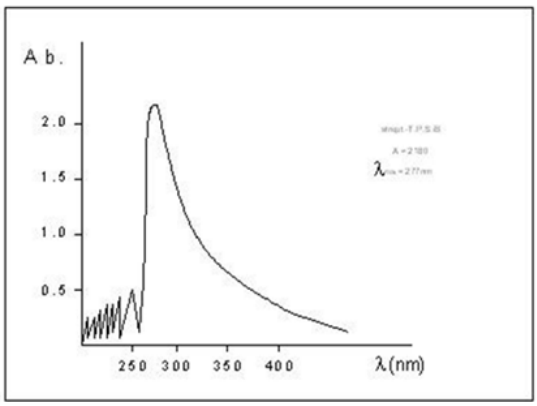


Figure (1): Uv spectrum of BZ.CL.

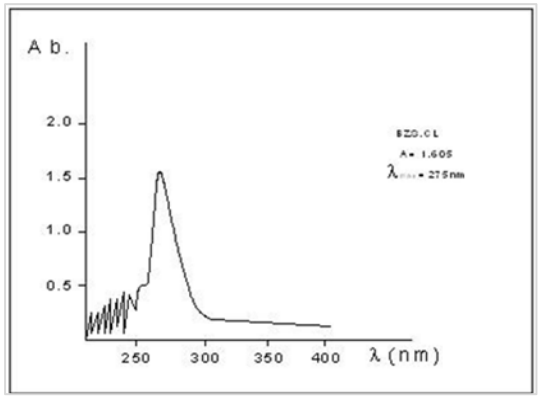


Figure (2): Uv spectrum of BZ.S.CL.

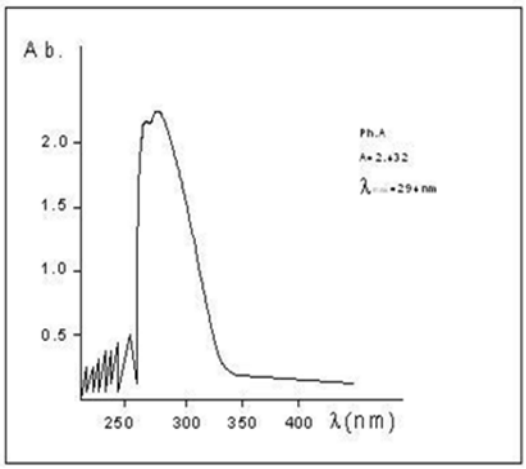


Figure (3): Uv spectrum of T.P.S.CL.

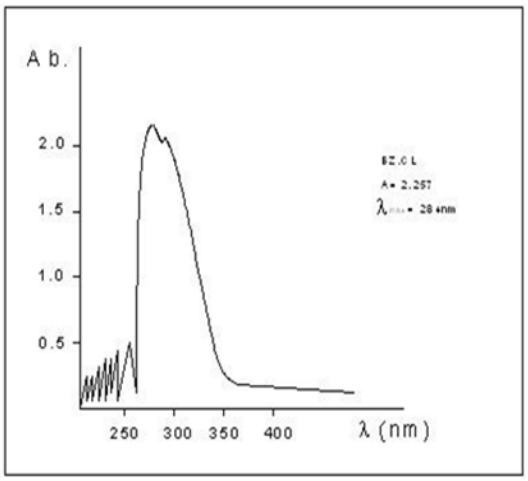


Figure (4): Uv spectrum of PH.A.

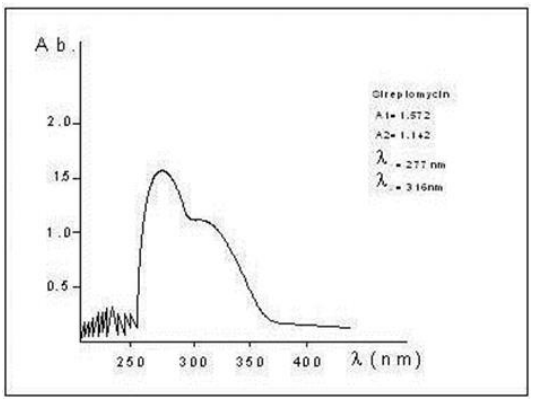


Figure (5): Uv spectrum of streptomycin.

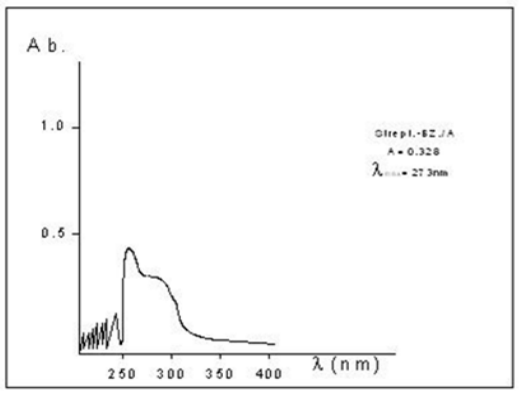


Figure (6): Uv spectrum of strept.

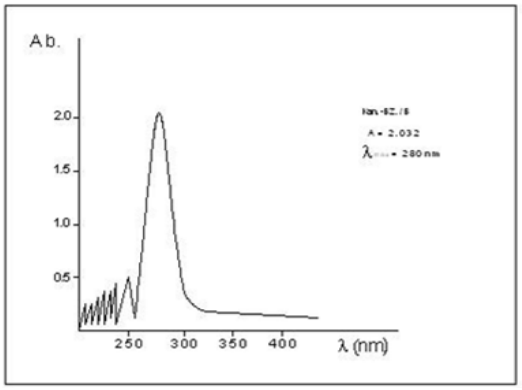
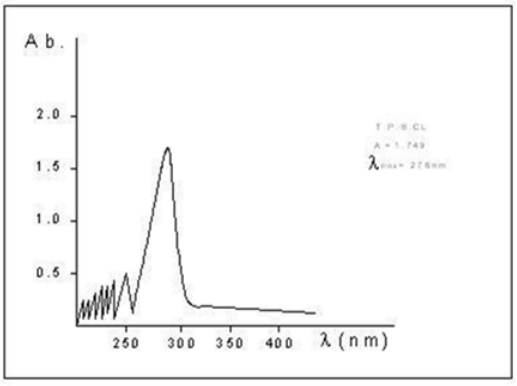


Figure (7): Uv spectrum of strept –T.P.S./B.



Figure(8): Uv spectrum of Kan. –BZ./B.

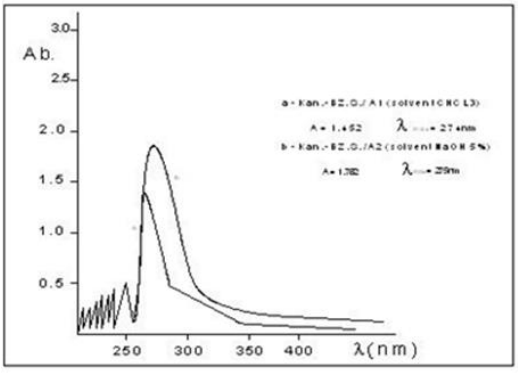


Figure (9): Uv spectrum of Kan.

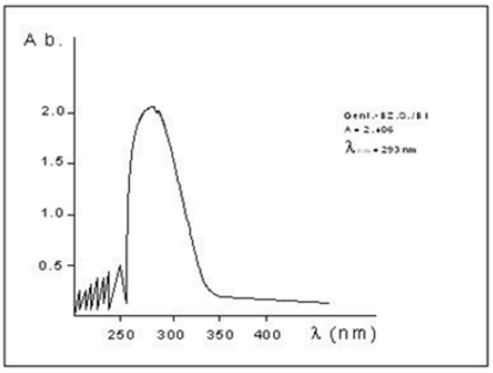


Figure (10): UV spectrum of Gent. –BZ.S./Bl.

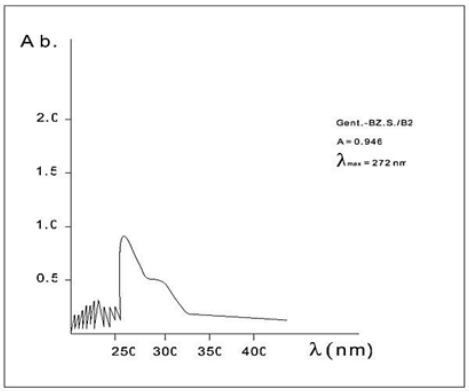


Figure (11): Uv spectrum of Gent.-BZ.S./B2.

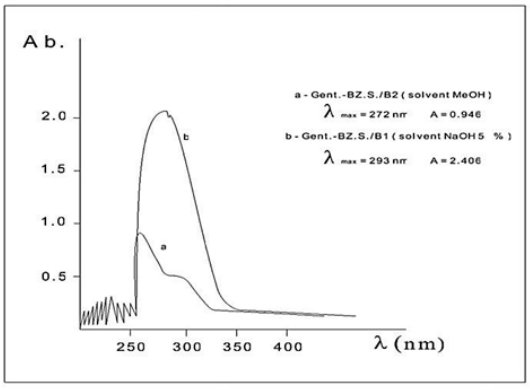


Figure (12): Uv spectrum of Gent.-BZ.S./B.

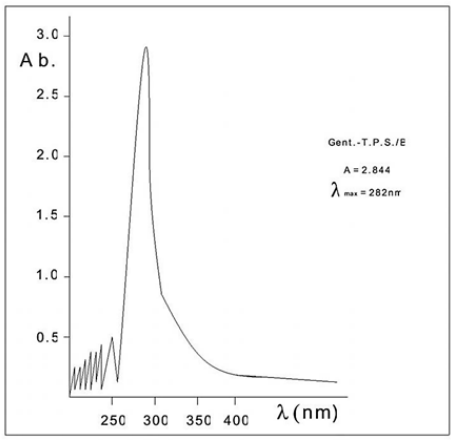


Figure (13): Uv spectrum of Gent.-T.P.S./B.

CONCLUSIONS

The reactivity in the formation of products and hydrolysis of antibiotic-derivatives esters and/ or amides are related to: i. the electrophilic character of the carbonyl carbon atom. ii. Steric hindrance on co planarity. iii. Stabilization of the carbonyl group by conjugation. The best solvent and/ or catalyst used in preparation of antibiotic-derivatives are pyridine. Uv-spectroscopy is very sensitive to distortion of the chromosphere and the consequently the steric repulsions which oppose the co planarity of conjugate π- electrons system. The reagent chromosphere absorbance are at λ_{max} (287±7) for pH. A, (280 ± 4) for BZ.Cl and (273 ± 2) .For BZ.S.Cl and T.P.S.Cl which ar reflected with antibiotic-derivatives as λ_{max} (276 ± 3 nm) for Kan.-BZ.,(276 ± 5) for Kan.-BZ.S. and (280 ± 4) for Kan.-T.P.S. The reagent that gives the higher absorptive (ε) is the best for the detection limits for example phthalic acid anhydride reagent in methanol $\lambda_{max.}$274. (ε =1290). While in benzoyl chloride reagent at 271 nm (ε =1000).The bathochromic or hypsochrome shift occurs with antibiotic- derivatives belong to different substituted functional groups on (─OH or─ NH_2) to gives esters which absorbed at higher wavelength (relative to absorption of corresponding to reagent) while in case of amide the wavelength displaced to short wavelength. This result gives the good indication in stability of the antibiotic- derivative (amide more stable than ester). The crystalline form for some of these antibiotic-derivatives, does not melts directly to a liquid phase but first passes through an intermediate stage (the Para- crystalline state).

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