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Efhema & Khalaf

المجلة العراقية لبحوث السوق وحماية المستهلك

RATE CONSTANT OF SOME AMINO DERIVATIVES DISSOCIATION

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ABSTRACT

Amino glycoside derivation including, Neomycin, Streptomycin, Kanamycin and Gentamycin with special reagents, which are benzoylchloride; benzene sulfonyl chloride and phthalic anhydride were made to enhance Uv-detectability for HPLC analysis. But there are many problems facing pre column derivation and in order to solve this, the conductivity of antibiotic derivatives were used to calculate the dissociation constant and the hydrolysis rate which determined concern type reaction. In addition the characteristics those controlling the hydrolysis of antibiotic-derivatives were investigated.

Keywords: Aminoglycosides, derivative reagents, conduct metric measurement, dissociation constant calculation.

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ثابت سرعة تحلل بعض مشتقات الامينوكلايكوسيدات

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الخلاصة

تتضمن مركبات الأمينوكلايكوسيد صيغ متعددة منها النيومايسينو الاستربتومايسينو الكاناميسينو الجنتاميسينو التي يتم معالجتها بكواشف معينة لتحسين تحديد امتصاصيتها بواسطة الأشعة فوقالنفسجية لمركبات الكواشف كالبنزويل كلوريد والبنزين باراسلفونيل كلوريد والتلوين بارا سلفونيل كلوريد والفثاليكانهيدريد من خلال الحصول على معقدات لتحديدها كيميائيا بواسطة تقنية كروماتوكرافيا السائل عالي الكفاءة، الا انه هناك مشكلة تواجه تلك الدراسة وهي تفكك المعقدات التي يتم حقنها في العمود، ولهذا وجد من الضروري معرفة استقراريه تلك المعقدات من خلال تعيين ثابت التفكك ومعدل التحلل لهما وذلك بدراسة توصيلية تلك المعقدات المتكونة، وكذلك تم تحديد العوامل التي تتحكم في عملية تحلل كل منهما.

الكلمات المفتاحية: الامينوكلايكوسيدات، العوامل الكاشفة، مقياس التوصيلية، حساب ثابت التحلل.

INTRODUCTION

Aminoglycoside are groups of antibiotics that are used to treat certain bacterial infection **(Macdonal, 1978)**. This group of antibiotics includes, Gentamycin, Kanamycin, Neomycin and Streptomycin. Some of these antibiotics are complex and consists of two major isomeric components. Streptomycin the first amino glycoside was isolated from streptomyces griseous in mid-1940S **(Macdonal,1978)**. This antibiotic effective against tuberculosis. Neomycin is analogous to streptomycin and isolated from *Streptomyces faradiae* **(Tsuji & Jenkins,1986)**, also Kanamycin produced by *Streptomyces kanamyeticusanti* microbial spectrum is similar to neomycin**(Sybil,1998)** Gentamycin's. Neomycin's, Kanamycin and streptomycin's are antibiotic belonging to the amino glycoside group and they are effective against wide variety of microorganisms**(Dely*et al.*, 1975)**. Because these antibiotics are not Uv-absorbent therefore we need to introduce a suitable organic reagent as chromosphere in pre column technique for high performance liquid chromatography (HPLC) to enhance solubility, separation and detectability. Its bactericidal power derives from the binding of the molecule to the protein of the bacterial subunit 30S, which disturbs protein synthesis **(Roberts *et al.*,2001)**.

The chemical modification has been made in order to increase sensitivity and selectivity for a variety of pharmaceutical compounds to some or all of the functional groups because no Uv-absorbance between 212-360 nmandchromatic characteristic of these compound can be improved **(Leach *et al.*,1951)**.

Some antibiotics have hydroxyl and amino functional groups (i.e, poly functional with two types of groups) which effected by reaction conditions **(2010; 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 baseas catalyst which enhance the reaction and neutralized the liberated acid **(Snyder & Kirkaland,1979; Furniss *et al.*,1984)**.

The effects of solvents and/ or catalysts play important role in types of products. i.e. complete or incomplete **(Ziadan, 1989)**.The problems facing amino characterization of glycosides (antibiotics) and their derivatives.

- The quantitative determination of antibiotics is one of the most difficult areas of pharmaceutical analysis **(Snyder & Kirkaland, 1979)**.
- Derivatization products recovery from solvent shows difficulty specially that used for recrystallization.

- The derivatives of aminoglycoside 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**(Dely*et al.*, 1975)**.
- Most amino glycoside derivatives have not sharp melting point **(Furniss*et al.*, 1984)**.

Derivation Reagents

Benzoyl chloride was suitable reagent for aliphatic derivatives and polyhydric alcohol as benzoate also for derivatives primary and secondary amine as Benz amide benzene sulfonyl chloride and P-toluene sulfonyl chloride used for primary and secondary amine gives benzene sulfonamide and P-Toluene sulfonamide respectively **(Furniss*et al.*, 1984)**.Phthalic anhydride used for primary amine and resolution of racemic alcohol as phthalate**(Dely*et al.*, 1975;Furniss*et al.*,1984)**. alcohol reacts with benzene sulfonyl chloride to from ester **(Morrison&Boyd,1995)**.The product called sulfonate**(Graham&Craig,2002)**.

Physical properties of antibiotic derivation

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 esterrespectively 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**(Morrison & Mosher,1971)**.

Benzoylation

An acid chloride is widely used as a derivatizing reagent to enhance delectability for isolation and quantification analysis**(Brooks *et al.*, 1953)**. The (BZ.Cl) used for separation of somecarbohydrates by HPLC with pre benzoylation**(Lehrfeld,1976)**.Some polyhydric alcohols were separated as -P-NO_2- benzoate **(SchwerZenback, 1977).** p-NO_2-benzoate used as a derivative from neomycin complex for separation by HPLC **(Ziadan,1989).**

Amidation

Acid chlorides were used also for amidation of amino groups to produce polyamine, it is a simple and rapid procedure and also**(Linskens&Jakson,1978)**.benzoyl chloride used for derivatization of amine in liquid chromatography (LC)**(Clark & Wells,1978)**.The reaction of acid chloride with primary and secondary amines also used**(Iwamori*et al.*, 1979)**.The BZ.Cl used for analysis of aminoglycoside antibiotics as benzoyl derivatives by HPLC andits application to quantitation of Neomycin in perilymph.The benzoyl chloride used for pre-column derivatization of neomycin complex **(Ziadan,1989)**. The molar conductivity of strong electrolytes vary linearly with square root of concentration. **(Crow,1994)**.

$$\Lambda = \Lambda_\infty - B\sqrt{C} \qquad \text{.....(1)}$$

This relation is called kohlrausch's law **(Bokris& Reddy, 1977)**.

Λ - equivalent conductance.

Λ_∞ - equivalent conductance at infinity dilution .

C-concentration.

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Weak electrolytes

Weak electrolytes are partially ionized in solution. They include weak Bronsted acids and bases. The molar conductivities arise from the displacementof equilibrium toward products at low molar.

$$HA\,(aq) + H_2O \rightleftharpoons H_3O^+ + A^- \quad \ldots\ldots\ldots\ldots (2)$$

The conductivity depends on the number of ions in solution and therefore on the degree of ionization α of electrolyte.

$$K_a = [H_3O^+][A^-] / [HA][H_2O] \quad \ldots\ldots\ldots\ldots (3)$$

$[H_3O] = \alpha C$, $[A^-] = \alpha C$, $[HA] = (1-\alpha)\, C$

$$K_a = \frac{\alpha^2 C}{1-\alpha} \quad \ldots\ldots\ldots\ldots (4)$$

The electrolyte is fully ionized at infinite dilution, and it's molar conductivity, Λ_∞ .**(Paul,2001)**.

$$\alpha = \frac{\Lambda}{\Lambda_\infty} \quad \ldots\ldots\ldots\ldots (5)$$

Substituting eq. (5) in eq. (4) we get eq. (6)

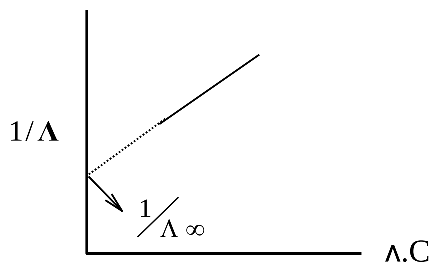
$$K_a = \frac{C\,\Lambda^2}{\Lambda_\infty(\Lambda_\infty - \Lambda)} \quad \ldots\ldots\ldots\ldots (6)$$

Which can be rearranged to give :

$$\frac{1}{\Lambda} = \frac{1}{K_a \Lambda_\infty^2}(\Lambda C) + \frac{1}{\Lambda_\infty} \qquad \text{(Ostwald dilution law)}$$

Plotting of $(1/\Lambda)$ versus (ΛC) give straight line with a slope of $(1/K_a\Lambda_\infty^2)$ $(1/\Lambda_\infty)$ can be determined by extrapolation to zero.

Slope = $1/K_a\Lambda_\infty^2$, intercept = $1/\Lambda_\infty$



Chemical Kinetics

Chemical kinetics concerns with study of reaction rates, the changes in concentrations of reactants (or products) as a function of time.Rate laws and rate constants.

The rate is a change in some variable per unit of time.

Consider the general reaction :

$$aA + bB = cC + dD \quad \ldots\ldots\ldots\ldots (7)$$

$$\text{Rate change of [C] is defined as rate} = \frac{1}{c} d[C]/dt \ldots\ldots\ldots(8)$$

The rate varies with time and is equal to some function of concentration **(Paul, 2001).**

$$\frac{1}{c} d[C]/dt = f[A], [B], [D]. \ldots \quad \ldots\ldots\ldots(9)$$

MATERIALS AND METHODS

Instruments

The main instruments used in the study were:

1. Conductivity meter. HANNA Instruments. HI 8733. Made Portugal.
2. Melting point apparatus. Philip Harris, Shinnston-England, serial No. B/A-211.
3. Weight balance; Sartorius analytic type. A200S. Made in Germany.

Experimentation of Drugs

Benzoyl chloride derivatization procedure**(Furnisss*et al.*,1989)**.

Sodium hydroxide (NaOH)

Dissolve 1.0g of drugs (*Gentamycin, Kanamycin,Neomycin* and *Streptomycin*) in 50mL of D.W in 150mL conical flask then add 12 mL of benzoylchloride and 30 mL of 10% NaOH solution. Stopper the flask and shake vigorouslyatfrequent intervals until the odor of benzoylchloride disappear (about 5 minutes) and crystalline product precipitates out. Collect the crystals by suction filtration and wash well with water. The crude product was recrystallized from ethanol and dried in oven.Benzensulfonyl chloride derivation procedure **(Furnisss*et al.*, 1989)**.

Sodium hydroxide (NaOH)

Dissolve 1.0g of drugs in 30mL of 10% aqueous NaOH solution In150mL conical flask and then add (3.0 mL of *benzene sulfonyl chloride* (toluene-p-sulfonyl chloride) in 13Ml cold acetone, cork the flask securely and shake the flask frequently for duration 15-20 minutes.Cool the flask in running water from the tap and then pour its contents into about 150mL water.Stir the aqueous mixture well and wash the crystal with H_2O and drain. *Recrystallize* the product by methylated spirit and dry on filter paper in the air.

Determination of OH groups

Put 1M phallic anhydride, 10mL equivalent D-glucose. 12.5mL of reagent and 1.8g D-glucose all in round bottom flask for 1hr on steam bath.12.5mL of reagent without sample as blank, heated on steam bath for 1hr,and 5 mL of distilled water to both and heat for 5min. Cooling then titrated both blank and sample with 0.5N NaOH using pH indicators.

Occupation method**: (Smith & March,2001)**.

Using the following equation: $1/\upsilon^- = 1/\upsilon^- n - K[A]/\upsilon^-$ .....(10)

[A] reagent concentration need for antibiotic substitution.

υ^-- average number of reagent molecule bonded to one antibiotic molecule., n. number of position available for occupation on antibiotic.

and K-equilibrium constant.

Kinetic study procedure

1. prepared 100 mL of 0.05M ester in methanol and 0.05M NaOH.
2. Take 25mL of NaOH and dilute to 50mL and measure the conductivity,C_o.
3. Add 25mL of 0.05M ester (form from reagents) and 25mL of 0.05M NaOH, start tomeasure conductivity after 2min. for first 10min and then 5min, C_t.
4. Take solution of ester and NaOH; after 1hr. to complete reaction, then measure the conductivity, C_∞.

Conductivity measurement

1. prepared 0.1Mof reagents benzoyl chloride (BZ.Cl), benzene sulphonyl chloride (BZ.S .Cl) Para toluene sulfonly chloride (T.P.S.Cl) and phtalic anhydride (ph.A.
2. Prepared stock solution for antibiotic-derivatives ($g.L^{-1}$) as in (Tables 5-16).
3. Makes different solution by dilution for (1,2) and measure the conductivity.

Determination of rate constant of ester hydrolysis catalyzed by sodium hydroxide.

The second order reaction when (a = b) is $k_2 t = \frac{x}{a(a-x)}$, using conductivity instead of concentration as:

C_o at $t = 0$, C_t at $t = t$, and C_∞ at $t = \infty$

Therefor $a = C_o - C_\infty$ and $x = C_t - C_\infty$.

$$k_2 = \frac{1}{a \cdot t} \cdot \frac{C_t - C_\infty}{C_0 - C_t} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots(11)$$

$$C_t = \frac{1}{a \cdot K_2} \frac{(C_o - C_t)}{t} + C_\infty \quad \ldots\ldots(12)$$

Plotting of $\frac{C_o - C_t}{t}$ against C_t gives a streetlight line with slope $\frac{1}{a \cdot k_2}$ and intercept at C_∞**(John& Ralph,1981)**.

RESULTS AND DISCUSSION

Conductivity measurement

(Tables 1-4) and (Figure 1-4) show dissociation constant(Ka) for several concentration for reagents(BZCl), (BZ.S.Cl), (T.P.S.Cl) and (Ph.A), while the (Tables5-16) and (Figure 5-11) gives the antibiotic-derivatives results.

Table (1): Dissociation constant of *BZ.CL* at several concentration from conductivity measurements (Solvent MeOH).

C ($M.L^{-1}$)	k ($ms.cm^{-1}$)	Λ ($ms.cm^2.mol^{-1}$)	1 / Λ	C. Λ
0.100	9.85	98.5000	0.001015	9.850
0.065	6.97	107.231	0.009330	6.970
0.048	5.68	118.333	0.008450	5.679
0.038	4.72	124.211	0.008051	4.720
0.032	4.27	133.4375	0.007490	4.270
Cal. Λ ∞=176.923 K_d =0.0661				

Table (2): Dissociation constant of *BZ.S.CL* at several concentration from conductivity measurements (Solvent MeOH).

C ($M.L^{-1}$)	k ($ms.cm^{-1}$)	Λ ($ms.cm^2.mol^{-1}$)	1 / Λ	C. Λ
0.100	9.01	90.1000	0.011100	9.01
0.065	5.68	87.3850	0.011440	5.68
0.048	4.49	93.5542	0.010690	4.49
0.038	3.71	97.6320	0.010243	3.71
0.032	3.16	98.7500	0.010130	3.16
Cal. Λ ∞= 111.44 K_d = 0.2423				

Table (3): Dissociation constant of *T.P.S.Cl*at several concentration from conductivity measurements (Solvent MeOH).

C ($M.L^{-1}$)	k ($ms.cm^{-1}$)	Λ ($ms.cm^2.mol^{-1}$)	1 / Λ	C. Λ
0.100	6.01	60.100	0.01664	6.01
0.065	4.32	66.462	0.01505	4.32
0.048	3.49	72.708	0.01375	3.49
0.038	2.93	77.105	0.01297	2.93
0.032	2.61	81.563	0.01226	2.61
Cal. Λ ∞= 109.61 K_d = 0.0643				

Table (4): Dissociation constant of (*Ph.A*) at several concentration from conductivity measurements (Solvent MeOH).

C ($M.L^{-1}$)	k ($\mu s.cm^{-1}$)	Λ ($\mu S.cm^2.mol^{-1}$)	1 / Λ	C. Λ
0.100	32.0	320.000	0.003125	32.0
0.065	20.5	315.385	0.003171	20.5
0.048	16.5	343.75	0.00291	16.5
0.038	14.1	371.053	0.002695	14.1
0.032	12.1	378.125	0.002645	12.1
Cal. Λ ∞ = 461.32 K_d = 0.124				

Table (5): Dissociation constant of *Kan.BZ.* at several concentration from conductivity measurements (Solvent MeOH).

C (g. L^{-1})	k ($\mu s.cm^{-1}$)	Λ ($\mu S.cm^2.mol^{-1}$)	1 / Λ	C. Λ
0.031000	11.0	354.8390	0.002818	11.0
0.002000	7.9	3950.000	0.0002532	7.9
0.001490	6.1	4093.960	0.0002443	6.1
0.001185	5.3	4472.574	0.0002236	5.3
0.000980	4.5	4577.820	0.0002184	4.5
Cal. Λ ∞ = 357.73 K_d = 0.0154				

Table (6): Dissociation constant of *Neo.BZ.* at several concentration from conductivity measurements (Solvent MeOH).

C (g. L^{-1})	k (µs.cm^{-1})	Λ (µS.cm^2.mol^{-1})	1 / Λ	C. Λ
0.026	5.4	207.692	0.00481	5.4
0.017	3.9	229.41	0.00436	3.9
0.012	3.1	258.34	0.00387	3.1
0.0098	2.9	295.92	0.00338	2.9
0.00813	2.6	325.0	0.00308	2.6
Cal. $\Lambda \infty = 652.13$ $K_d = 0.00353$				

Table (7): Dissociation constant of *Gent.BZ* at several concentration from conductivity measurements (Solvent MeOH).

C (g. L^{-1})	k (µs.cm^{-1})	Λ (µS.cm^2.mol^{-1})	1 / Λ	C. Λ
0.0288	7.5	260.417	0.00384	7.5
0.01872	5.7	304.487	0.00328	5.7
0.0139	4.6	330.935	0.00302	4.6
0.0110	4.2	381.818	0.00262	4.2
0.009	3.6	400.0	0.0025	3.6
Cal. $\Lambda \infty = 1222.67$ $K_d = 0.0015$				

Table (8): Dissociation constant of *Gent.T.P.S.* at several concentration from conductivity measurements (Solvent MeOH).

C (g. L^{-1})	k (µs.cm^{-1})	Λ (µS.cm^2.mol^{-1})	1 / Λ	C. Λ
0.069	64	927.54	0.0011	64
0.045	49	1088.9	0.00092	49
0.033	37.5	136.4	0.00088	37.5
0.027	29.3	1085.2	0.00092	29.3
0.022	25.2	1145.5	0.000873	25.2
Cal. $\Lambda \infty = 1456.7$ $K_d = 0.069$				

Table (9): Dissociation constant of *Streptomycin* at several concentration from conductivity measurements (Solvent MeOH).

C (M. L^{-1})	k (ms.cm^{-1})	Λ (mS.cm^2.mol^{-1})	1 / Λ	C. Λ
0.01	2.89	289.0	0.00346	2.89
0.0087	2.48	285.1	0.003508	2.48
0.007	2.16	308.57	0.003241	2.16
0.0061	1.92	314.75	0.003177	1.92
0.0053	1.76	332.075	0.003011	1.76

Table (10): Dissociation constant of *Strept.BZ.* at several concentration from conductivity measurements (Solvent MeOH).

C (g. L^{-1})	k (µs.cm^{-1})	Λ (µS.cm^2.mol^{-1})	1 / Λ	C. Λ
0.018	2.4	133.33	0.0075	2.4
0.014	2.2	157.14	0.00636	2.2
0.011	2.1	190.91	0.00524	2.1
0.009	2.1	233.33	0.00429	2.1
0.008	2	250.00	0.0040	2
Cal. $\Lambda \infty = 66.49$ $K_d = 0.024$				

Table (11): Dissociation constant of *Strept.BZ.S.*at several concentration from conductivity measurements (Solvent MeOH).

C (g. L^{-1})	k (µs.cm^{-1})	Λ (µS.cm^2.mol^{-1})	1 / Λ	C. Λ
0.026	9.2	353.85	0.00283	9.2
0.02	7.9	395.0	0.00253	7.9
0.016	6.9	431.25	0.00232	6.9
0.013	6.2	476.92	0.0021	6.2
0.011	5.7	518.18	0.0019	5.7
Cal. Λ ∞= 2125.056 K_d = 0.000485				

Table (12): Dissociation constant of *Strept.T.P.S.* at several concentration from conductivity measurements (Solvent MeOH).

C (g. L^{-1})	k (µs.cm^{-1})	Λ (µS.cm^2.mol^{-1})	1 / Λ	C. Λ
0.021	9.2	438.095	0.00228	9.2
0.016	7.5	468.750	0.00213	7.5
0.013	6.2	476.923	0.00210	6.2
0.011	5.2	472.727	0.00212	5.2
0.009	4.4	488.89	0.00205	4.4
Cal. Λ ∞=556.1 K_d= 0.0065				

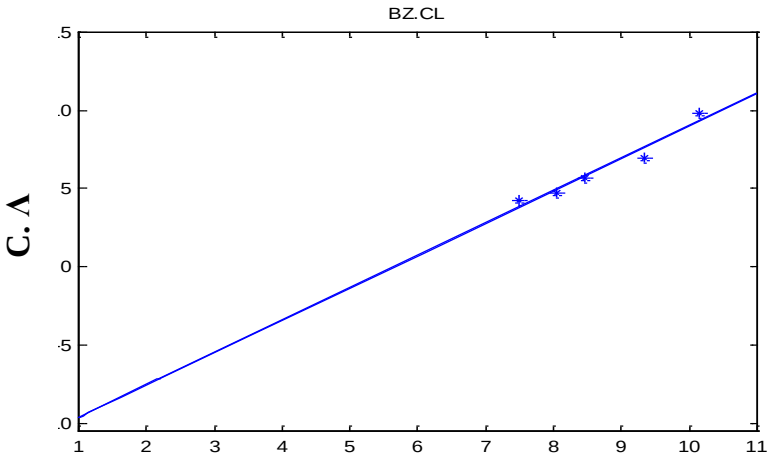
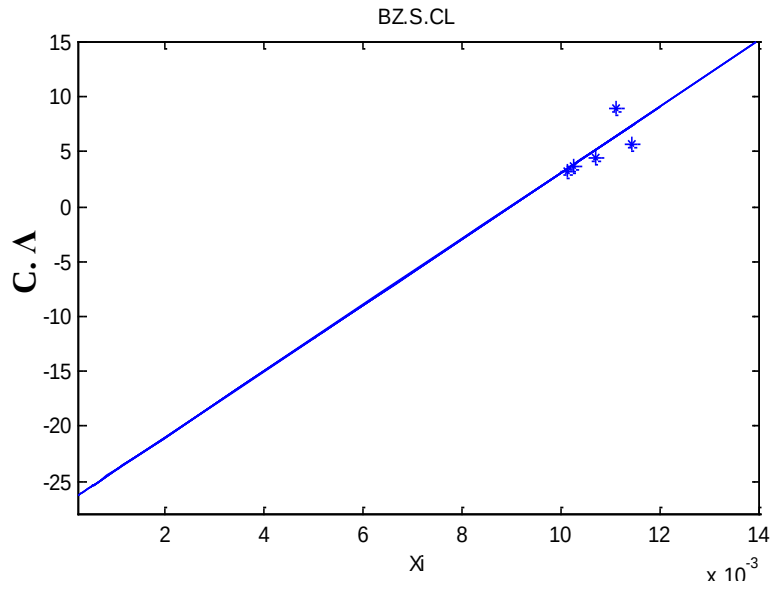


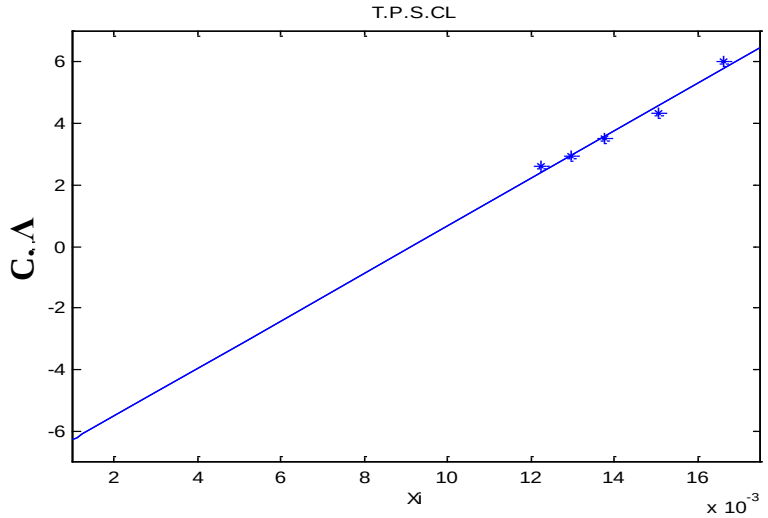
Figure (1): Determination of dissociation constant (K_d) from plotting (1/Λ) against (C. Λ) for *BZ.CL*.



1 / Λ

Slope = 3008.9 b = -27.0

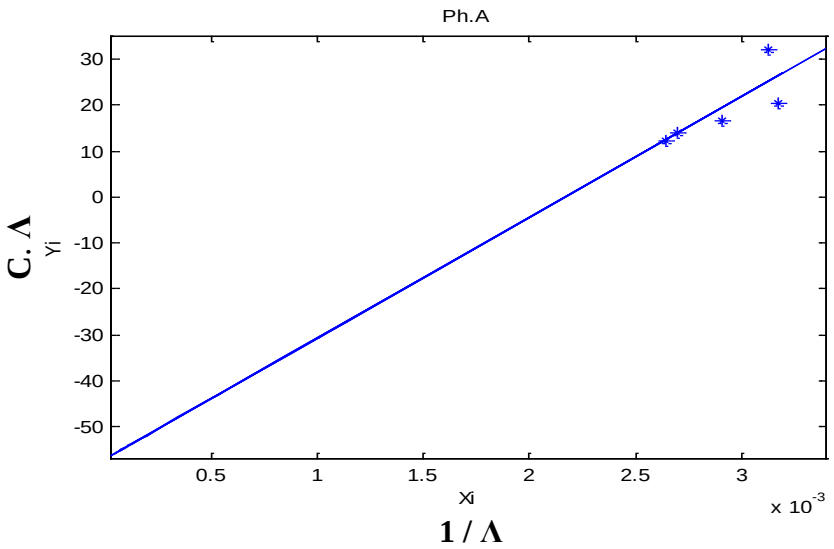
Figure (2): Determination of dissociation constant (K_d) from plotting (1/Λ) against (C. Λ) for *BZ.S.CL*.



1 / Λ

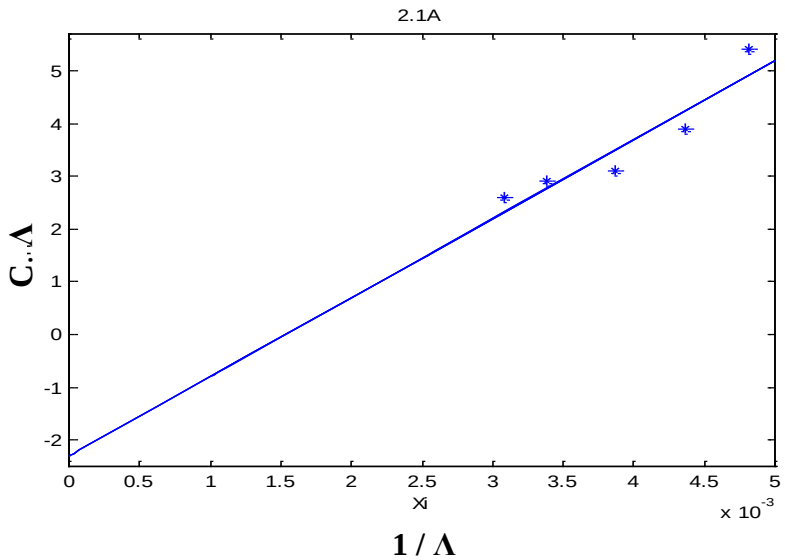
Slope = 772.778 b = -7.0504

Figure (3): Determination of dissociation constant (K_d) from plotting (1/Λ) against (C. Λ) for *T.P.S.CL*.



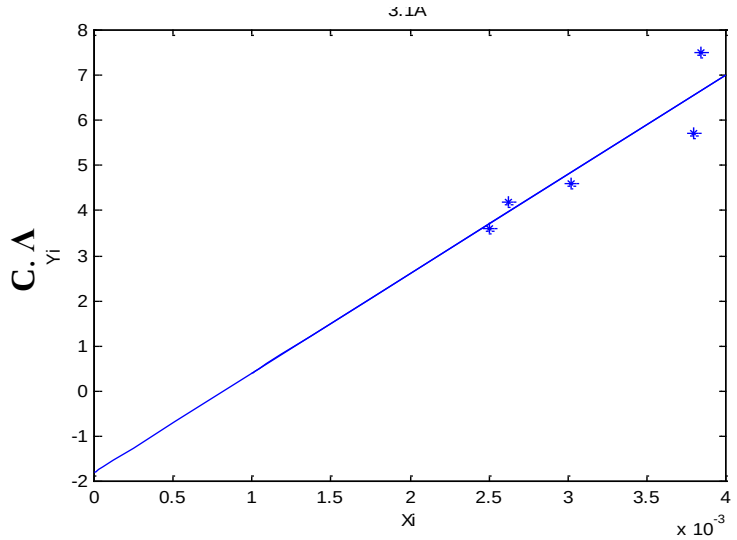
Slope=26295.0 **b=-57.0**

Figure (4): Determination of dissociation constant (K_d) from plotting (1 / Λ) against (C. Λ) for *Ph.A.*



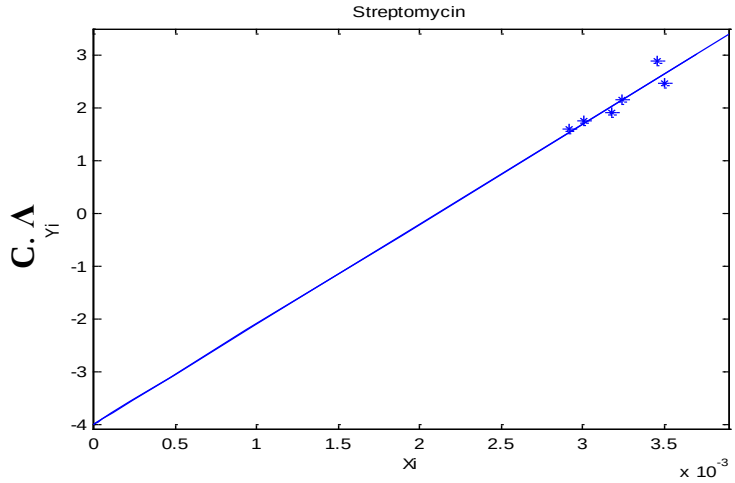
Slope = 1499.9 **B = -2.3**

Figure (5): Determination of dissociation constant (Kd) for *Neo.BZ. /A* from plotting (1 / Λ) against (C. Λ).



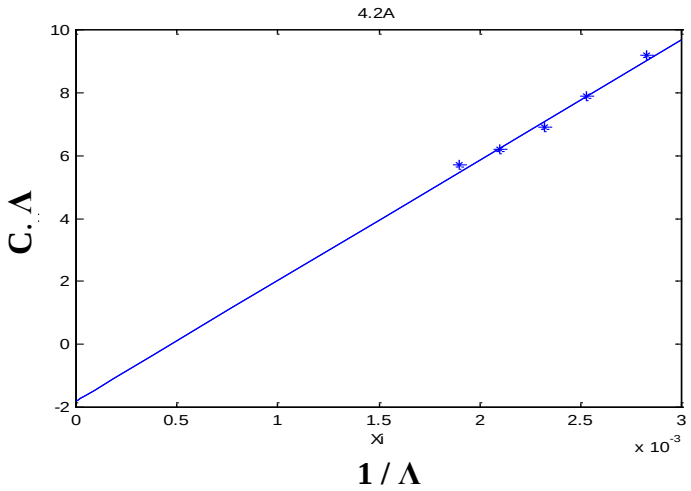
1 / Λ
Slope = 2200.8 b = -1.8

Figure (6): Determination of dissociation constant (Kd) for *Gent.BZ.* from plotting (1 / Λ) against (C. Λ).



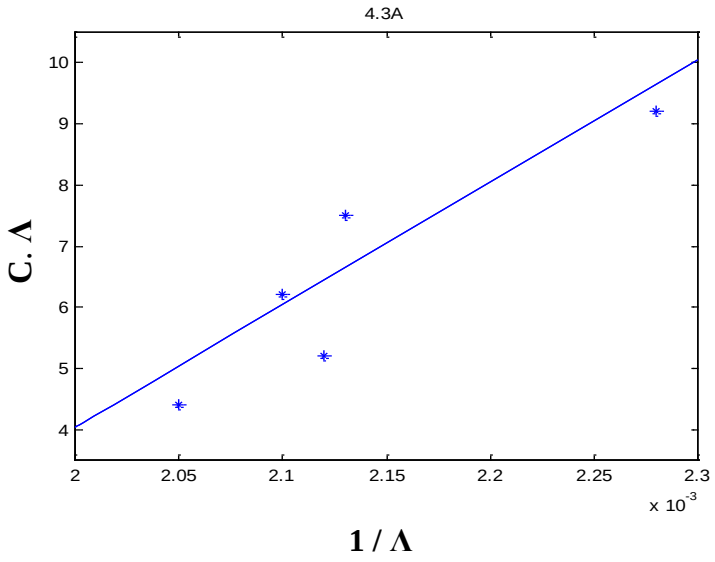
1 / Λ
Slope =1900.8 b = -4.0

Figure (7): Determination of dissociation constant (K_d) for *Streptomycin* from plotting (1 / Λ) against (C. Λ).



Slope = 3825.1 **b = -1.8**

Figure (8): Determination of dissociation constant (K_d) for *Strept.BZ.S.* from plotting (1 / Λ) against (C. Λ).



Slope = 20020 **b = -36.0**

Figure (9): Determination of dissociation constant(K_d) for *Strept.T.P.S* from plotting (1 / Λ) against (C. Λ).

Conductivity measurements

Dissociation constant(K_d)

The conductivity measurements to calculate dissociation constant(K_d) through dilution for reagents as in (Tables1-4) and for antibiotic-derivatives as in (Tables4-12).The dissociation constant(K_d) for reagents follow the order T.P.S.Cl (0.0643)<BZ.Cl(0.0661)<Ph.A(0.124) <BZ.S.Cl (0.2423).

Substituted reagent on the Kanamycin not the same process to give antibiotic-derivative product. In the (Figures 1-9) the molar conductivity at infinite dilution (Λ_∞), is differ for antibiotic-derivatives which leads to give various values of dissociation degree. From (Table9) the value of Λ_∞, for Gen.BZ.S. is (1601 $\mu S.cm^2 .mol^{-1}$) which give dissociation degree (α) is equal to (0.212).

Table (13): Kinetic study of *D-glucose-phthalic anhydride* derivatives.

Time (min.)	Vol. Of NaOH (mL)	ύ	1/ ύ	[A]/ ύ
5	15.0	0.300	3.34 0	0.9000
10	17.4	0.348	2.870	0.7760
15	20.0	0.400	2.500	0.6750
20	21.5	0.430	2.326	0.6280
30	21.7	0.434	2.304	0.6220
40	23.7	0.462	2.165	0.5840
50	42.8	0.856	1.168	0.3154
60	44.0	0.880	1.136	0.3068

υ^-- average number of reagent molecule bonded to one antibiotic olecule.

Table (14): Determination of rate constant *Neo.BZ.* by conductivity Measurement (MeOH).

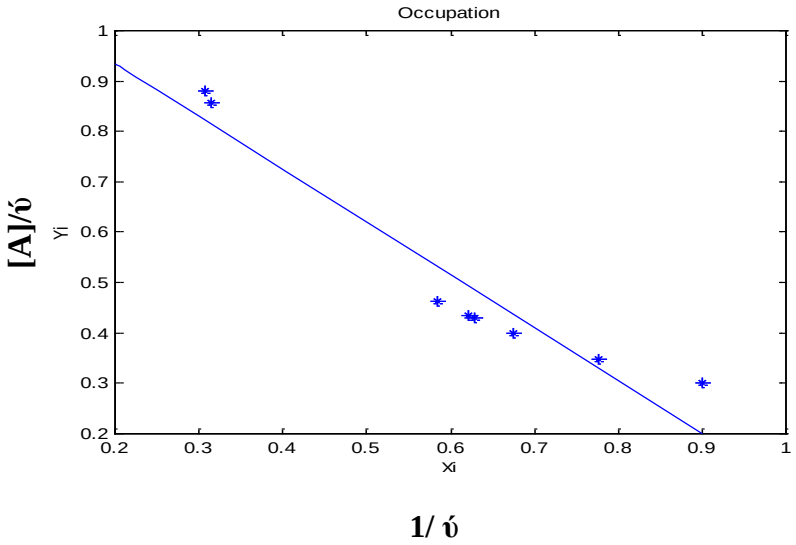
t (min).	$(C_0- C_t)/t$	C t ($\mu s.cm^{-1}$)	K ($\mu s.cm^{-1}. min^{-1}$)
0	∞	69.3	-
2	20.85	27.6	926.67
4	10.4	27.7	416.0
6	6.92	27.8	251.52
8	5.175	27.9	172.5
13	3.185	27.9	106.154
18	2.3	27.9	76.852
34	0.99	26.7	-
Cal.	Rate constant (k)=4301.08		Average=350.59

Table (15): Determination of rate constant *Strept.BZ.S.* by conductivity easurement (MeOH).

t (min)	$(C0- C_t)/t$	C t ($\mu s.cm^{-1}$)	K ($\mu s.cm^{-1}. min^{-1}$)
0	∞	69.3	-
4	-6.4	94.9	54.074
6	-4.58	96.8	21.695
8	-3.55	97.7	13.382
13	-2.2	98.3	9.73
18	-1.63	98.6	5.87
23	-1.27	98.5	4.2
28	-1.03	98.1	3.298
33	-0.87	98.1	2.298
38	-0.74	97.4	2.068
43	-0.32	83.1	-
Cal.	Rate constant (k)=111.02		Average=13.033

Table (16): Determination of rate constant *Strept.T.P.S* by conductivity measurement (MeOH).

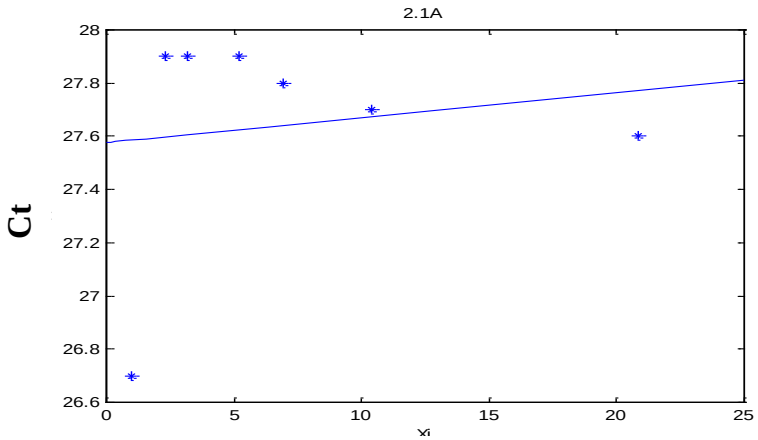
t (min).	$(C_0- C_t)/t$	C t ($\mu s.cm^{-1}$)	K ($\mu s.cm^{-1}. min^{-1}$)
0	∞	69.3	-
2	17.9	33.5	397.78
4	8.975	33.4	211.176
6	5.95	33.6	125.263
8	4.463	33.6	93.95
13	2.76	33.4	64.796
18	2.0	33.3	50.0
23	1.57	33.2	41.86
50	0.752	31.7	-
Cal.	Rate constant (k)=863.93		Average=140.689



1/ ύ

Slope = -1.0502 [A]/ ύ **= 1.1448**

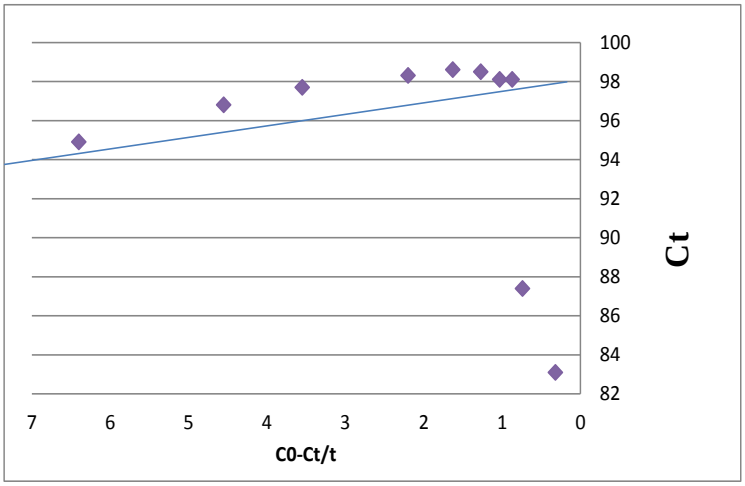
Figure (10): The relation between (1/ ύ) and [A]/ ύ.



$(C_0- C t)/t$

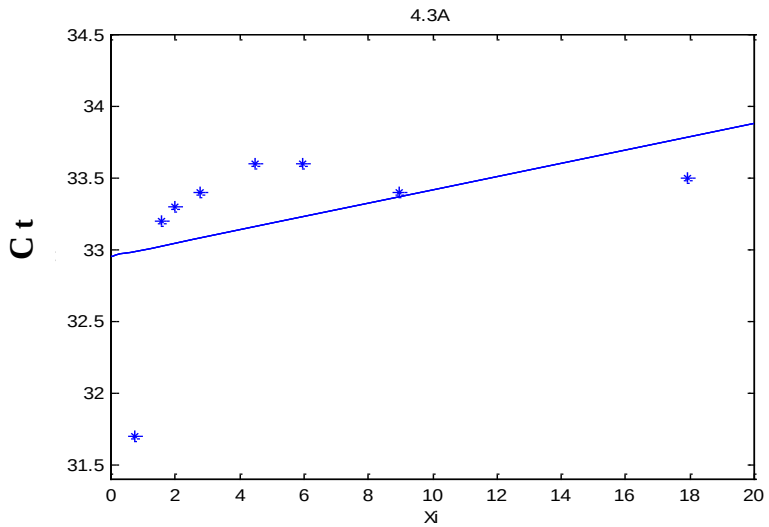
Slope = 0.0093 **C_∞= 27.5767**

Figure (12): Curve of (C0- C t)/t vs. C t for *Neo.BZ. /A.*



$(C_0 - C\ t)/t$
Slope = 0.3603 **C_∞= 95.3361**

Figure (13): Curve of $(C_0 - C\ t)/t$ vs. Ct for *Strept.BZ.S. /A.*



$(C_0 - C\ t)/t$
Slope = 0.0463 **C_∞= 32.956**

Figure (14): Curve of $(C_0 - C\ t)/t$ vs. C t for *Strept.T.P.S. /A.*

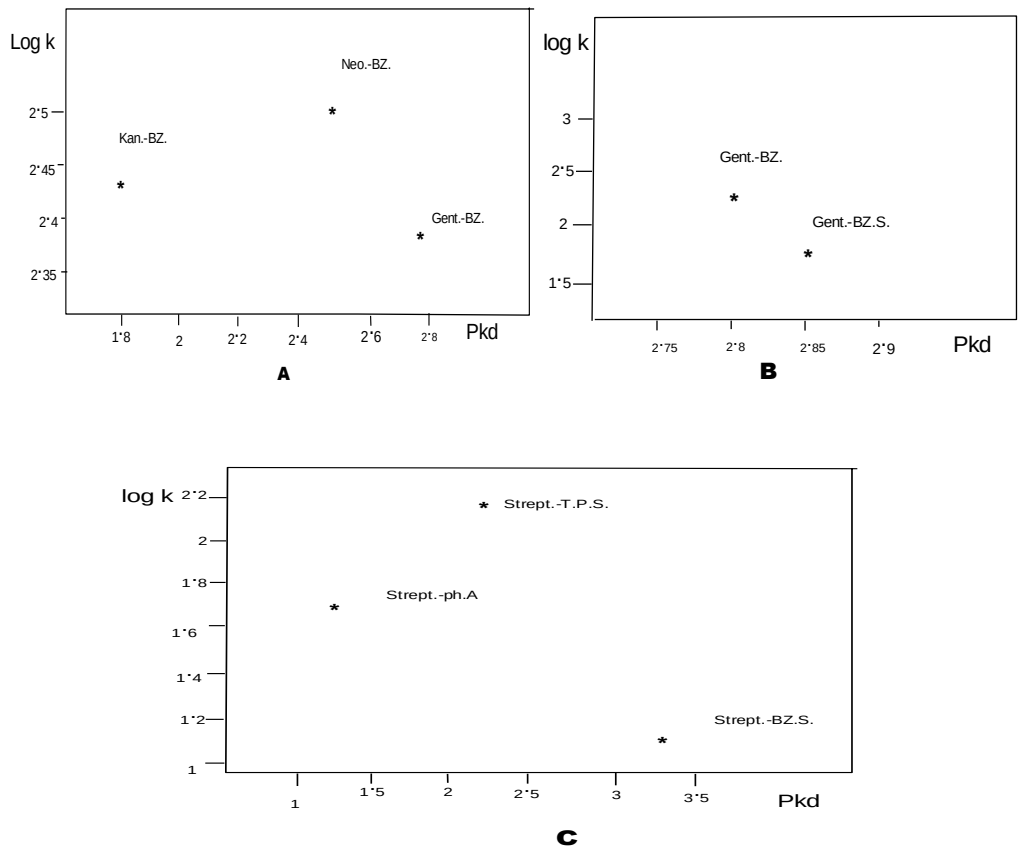


Figure (15):A, B, C plots log k against pkd.

Rate constant (k)

Determination the rate constant(k) of alkaline (NaOH)hydrolysis of antibiotic-derivatives (as ester), the formation of benzoate or sulfonate ion and alcohol, as in general:

O O
IIII
R-C-OR + OH^- → R-C-O^- + R -OH

The rate formation of acetate ion proportional both to the concentration of ester and to the concentration of hydroxide ion **(Frost & Ralph,1963)**.The rate constant represented in (Tables13-16) for antibiotic-derivatives, from theoretical view of these types of reaction should be follow second-order reaction, but the experimental values (Tables and Figures11-14) of derivatives not follow second-order rule because it gives different values for rate constant(k) when integrated rate.

This means that this type of hydrolysis for antibiotic-derivatives are not simple but it follow complex type reaction specially concurrent reaction type of different reactions produce a common product. The experimental results of antibiotic-derivatives, look like to be more convenient with theoretical concept and expression of thistype of reaction**(Gorden,1978)**.This hydrolysis reaction for antibiotic derivatives is composite reaction due to presence of more than one type of functional groups(ester and amide) as well as the position of the same and functional groups where differ **(Bluesone& Yan,1995)**.There are three ways in which structure

of the reacting ester can influence the rate of attack by hydroxide ions. These three ways relate to:

Electrophilic character of carbonyl carbon atom. Steric hindranceand stabilization of the carbonyl group by conjugation.The steric hindrance around the carbon site antibiotic molecule make difference in the rate of the SN^2 reaction. The reaction of the antibiotic-derivatives strongly depended on stability of the central carbon (electrophilic) and on nucleophile reagent**(Jack,1962),** (Figures 15-A,B and C) show the relation between rate constant,

logK and dissociation constant reagent pK_din (Figure 15-A) reagents for the derivatization are constant while for antibiotic-derivatives are variant, the points are seen to deviate markedly from any possible straight line.This give indication that the antibiotic(Kanamycin,Gentamycin,Neomycin) are structural character control hydrolysis of (Kan.-BZ.,Gen.-BZ. and Neo.-BZ.), while in (Figure 15-B and C) the predominant factor control the hydrolysis(Gen.-BZ and Gen.-BZ.S.) also (Strept.-BZ.S)., (strept.-ph.A.) and (strept. T.P.S.) are reagent character**(Smith & March, 2001; Martin,2003).**

CONCLUSIONS

The reactivity in the formation of products and hydrolysis of antibiotic-derivatives esters and/ or amides.The central carbon atom in reactant and product is tetrahedral, whereas carbon in the transition state is bonded to five atoms or groups, therefore, there will be anincrease in crowding on going from starting substrate to the transition state.The more crowded the transition state relative to substrate the higher its energy will be, and the slower it will be formed.The rate of SN^2 reaction is strongly dependent on the nature of nucleophilic reagentused, it increases with nucleophilic strength of the incoming. The rate of reaction is dependent on the nature of the solvent. The rate of reaction increases with increase solvent due tostabilized transition state ofthe reaction.The crystal form for some of these antibiotic-derivatives, does not melt directly to a liquid phase but firstpasses through an intermediate stage (the Para-crystalline state) which only at higher temperature undergoes transition to the liquid state. These intermediate states have been called liquid crystals, since they, display some properties of both liquid and crystals. Liquid crystaltend to occur when molecules aremarkedly unsymmetrical in shape. Also liquid crystal polymer dose not melt, it decomposed. The chemical kinetics of antibiotic-derivatives hydrolysis follow parallels type of reaction (different reactants amide and ester produce a common product).

$$A \xrightarrow{K_1} C \quad \text{and} \quad B \xrightarrow{K_2} C$$

Our experimental result indicate this phenomena, the curvature of most curve of antibiotic-derivatives during the initial part of the reaction and after a sufficient length of time the curve becomes liner which means the reaction mechanisms are proceed through different reaction order.

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