AL-TAHADI UNIVERSITY
FACULTY OF SCIENCE
DEPARTMENT OF CHEMISTRY

PHYSICAL CHARACTERISTICS OF SOME AMINOGLYCOSIDE DERIVATIVES (ANTIBIOTICS)

A THESIS SUBMITTED FOR PARTIAL FULFILLMENT FOR THE REQUIREMENTS OF THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY BY.

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AL-TAHADI UNIEVERSITY
(2004 – 2005)

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Title of Thesis

((Physical Characcteristic Of Aminoglycoside derivatives - Antibiotics))

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

ٱلرَّحْمَٰنُ ﴿١﴾ عَلَّمَ ٱلْقُرْءَانَ ﴿٢﴾ خَلَقَ ٱلْإِنسَٰنَ ﴿٣﴾
عَلَّمَهُ ٱلْبَيَانَ ﴿٤﴾ ٱلشَّمْسُ وَٱلْقَمَرُ بِحُسْبَانٍ ﴿٥﴾ وَٱلنَّجْمُ
وَٱلشَّجَرُ يَسْجُدَانِ ﴿٦﴾ وَٱلسَّمَآءَ رَفَعَهَا وَوَضَعَ ٱلْمِيزَانَ

صَدَقَ اللهُ العَظِيمُ ... سورة الرحمن"

To memory of my mother.

My love for father, brothers, sisters and

for all friends.

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ABSTRACT

Aminoglycoside derivatization including, *Neomycin*, *Streptomycin*, and *Gentamycin* with special reagents, which are benzoylchloride; benzene sulfonyl chloride and phthalic anhydride were made.

Ultraviolet spectrophotometric studies for antibiotic – derivatives were followed through measurements of the uv-absorbance (A) from which the absorpitivity (ε) of the complexes was deduced .

the Conductivity of antibiotic derivatives were used to calculate the dissociation constant and *the hydrolysis rate* which determined concurrent type reaction .

In addition the characteristics those controlling the hydrolysis of antibiotic-derivatives were investigated.

CONTENTS

List of Abbreviations needed

- Kan. .... Kanamycin .
- Neo. .... Neomycin .
- Gent. .... Gentamycin .
- Strept. .... Streptomycin .
- BZ.Cl .... Benzoylchloride .
- BZ.S.Cl .... Benzene sulfonyl chloride .
- T.P.S.Cl .... Toluene-p-sulfonyl chloride .
- Ph.A .... Phthalic anhydride .
- A .... Sodium hydroxide .
- B .... Pyridine .
- MeOH .... Methanol .
- EtOH .... Ethanol .
- G .... D-glucose .
- Refl. .... Reflux .

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INTRODUCTION AND LITERATURE REVIEW

1. Introduction

Aminoglycoside are groups of antibiotics that are used to treat certain bacterial infection[1,2].

This group of antibiotics includes, *Gentamycin, Kanamycin, Neomycin* and *Streptomycin*. Some of these antibiotics are complex and consists of two (or three) major isomeric components.

Streptomycin the first aminoglycoside was isolated from streptomyces griseous in mid-1940S [2]. This antibiotic effective against tuberculosis. Neomycin is analogous to streptomycin and isolated from streptomyces faradiae [3], also Kanamycin produced by streptomyces kanamyeticus anti microbial spectrum is similar to neomycin [4,5]. Gentamycins, Neomycin's, Kanamycins and streptomycin's are antiantibiotic belonging to the aminoglycoside group and they are effective against wide verity of microorganisms [6-9]. Because these antibiotics are not uv-absorbent so we need to introduce a suitable organic reagent as chromophore in pre-column derivataization 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)[10].

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 nm[11] and chromatic characteristic of these compound can be improved.

Some antibiotic have hydroxyl and amino functional groups (i.e, poly functional with two types of groups) which effected by reaction conditions [12, 13] . For trace analysis acid chloride the suitable reagent for quantification quantification by HPLC .

The reaction of aminosugare with acid chloride is effected by base as catalyst which enhance the reaction and neutralized the librated acid[14, 15] .

The effects of solvents and / or catalysts play important parts in types of products. (i.e. complete or incompletes)[16] .

1.1. Literature review

1.1.1. Amino glycosides (amino sugar glycosides) Antibiotics.

In (1944) *Streptomycin* was isolated by *Waksman* et al from the cultures of *streptomyces griseous*. This antibiotic is very effective in treatment of tuberculosis, meningitis and pneumonia[1, 2].

Neomycin is analogous to that of *Streptomycin*. It was isolated from cultures of *streptomyces faradiae*[3] and used topically in treatment of bacteria infections of the digestive track, as well as *kanamycin*, which produced by *streptomyces kanamyeticus*[2] Antimicrobial spectrum is similar to Neomycin[4, 5].

Gentamycin, is useful in the treatment of skin infections, acute and chronic urinary track Infection[4, 5].

Streptomycins, Neomycins (B , C and A), Kanamycins (A , B and C) and

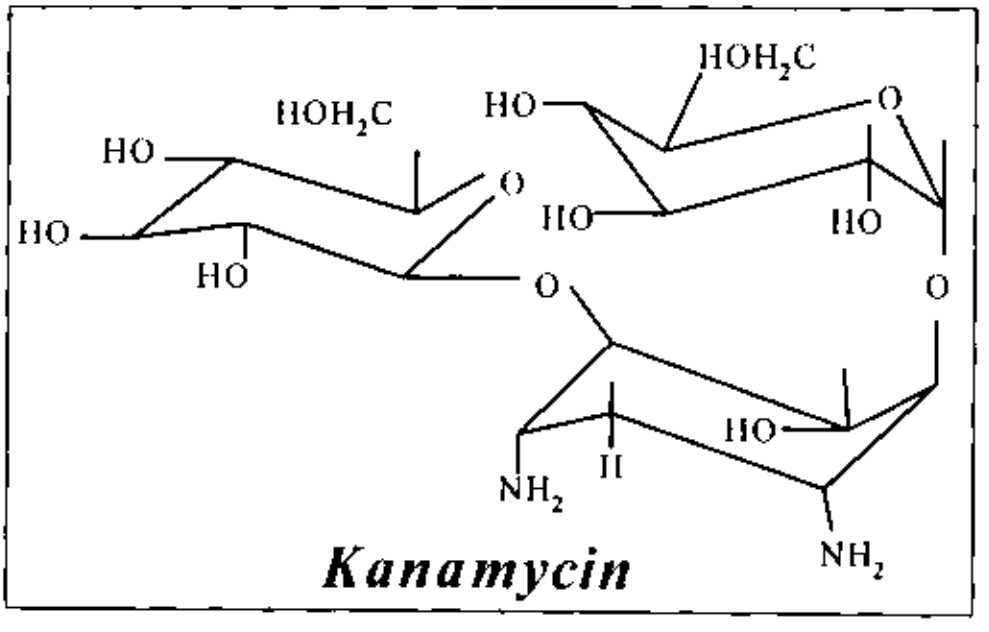
Gentamicins (C_1, C_1a and C_2),[6] are carbohydrate antibiotics. They are amino sugars. Each contains sugar part joined by glycoside – link[7-9].

The characteristic of these antibiotics was bactericidal against aerobic and facultative bacteria, binds to the 30S ribosomal subunit (protein) blocking the initiation of translation and causing the misreading of mRNA[10].

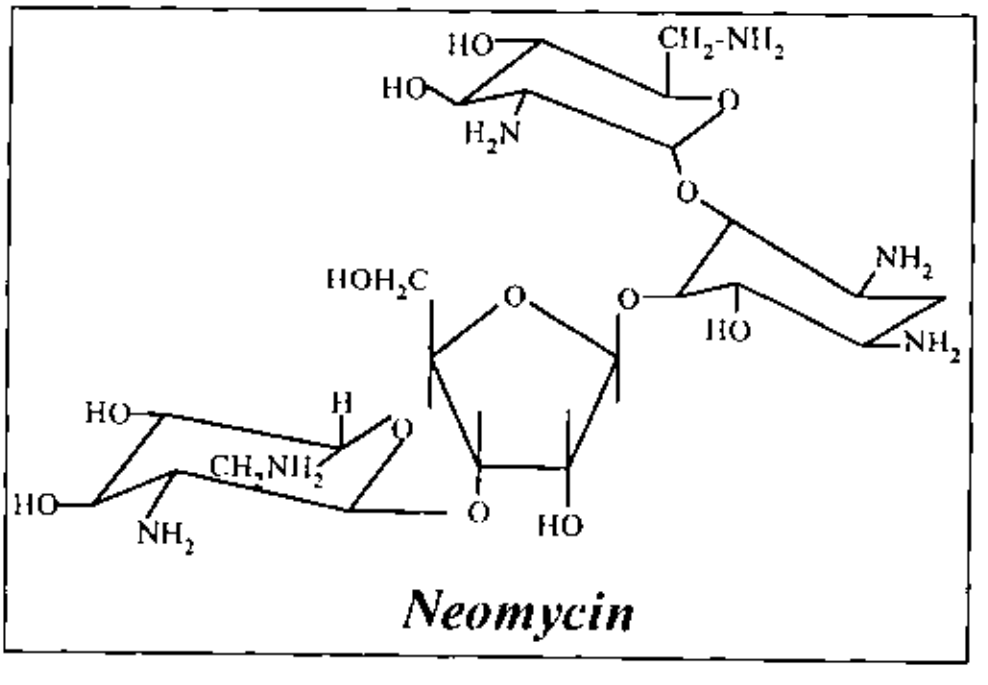
Neomycin, Kanamycin and *Gentamycin* does not show any uv absorbance between 212 – 360 nm[11].

1.1.2. Chemical structure of antibiotics.

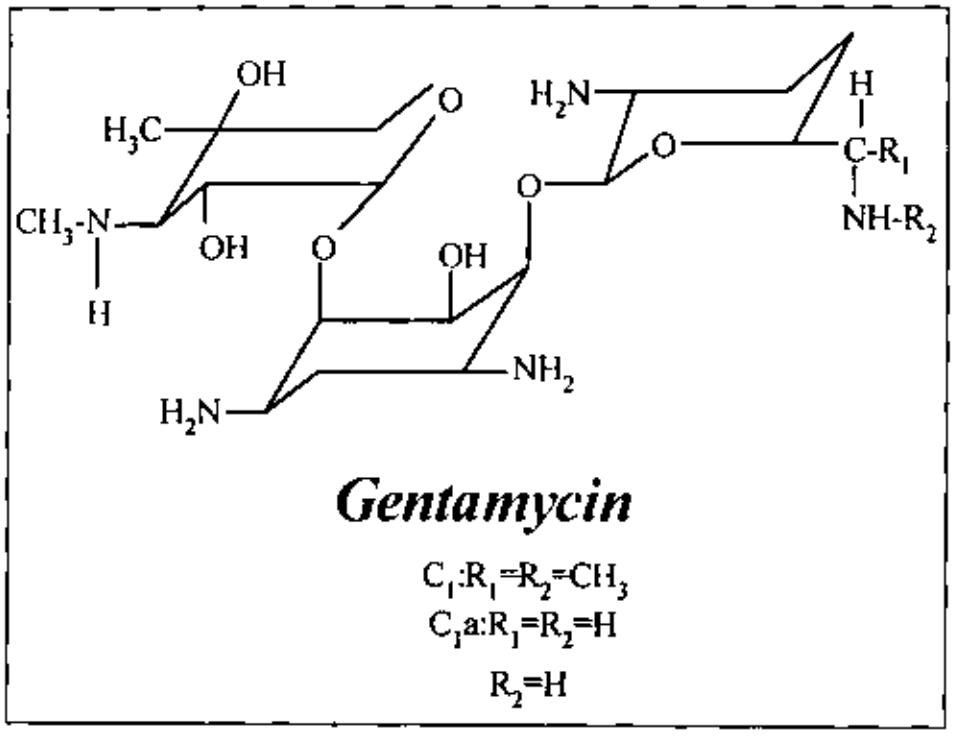
The chemical structure of antibiotics are as the following .



Kanamycin



Neomycin

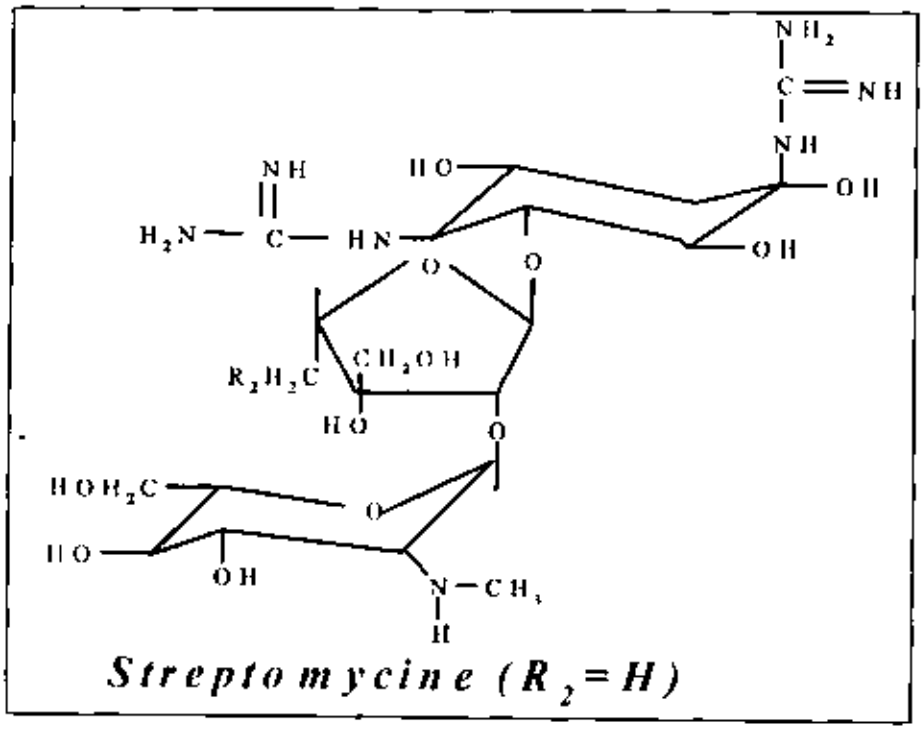


Gentamycin

C_1:R_1=R_2=CH_3

C_1a:R_1=R_2=H

R_2=H



Streptomycine (R_2 = H)

1.1.3. Physical properties of antibiotics[12, 13].

Antibiotics	Molecular formula	M.Wt	M.P (C°)	Solubility
Kanamycin	$C_{18}H_{35}N_4O_{11}$	483	-	H_2O, MeOH
Neomycin	$C_{23}H_{46}N_6O_{13}$	614	324 - 336	-
Gentamycin	$C_{21}H_{43}N_5O_7 = C_1$ $C_{19}H_{34}N_5O_7 = C_1a$ $C_{20}H_{41}N_5O_7 = C_2$	477 444 463	94 – 100 124 – 136 107 – 124	H_2O, Pyridine, MeOH, EtOH, Benzene
Streptomycin	$C_{21}H_{39}N_7O_{12}$	581.58	-	H_2O, MeOH, Isopropanol

1.1.4. Problems facing amino glycoside (antibiotics) characterization and their derivatives.

1- The quantitative determination of antibiotics is one of the most difficult areas of pharmaceutical analysis[14].

2- Derivatization products recovery from solvent shows difficulty specially that used for recrystallization.

3-The derivatives of aminoglycoside under investigation which gives more than one type of products (ester and / or amide)[16] and this made difficulty to find a suitable solvent to give precise uv- result[6].

4- Most aminoglycoside derivatives have not sharp melting point[15, 17].

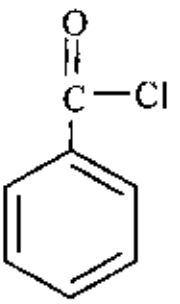
1.2.1. Derivatization Reagents.

Benzoyl chloride was suitable reagent for derivatized aliphatic and polyhydric alcohol as benzoate also for derivatized primary and secondary amine as benzamide benzene sulfonyl chloride and *P.Tolunene sulfonyl chloride* used for primary and secondary amine gives benzene sulfonamide and P-Toluene sulfonamide respectively[15, 19].

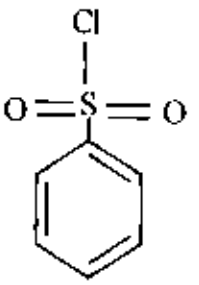
Phthalic anhydride used for primary amine and resolution of racemic alcohol as phthalate[6, 15, 19].

alcohol reacts with *benzene sulfonyl chloride* to from ester[20]. The product called sulfonate[21].

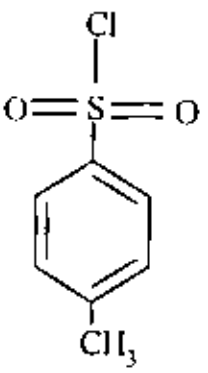
1.2.2. Chemical structure of reagents.



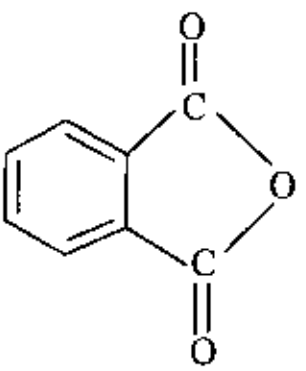
Benzoyl chloride
(BZ.Cl)



Benzene sulfonyl chloride
(BZ.S.Cl)



Toluene-p-sulfonyl chloride
(T.P.S.Cl)



Phthalic anhydride
(Ph.A)

1.2.3. Physical properties of reagents[13].

Reagents	Molecular formula	M.Wt	M.P (C°)	b.p(C°)	ρ(g/cm³)	Solubility
Benzoyl chloride	C_7H_5ClO	140.57	-1.0	197.2	1.212	Ether, Benzene, CCl_4
Benzene sulfonyl chloride	$C_6H_5ClO_2S$	176.5	13-14	251	1.370	EtOH, Ether, CCl_4
Toluene-P-Solfonyl Chloride	$C_7H_7ClO_2S$	190.5	71	145	-	EtOH, Ether, Benzene
Phthalic anhydride	$C_8H_4O_3$	148.12	130.8	295	1.527	EtOH, acetone

1.2.4. 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: -

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

$$R\text{-}OH + BZ.Cl \xrightarrow{NaOH} R\text{-}O\text{-}C(=O)\text{-}Ph + NaCl + H_2O$$

These are simple molecules, but for complex molecule such as *Neomycin, Kanamycin, Gentamycin* and *Streptomycin* the product is dependent on reaction conditions[22, 24].

1.2.4.1. Benzoylation.

An acid chloride is widely used as aderivatizing reagent to enhance delectability for isolation and quantification analysis [25]. The BZ.Cl used for separation of some carbohydrates by HPLC with pre-benzoylation[26]. Some polyhydric alcohols as were separated as -P-NO2- benzoate[27]. p-NO2-benzoate used as a derivative from neomycin complex for separation by HPLC[28].

1.2.4.2. Amidation.

Acid chlorides were used also for amidation of amino groups to produce polyamine, it is a simple and rapid procedure and also[29] benzoylchloride used for derivatization of amine in liquid chromatography (LC)[30]. The reaction of acid chloride with primary and secondary amines also used[31]. The BZ.Cl used for analysis of aminoglycoside antibiotics as benzoyl derivatives by HPLC and it's application to quantitation of Neomycin in perilymph[32]. The benzoyl chloride used for pre-column derivatization of neomycin complex[16].

1.3.1. Electronic spectra.

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 [33].

$$\Delta E = E_2 - E_1 = h\nu$$

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[34, 35].

Absorption of ultraviolet radiation by an organic molecule leads to electronic excitation among various energy level within the molecule .

The transition generally occur in between a bonding or lone – pair orbital and occupied non – bonding or antibionding orbital.

The ultraviolet visible spectronic regions are as following :-

Spectrum	Region (λ)
Ultraviolet	200 – 400 nm
Visible	400 – 750 nm
uv and visible	200 – 750 nm

1.3.3. Important useful terms concerning ultraviolet spectroscopy.

Chromophores .

The coloured substances owe their colour 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 n electrons[36].

Auxochromes.

An auxochrome is an auxillary 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 colour; its presence causes a shift in the UV or visible absorption maximum to a longer wavelength[34 , 36].

Bathochroice effect (Red shift).

Shift of an absorption maximum to longer wavelength is called Bathochromic shift. It is produced by a change of medium ($\pi \rightarrow \pi^*$

transitions undergo bathochromic 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 [36].

Hypos chrome effect (blue shift).

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)[36].

Ahyperchromic effect.

It is the effect leading to increased absorption intensity. For example, the intensities of primary and secondary bands of phenol are increased in phenolate.

hypochromic effect.

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[34 , 36].

The most important factors affecting the position of uv – bands.

1- Effect of steric hindrance on coplanarity (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 [36] .

1.3.4. The effect of solvent.

Solvent effects of carbonyl compounds my be observed up to 20 nm . Thus the ($n \longrightarrow \pi^*$) absorption of acetone occur at 279 nm in hexane; In water λ_{max} is 264.5 nm [37 - 38]. 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[39]. As the solvent polarity increases, blue shift is observed in the maximum absorption wavelength (λ_{max}) (hypsochromic shift)[36].

1.3.5. 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[40, 41] .

1.4. Electronic conduction.

Experimental determinations of conducting properties of electrolytes solutions are important essentially in two respects.

1- To study quantitatively the effects of intrinsic forces, degrees of dissociation and the extent of ion-pairing.

2- Conductance values may be used to determine quantities such as solvability's of sparingly soluble salts, ionic products of self- ionizing solvents, dissociation constants of weak acids and to form the basis for conduct metric titration methods[42 , 43] .

The resistance of an electrolytic solution may be defined: -

$$R = \rho \cdot \frac{\ell}{a} \qquad \text{.....(1)}$$

ρ – specific resistance or resistively.

ℓ and **a** the length and area respectively of the portion of solution studied.

The reciprocal of, **ρ**, known as conductivity, **K**,

$$K = \frac{1}{\rho} \qquad \text{.....(2)}$$

The reciprocal of resistance, **R**, is known as conductance, L .

$$L = \frac{1}{R} \qquad \text{..... ...(3)}$$

$$\text{The cell constant, } k_{cell} = \frac{\ell}{a} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

$$K = \frac{1}{\rho} = \frac{\ell}{R\,a} = L.\,k_{cell} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

The unit of K is $\Omega^{-1}.\,Cm^{-1}$

1.4.1. Molar conductivity.

This is given by conductivity, K, multiplied by the volume, which contains one mole of electrolyte. i.e. :-

$$\Lambda = \frac{K}{C} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

C- The concentration in equivalent per cubic centimeter[42 - 44].

The conductivity of a solution depends on the number of ions present, and normal to introduce the molar conductivity, **Λ**, and classes of electrolytes.

1.4.2. Strong electrolyte.

The molar conductivity of strong electrolytes vary linearly with square root of concentration.

$$\Lambda = \Lambda_{\infty} - B\sqrt{C} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

This relation is called kohlrasch's law.

Λ - equivalent conductance.

Λ_{∞} - equivalent conductance at infinity dilution .

1.4.3. Weak electrolytes.

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

$$HA\,(aq) + H_2O \rightleftharpoons H_3O^{+} + A^{-} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (8)$$

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

$$K_a = [H_3O^{+}][A^{-}] / [HA][H_2O] \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (9)$$

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

$$K_a = \frac{\alpha^2 C}{1-\alpha} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (10)$$

The electrolyte is fully ionized at infinite dilution, and it's molar conductivity, Λ_{∞} [45, 46].

$$\alpha = \frac{\Lambda}{\Lambda_{\infty}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (11)$$

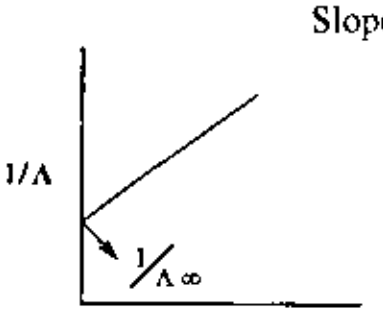
Substituting eq. (11) in eq. (10) we get eq. (12) .

$$K_a = \frac{C \Lambda^2}{\Lambda_\infty (\Lambda_\infty - \Lambda)} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (12)$$

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 .



1.5.1. Chemical Kinetics.

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

1.5.1.1. Rate laws and rate constants.

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

Consider the general reaction :

$$aA + bB = cC + dD \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (13)$$

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

This rate varies with time and is equal to some function of concentrations:

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

The time rates of change for the concentration of other species in the reaction are related to that of the first species by the stoichiometry of the reaction. For the example presented above. We find that:

$$\frac{1}{c}\frac{d[C]}{dt} = \frac{1}{d}\frac{d[D]}{dt} = \frac{1}{a}\frac{d[A]}{dt} = \frac{1}{b}\frac{d[B]}{dt} \quad \ldots\ldots\ldots\ldots (14)$$

The equation:

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

Is called the rate law for the reaction. While f **([A] , [B] , [D])** might in general be a complicated function of the concentrations. It often occurs if that can be expressed as simple product of a rate constant , k , and the concentration each raised to some power .

$$\frac{1}{c}\frac{d[C]}{dt} = k[A]^m[B]^n[C]^o[D]^p \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(15)$$

The overall order of the reaction as the sum of the powers, i.e.

overall order, $q = m + n + o + p$.

The rate constant will have units of time^{-1} concentration$^{-(q-1)}$ [47].

1.5.3. Types of reactions .

1- simple reactions (low order reaction): -

These are, first, second and third order reactions.

2- complex reactions: -

These are opposing, parallel, consecutive and free radical reactions[48].

1.5.3.1. First order reaction .

$$A \longrightarrow \text{products}$$

Let **a** = initial concentration of A

x = decrease in A at time t.

a - x = concentration of A at time t .

The rate equation: -

$$\frac{dx}{dt} = k_1 (a - x) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (16)$$

$$\frac{dx}{(a - x)} = k_1 t \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (17)$$

Integrating of equation (17) gives :

$$\int \frac{dx}{a - x} = k_1 \int dt$$

$$-\ln (a - x) = k_1 t + C \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (18)$$

Where **C** is the constant of integration.

When **x = 0** at **t = 0**

$$C = -\ln a \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (19)$$

And equation (18) becomes: -

$$\ln \frac{a}{a - x} = k_1 t \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (20)$$

1.5.3.2. Second order reaction .

Consider the reaction:

$$A + B \longrightarrow \text{products}$$

Either: $[A] = [B]$

Or: $[A] \neq [B]$

When $[A] = [B]$, $\dfrac{dx}{dt} = k_2 (a - x)^2$ .... (21)

$$\frac{dx}{(a - x)^2} = k_2\, dt \quad \text{..... ....(22)}$$

Integration of equation (22) gives :

$$\frac{1}{a - x} = k_2 t + C \quad \text{..... ..... . (23)}$$

When $t = 0$, $x = 0$ so that $C = a^{-1}$

By substitution of C in equation (23) gives :

$$\frac{x}{a(a - x)} = k_2 t \quad \text{..... ..... (24)}$$

And when $[A] \neq [B]$:

Let: a = initial concentration of A.

b = initial concentration of B.

x = decrease in A or B at time t = amount of product at time t.

a - x = concentration of A at time t.

b - x = concentration of B at time t.

The differentiated second order rate equation would then be:

$$\frac{dx}{dt} = k_2 [A][B] = k_2 = k_2 (a - x)(b - x) \quad \ldots\ldots\ldots\ldots (25)$$

Or

$$\frac{1}{(a - x)(b - x)} = k_2 t \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (26)$$

Integration by using the algebraic of partial functions.

The partial function:

$$\frac{A}{(a - x)} + \frac{B}{(b - x)} + \ldots\ldots\ldots\ldots\ldots\ldots\ldots (27)$$

$$\frac{1}{(a - x)(b - x)} = \frac{1}{a - b}\left(-\frac{1}{a - x} + \frac{1}{b - x}\right) \quad \ldots\ldots\ldots\ldots (28)$$

This leads to integral:

$$\frac{1}{(a - b)} \int_0^x \left(-\frac{dx}{(a - x)} + \frac{dx}{(b - x)}\right) = k_2 \int_0^t dt \quad \ldots\ldots\ldots\ldots (29)$$

On integration gives:

$$\frac{1}{a - x}\left[\ln (a - x) - \ln (b - x)\right]_0^x = k_2 t \quad \ldots\ldots\ldots\ldots (30)$$

Inserting the limits and rearrangement give, finally[3, 48].

$$\frac{1}{a - b} \ln \frac{b(a - x)}{a(b - x)} = k_2 t \quad \ldots\ldots\ldots\ldots\ldots\ldots (31)$$

1.5.3.3. Complex reactions .

Have mechanism that involves several elementary processes or reaction steps.

- Parallel reactions (side reactions).

If the mechanism involves a reactant that can undergo two or more reaction independently and concurrently.

Let the mechanism be:

$$A \xrightarrow{k_1} u$$

$$A \xrightarrow{k_2} v$$

$$A \xrightarrow{k_3} w$$

$$-\frac{d[A]}{dt} = k_1A + k_2A + k_3A = (k_1 + k_2 + k_3)\,A = kA \quad \ldots\ldots\ldots\ldots\ldots (32)$$

$$\text{And} \quad \ln [A]_o / [A] = kt \quad \ldots\ldots\ldots\ldots\ldots (33)$$

The reaction is simple first order as A is concerned.

Also: -

$$\frac{du}{dt} = k_1A = k_1 [A]_o e^{-kt} \quad \ldots\ldots\ldots\ldots\ldots (34)$$

$$\text{And} \quad u = \frac{-k_1 [A]_o}{k} e^{-kt} + \text{constant} \quad \ldots\ldots\ldots\ldots\ldots (35)$$

$$\text{Or} \quad u = u_o + k_1 \frac{[A]_o}{k} (1 - e^{-kt}) \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (36)$$

$$v = v_o + k_2 \frac{[A]_o}{k} (1 - e^{-kt}) \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (37)$$

$$w = w_o + k_3 \frac{[A]_o}{k} (1 - e^{-kt}) \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (38)$$

If $\mathbf{u_o = v_o = w_o = 0}$ the equation can be simplified to give :

$$\frac{v}{u} = \frac{k_2}{k_1} \quad , \quad \frac{w}{u} = \frac{k_3}{k_1}$$

The products are in a constant ratio to each other,

of time and initial concentration of reactant A[49] .

1.5.4. Acid - base catalysis.

There are several types, which involve a proton transfer in at least one step. These are treated in general terms of substrate S or SH on which the acids or Bases work ,S^- the conjugate base of the substrate SH , B a general base BH^+ it is conjugate acid, HA general acid and A^- it's conjugate base .

B and A^- may or may not be the same as may BH and HA . R is some other reactant not acting as abase or acid .

A slow ionization of the substrate followed a rapid reaction of anion to give the products[49].

$$HS + B \xrightarrow{\text{Slow}} S^- + BH^+ \quad \text{..... (39)}$$

$$S^- + R \xrightarrow{\text{fast}} \text{products} \quad \text{..... (40)}$$

$$\text{Rate} = k\,[HS][B] \quad \text{..... (41)}$$

Transesterification (alcoholsis) is catalyzed by acids or bases (identical with ester hydrolysis)[50] .

$$R{-}\overset{\overset{O}{\|}}{C}{-}OR + R^-{-}OH \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} R{-}\overset{\overset{O}{\|}}{C}{-}OR^- + R{-}OH$$

1. 6. Aim of study.

To introduce a suitable chromophores to the antibiotic molecules (*Neomycin, Gentamycin, Kanamycin* and *Streptomycin*) *by reaction with Benzoylchloride , Benzenesulphonylchloride, P-Toluenesulphonyl chloride* and *Phthalicanhydride* in order to improve detectability of antibiotic-derivatives through uv- absorption spectrum as well as reducing of the polarity which are merit object in pre-column high pressure liquid chromatography (HPLC) for better separation and quantitation of those antibiotics of aminoglycoside type.

Also to predict the type of chemical reaction of the antibiotic-derivatives kinetically through alkali hydrolysis and determination of dissociation constant which gives a good indication about the stability of the antibiotic-derivatives compound and derivatives.

EXPERIMENTAL

2.1. Instruments.

The main instruments used in the study were :

1-uv /vis. Spectrometer, double-beam UVE – unicam , ATI UNICAM. Unicam uv series, prism soft ware. (u.k). uv – sample cells path length 4 cm (10 ml).

2 - Conductivity meter. HANNA Instruments. HI 8733. made Portugal.

3 - Melting point apparatus. Philip Harris, shenston - England, serial No. B/A-211.

4 - Weight balance; sartorius analytic type. A200S. Made in Germany.

2.2. The chemical compounds used in this study.

The following table shows chemical compounds and company supplier.

Compounds	M.W	Structure formula	Company supplier
Kanamycin mono sulfate	582.6	$C_{18}H_{36}N_4O_{11}.H_2SO_4$	Sigma Chemical Germany
Streptomycin mono sulfate	559	$C_{21}H_{39}N_7O_{12}$	GMbH Hamburg Germany
Gentamycine	477	$C_{21}H_{34}N_5O_7$	Rotex medica Germany
Neomycin	614	$C_{23}H_{46}N_6O_{13}$	Oxide limited England
Benzoylchloride	140.57	C_6H_5COCl	Aldrish-europe Belgium
Benzene sulfonyl chloride	212	$C_6H_5SO_2Cl$	Merck-schuchardt Germany
Toluol-4-sulfonylchloride	190.65	$4\text{-}(CH_3)C_6H_4SO_2Cl$	Merck-schuchardt Germany
Phthalic anhydride	148.12	$C_6H_4(CO)_2O$	Farm Italia carloerba
Sodium hydroxide	40	NaOH	Carloerba millano
Pyridine	81	C_5H_7N	Riedel-De Haenag seelze-hannover germany
Methyl alcohol	32.042	CH_3OH	Carloerba millano
Chloroform	119.32	$CHCl_3$	Carloerba millano
Acetone	58.08	$(CH_3)_2CO$	Tim star laboratory England
Ethyl alcohol	46.07	C_2H_5OH	Carloerba millano
Anhydrous sodium sulphate	142.04	Na_2SO_4	T.Baker lab chemicals. INDIA
Sodium hydrogen carbonate	84.007	$NaHCO_3$	Carloerba millano
Hydrochloric acid	36.5	HCl	Carloerba millano

2.3.1. Benzoylchloride derivatization procedure[15, 19].

2.3.1A. 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 *benzoylchloride* and 30 ml of 10 % NaOH solution. Stopper the flask and shake vigorously at frequent 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 recrystllized from ethanol and dried in oven .

2.3.1B. Pyridine: -

Dissolve 1.0 g of drugs in 30 ml pyridine and 15 ml of chloroform in 150 ml conical flask . Then 12 ml *benzoylchloride* was added to the mixture. Stopper the flask and shake vigorously for about 10 minutes. Leave the the reaction 24 hours at 0C° temperature.
Dilute the reaction mixture with 75 ml $CHCl_3$ in separating funnel (500 ml), wash with 2 M sulfuric acid (H_2SO_4), water (H_2O), sodium hydrogen carbonate ($NaHCO_3$), then dry over anhydrous sodium sulfate, remove $CHCl_3$, dry the product.

2.3.2. Benzensulfonyl chloride derivatization procedure[15, 19].

2.3.2A. Sodium hydroxide (NaOH): -

Dissolve 1.0 g of drugs in 30 ml of 10 % aqueous NaOH solution In150 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 minutes. Cool the flask in running water from the tap and then pour it's contents into about 150 ml water. Stir the aqueous mixture well and wash the crystal with H_2O and drain. *Recrystallize* the product from methylated spirit and dry on filter paper in the air.

2.3.2B. 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 minutes Leave the reaction over night at 0C° temperature.

Dilute these contain with 75 ml $CHCl_3$ in separating funnel (500 ml),wash with 2 M sulfuric acid (H_2SO_4), water (H_2O), sodium hydrogen carbonate ($NaHCO_3$). then dry over anhydrous sodium sulfate, remove $CHCl_3$, dry the product.

2.3.3.1. Phthalic anhydride derivatization procedure.

Heat mixture of 1.0g of drugs, 7.5 g of *phthalic anhydride* and 20 ml of dry *pyridine* on water bath for 1 hour 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 hour.

If the resulting is semi-solid or pasty transfer to large flask and dry the product [15, 19].

2.3.3.2. Determination of OH groups procedure[19].

1 - a- 1 M phthalic anhydride.

b- 10 mill 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.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.ph and titration with 0.1 N NaOH.

b - Repeat (a) at different time.

2.3.3.3. Kinetic study procedure.

1- prepare 100 ml of 0.05 M ester in methanol and 0.05 M NaOH.

2- Take 25 ml of NaOH and dilute to 50 ml and measure the conductivity, C_o.

3- 25 ml of 0.05 M ester and 25 ml of 0.05 M NaOH, start to measure conductivity after 2 min. for first 10 min and then 5 min, C_t.

4-Take solution of ester and NaOH; after 1 hr. to complete reaction, then measure the conductivity, C_∞.

2.3.3.4.Conductivity measurement.

Procedure: -

1- prepare 0.1 $M.L^{-1}$ of reagents BZ.Cl, BZ.S.Cl, T.P.S.Cl and ph.A.

2- Prepare stock solution for antibiotic – derivatives ($g.L^{-1}$) as in tables (35 - 46).

3- Makes different solution by dilution for (1,2) and measure the conductivity.

2.4.Theoretical treatment for experimental data.

2.4.1.The number of reagents.

The possible number of reagents (BZ.- , BZ.S.- , Ph.A, T.P.S.-) were substituted on sample drugs calculated by two methods according to uv-measurement.

2.4.1.1. Concentration method (n_c)[33] .

By considering the following :

$$A_R = \varepsilon_R \cdot C_R \cdot L \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (42)$$

$$A_{S-R} = \varepsilon_{S-R} \cdot C_{S-R} \cdot L \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (43)$$

$\varepsilon_{S\text{-}R} = \varepsilon_R$ when **S** not uv.absorbent .

R is the reagent , **S** is the sample.

ε_R – calculated from eq.42 by plotting A_R vs C_R .L

And by measuring $A_{S\text{-}R}$ for known concentration ($C_{S\text{-}R}$)(experimental).

The $C_{S\text{-}R}$ are calculated: $$C_{S\text{-}R} = \frac{A_{S\text{-}R}}{\varepsilon_R \,.\, L} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (44)$$

Therefore:

$$n_c = \frac{(C_{S\text{-}R})\ \text{cal.}}{(C_{S\text{-}R})\ \text{exp.}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (45)$$ (n- number of reagent)

2.4.1.2. Absorpitivity ratio method. (n_ε).

Using the following expression.

$$n_\varepsilon = \frac{\varepsilon_{S\text{-}R}}{\varepsilon_R} \,.\, M_R\,\%, \quad (\varepsilon_S = 0 \text{ , S not uv.absorbent})$$

Where:

$\varepsilon_{S\text{-}R}$ = Absorpitivity of sample – reagent complex .

ε_R = Absorpitivity of reagent .

M_R = molecular weight of reagent.

2.4.2.1. Occupation method[50] .

Consider:

Vacante site (antibiotic) + [A] (reagent) $\rightleftharpoons$ occupied site ..... (46)

$$K = \frac{[\text{Occupied site}]}{[\text{Vacant site}][A]} = \frac{\acute{\upsilon}}{[n - \acute{\upsilon}]\,[A]} \quad \text{..... (47)}$$

Simplify eq. 47 gives:

$$\frac{1}{\acute{\upsilon}} = \frac{1}{n} - K\,\frac{[A]}{\acute{\upsilon}} \quad \text{..... (48)}$$

[A] is Concentration of reagent required to substituted on antibiotic molecule .

$\acute{\upsilon}$ is Average number of reagent molecule bonded to one antibiotic molecule .

n is Number of position available for occupation on antibiotic.

K is Equilibrium constant .

2.4.2.2. Determination of – OH groups[19].

Calculated by:

$$OH\,\% = \frac{(V_1 - V_2) \times N \times 17.01 \times 100}{\text{Wt. of sample} \times 1000}$$

N is Normality of NaOH.

V_1 is Volume of NaOH equivalent to (ph.A + D.glucose).

V_2 is Volume of NaOH equivalent to blank (ph.A).

By comparing of this percentage with theoretical value (D.glucose + 5 ph. A) to predict the number substituted of reagent.

2.4.3. 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 $t = t$, C_∞ at $t = \infty$

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

$$k_2 = \frac{1}{a \cdot t} \cdot \frac{C_o - C_t}{C_t - C_\infty}$$

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

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_∞.

RESULTS AND DISCUSSION

Table (1A) derivatives condition and characteristics

Antibiotics	Reagents	Reaction conditions				Weight of subs.		Physical properties			
		Medium	T(C°)	Time (min)	Solvent Recry.	In put	Out put(g)	Phase	M.P (C°)	Col-Shape	Solubility
Kanamycin	BZ.Cl	NaOH	25	10-15	EtOH	1.00 g	2.50 g	Solid	90 C°	Pale brown-powder	MeOH
		Pyridine	25	10-15	* $CHCl_3$	1.00 g	0.28 g	Solid	105 C°	White- powder	MeOH
	BZ.S.Cl	NaOH	25	30	EtOH	0.80 g	1.20 g	Solid	150 C°	Golden-powder	$CHCl_3$
		Pyridine	25	30	* $CHCl_3$	0.64 g	1.20 g	Oily	-	Brown	MeOH
	T.P.S.Cl	NaOH	25	10-15	EtOH	0.80 g	0.90 g	Solid	85 C°	Pale brown-powder	$CHCl_3$
		Pyridine	25	10-15	* $CHCl_3$	0.80 g	0.60 g	Oily	-	Brown	MeOH
	Ph.A	Pyridine	Refl.	60	* H_2O	1.00 g	3.80 g	Solid	165 C°	White- powder	NaOH
Gentamycin	BZ.Cl	NaOH	25	10-15	EtOH	0.25 g	1.50 g	Oily	-	White	MeOH
		Pyridine	25	10-15	* $CHCl_3$	0.25 g	1.00 g	Solid	75 C°	White- powder	MeOH
	BZ.S.Cl	NaOH	25	30	EtOH	0.02 g	0.01 g	Solid	165 C°	White- powder	NaOH
		Pyridine	25	30	* $CHCl_3$	0.40 g	-	Oily	-	Brown	MeOH
	T.P.S.Cl	NaOH	25	10-15	EtOH	0.03 g	0.002 g	Solid	95 C°	White-crystal	$CHCl_3$
		Pyridine	25	10-15	* $CHCl_3$	0.16 ml	0.012 g	Oily	-	Yellow	MeOH
	Ph.A	Pyridine	Refl.	60	* H_2O	0.16 ml	2.60 g	Solid	184 C°	White- powder	MeOH
Neomycin	BZ.Cl	NaOH	25	10-15	EtOH	0.0015 g	2.60 g	Solid	112 C°	Pale brown-powder	MeOH
		Pyridine	25	10-15	* $CHCl_3$	0.0015 g	1.10 g	Solid	105 C°	Greenish-powder	MeOH
	T.P.S.Cl	NaOH	25	10-15	EtOH	0.0015 g	0.018 g	Solid	135 C°	Pale brown-powder	$CHCl_3$
	Ph.A	Pyridine	Refl.	60	* H_2O	0. 030 g	0.92 g	Solid	164 C°	White-powder	MeOH
Streptomycin	BZ.Cl	NaOH	25	10-15	EtOH	1.00 g	1.95 g	Oily	-	Brown	MeOH
		Pyridine	25	10-15	* $CHCl_3$	1.00 g	0.95 g	Oily	-	Yellow	MeOH
	BZ.S.Cl	NaOH	25	30	EtOH	1.00 g	6.90 g	Solid	190 C°	Brown- crystal	MeOH
		Pyridine	25	30.	* $CHCl_3$	1.00 g	2.90 g	Oily	-	Brown	MeOH
	T.P.S.Cl	NaOH	25	10-15	EtOH	1.00 g	0.30 g	Solid	95 C°	Pale brown-powder	MeOH
		Pyridine	25	10-15	* $CHCl_3$	1.00 g	1.49 g	Oily	-	Brown	MeOH
	Ph.A	Pyridine	Refl.	60	* H_2O	1.00 g	3.02 g	Solid	165 C°	White- powder	MeOH

• → Recover from

Table (1B) the solubility of derivatives :-

Antibiotic	Reagents	Medium	Water	Methanol	Chloroform	Ether	Pyridine	NaOH 5%	HCl	Acetone
Kanamycin	BZ.Cl	NaOH	-	+	-	-	-	±	-	-
		Pyridine	-	+	-	-	-	±	-	-
	BZ.S.Cl	NaOH	-	-	+	+	+	+	+	+
		Pyridine	+	+	+	+	+	+	+	+
	T.P.S.Cl	NaOH	-	-	+	-	-	±	±	+
		Pyridine	+	+	+	+	+	+	+	+
	Ph.A	Pyridine	-	-	±	-	-	+	-	-
Gentamycin	BZ.Cl	NaOH	+	+	+	+	+	+	+	+
		Pyridine	-	+	-	-	-	+	-	-
	BZ.S.Cl	NaOH	-	±	±	-	-	+	-	-
		Pyridine	+	+	+	+	+	+	+	+
	T.P.S.Cl	NaOH	-	-	+	-	-	±	±	-
		Pyridine	+	+	+	+	+	+	+	+
	Ph.A	Pyridine	-	+	-	-	-	+	+	-
Neomycin	BZ.Cl	NaOH	-	+	-	-	-	+	-	-
		Pyridine	-	+	-	-	-	+	-	-
	T.P.S.Cl	Pyridine	-	-	+	-	-	±	-	-
	Ph.A	Pyridine	-	+	-	-	-	+	-	-
Streptomycin	BZ.Cl	NaOH	±	+	-	-	-	-	-	-
		Pyridine	-	+	-	-	-	-	-	-
	BZ.S.Cl	NaOH	+	+	-	-	-	-	-	-
		Pyridine	-	+	-	-	-	-	-	-
	T.P.S.Cl	NaOH	±	+	-	-	-	-	-	-
		Pyridine	-	+	-	-	-	-	-	-
	Ph.A	Pyridine	=	+	-	-	-	-	-	-

(+) soluble , (-) insoluble

3.2.1 .Ultraviolet measurement results :-

The tables (**2 – 5**) and Fig.s (**14 – 17**) show the variation of absorbance with the concentration in methanol for reagents (BZCl, BZ.S.Cl,T.P.S.Cl, and Ph.A) as well as calculated ε_{max} while tables (**6 – 28**) and Fig.s (**18 – 32, 34 – 40**) 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 (**29**) show the possible number of substituted reagent on antibiotic derivatives calculated from uv data (n_c , n_ε) .

The methods that are used to test the validity of theoretical expression which applied to calculate n_c and n_ε Table (**29**) to small molecule (D – glucose) represented in the table (**30**).

This table contains glucose – Ph.A derivative which was prepare in pyridine using titration methods (OH – groups and occupation, table (**47**), Fig. (**57**)) compare with that calculated from uv- data.

Also glucose – BZ. derivative in aq. NaOH medium by comparing penta-benzoate derivative as given in literature with that calculated from uv- data .

Fig.s (**1 – 4**) shows the uv- spectrum of reagents (BZCl, BZ.S.Cl, T.P.S.Cl, and Ph.A) . Fig.s (**5 –7**) are the uv- spectrum of streptomycin and Strept – derivatives, while Fig.s (**8 – 13**) are the uv- spectrum of (Kan. And Gent.) derivatives .

Table (2) Absorbance-concentration change of ***BZ.Cl***

at (λ_{max} = **280 ± 4 nm**), solvent; **methanol.**

C (M. L^{-1})	CL (M.L^{-1}.cm)	A
0.400	1.60	2.289
0.200	0.80	2.257
0.100	0.40	1.999
0.020	0.08	1.780
0.010	0.04	1.560
0.005	0.02	0.780
Cal.	ε_{max} **= 0.655**	

Table (3) Absorbance-concentration change of ***BZ.S.Cl***

at (λ_{max} = **273 ± 2 nm**), solvent; **methanol.**

C (M. L^{-1})	CL (M.L^{-1}.cm)	A
0.400	1.851	1.60
0.200	1.605	0.80
0.100	1.049	0.40
0.020	0.619	0.08
0.010	0.190	0.04
0.005	0.063	0.02
Cal.	ε_{max} **= 1.084**	

Table (4) Absorbance-concentration change of ***T.P.S.CL***

at (λ_{max} = **273 ± 2 nm**), solvent; **methanol.**

C ($M.L^{-1}$)	CL ($M.L^{-1}.cm$)	A
0.400	1.60	1.865
0.200	0.80	1.749
0.100	0.40	1.523
0.020	0.08	1.297
0.010	0.04	0.925
0.005	0.02	0.743
Cal.	ε_{max} **= 0.611**	

Table (5) Absorbance-concentration change of ***Ph.A***

at (λ_{max} = **287 ± 7 nm**), solvent; **methanol.**

C ($M.L^{-1}$)	CL ($M.L^{-1}.cm$)	A
0.40	1.60	2.587
0.20	0.80	2.432
0.10	0.40	2.315
0.02	0.08	1.014
0.01	0.04	0.871
Cal.	ε_{max} **= 1.048**	

Table (6) Absorbance-concentration change of ***Kan.-BZ. /A***

at (λ_{max} = **276 ± 3 nm**) solvent; **methanol.**

C (g. L^{-1})	CL (g. L^{-1}.cm)	A
0.05828	1.850	0.233
0.03643	1.286	0.146
0.02649	1.196	0.106
0.02081	1.001	0.083
0.01714	0.855	0.069
Cal.	ε_{max} **= 5.728**	

Table (7) Absorbance-concentration change of ***Kan.-BZ. /B***

at (λ_{max} = **276 ± 4 nm**), solvent; **methanol.**

C (g. L^{-1})	CL (g. L^{-1}.cm)	A
0.12000	0.480	2.059
0.07500	0.300	1.440
0.05455	0.218	1.020
0.04286	0.171	0.856
0.03530	0.141	0.664
Cal.	ε_{max} **= 3.6224**	

Table (**8**) Absorbance-concentration change of ***Kan.-BZ.S. /B***

at (λ_{max} **= 276 ± 5 nm**), solvent; **methanol.**

C (g. L^{-1})	**CL (g. L^{-1}.cm)**	**A**
0.14500	0.5800	2.222
0.12960	0.5184	1.190
0.10990	0.4396	1.068
0.08850	0.3540	0.974
0.06845	0.2738	0.880
Cal.	ε_{max} **= 3.5574**	

Table (**9**) Absorbance-concentration change of ***Kan.-T.P.S. /A***

at (λ_{max} **= 280 ± 4 nm**), solvent; **chloroform.**

C (g. L^{-1})	**CL (g. L^{-1}.cm)**	**A**
0.1200	0.480	2.552
0.0750	0.300	2.423
0.05455	0.218	2.250
0.0300	0.120	2.026
0.0231	0.092	1.844
0.0182	0.073	1.670
Cal.	ε_{max} **= 2.0322**	

Table (**10**) Absorbance-concentration change of ***Kan.-T.P.S./B***

at (λ_{max} **= 273 ± 1 nm**), solvent; **methanol**.

C (g. L^{-1})	CL (g. L^{-1}.cm)	A
0.07340	0.2937	1.439
0.04589	0.1836	1.154
0.02086	0.0834	0.987
0.00745	0.0298	0.854
0.00673	0.0263	0.735
Cal.	ε_{max} **= 2.365**	

Table (**11**) Absorbance-concentration change of ***Neo. -BZ. /A***

at (λ_{max} **= 276 ± 3 nm**), solvent; **methanol**.

C (g. L^{-1})	CL (g. L^{-1}.cm)	A
0.12000	0.480	1.895
0.07500	0.300	1.436
0.05455	0.218	1.180
0.04286	0.171	0.977
0.03530	0.141	0.836
Cal.	ε_{max} **= 2.7174**	

Table (**12**) Absorbance-concentration change of ***Neo. -BZ. /B_1*** at (λ_{max} **= 277 ± 3 nm**),solvent; **methanol.**

C (g. L^{-1})	**CL (g. L^{-1}.cm)**	**A**
0.1200	0.480	2.222
0.0750	0.300	1.852
0.0546	0.218	1.515
0.0429	0.171	1.321
0.0353	0.141	1.176
Cal.	ε_{max} **= 3.0559**	

Table (**13**) Absorbance-concentration change of ***Neo. -BZ. /B_2*** at (λ_{max} **= 278 ± 4 nm**), solvent; **methanol.**

C (g. L^{-1})	**CL (g. L^{-1}.cm)**	**A**
0.1200	0.480	2.400
0.0750	0.300	1.541
0.0546	0.218	1.209
0.0429	0.171	0.968
0.0353	0.141	0.828
Cal.	ε_{max} **= 4.6093**	

Table (**14**) Absorbance-concentration change of ***Neo. -T.P.S./A***

at (λ_{max} **= 282 ± 2 nm**), solvent; **chloroform**.

C (g. L^{-1})	CL (g. L^{-1}.cm)	A
0.1200	0.480	2.471
0.0750	0.300	2.454
0.0353	0.141	2.346
0.0261	0.104	2.309
0.0171	0.069	2.221
0.0128	0.051	2.136
Cal.	ε_{max} **= 0.6841**	

Table (**15**) Absorbance-concentration change of ***Gent. -BZ. /A***

at (λ_{max} **= 282 ± 2 nm**), solvent; **methanol**.

C (g. L^{-1})	CL (g. L^{-1}.cm)	A
0.1320	0.5280	2.352
0.1300	0.5200	2.323
0.1279	0.5116	2.286
0.1244	0.4976	2.223
0.0210	0.0840	1.994
Cal.	ε_{max} **= 0.714**	

Table (**16**) Absorbance-concentration change of ***Gent. -BZ. /B***

at (λ_{max} **= 277 ± 3 nm**),solvent; **methanol** .

C (g. L^{-1})	**CL (g. L^{-1}.cm)**	**A**
0.1200	0.480	2.144
0.0750	0.300	1.459
0.0546	0.218	1.194
0.0429	0.171	0.995
0.0353	0.141	0.897
Cal.	**ε_{max} = 3.6737**	

Table (**17**) Absorbance-concentration change of ***Gent. -BZ.S. /B***

at (λ_{max} **= 271 ± 1 nm**), solvent; methanol.

C (g. L^{-1})	**CL (g. L^{-1}.cm)**	**A**
0.06178	0.24712	0.946
0.05173	0.20692	0.792
0.04150	0.16600	0.635
0.03455	0.13820	0.529
0.03050	0.12200	0.467
Cal.	**ε_{max} = 3.8285**	

Table (**18**) Absorbance-concentration change of ***Gent. -T.P.S. /A***

at (λ_{max} **= 280 ± 5 nm**) , solvent; **chloroform.**

C (g. L^{-1})	**CL (g. L^{-1}.cm)**	**A**
0.1200	0.480	2.75
0.0750	0.300	2.63
0.0429	0.171	2.49
0.0300	0.120	2.31
0.0231	0.092	2.15
0.0116	0.046	1.42
0.0058	0.023	0.97
Cal.	ε_{max} **= 3.2107**	

Table (**19**) Absorbance-concentration change of ***Gent. -T.P.S./B***

at (λ_{max} **= 279 ± 3 nm**), solvent; **methanol.**

C (g. L^{-1})	**CL (g. L^{-1}.cm)**	**A**
0.1391	0.556	2.844
0.0869	0.348	2.581
0.0632	0.253	2.380
0.0497	0.199	2.242
0.0348	0.139	2.053
0.0268	0.107	1.900
Cal.	ε_{max} **= 2.0268**	

Table (**20**) Absorbance-concentration change of ***Gent. -Ph.A /B*** at (λ_{max} **= 283 ± 1 nm**), solvent; **methanol**.

C (g. L^{-1})	CL (g. L^{-1}.cm)	A
0.1200	0.4800	2.350
0.0750	0.3000	2.129
0.0429	0.1710	1.445
0.0300	0.1200	1.049
0.0261	0.1044	0.965
Cal.	**ε_{max} = 3.7957**	

Table (**21**) Absorbance-concentration change of ***Streptomycin*** at (λ_1 **= 278 ± 1 nm**) and (λ_2 **= 315 ±1 nm**), solvent; **methanol**.

C (M.L)	CL (M.L .cm)	A (-)	A (+)
10.0	40.00	2.304	1.701
6.67	26.68	1.572	1.420
5.00	20.00	1.120	0.811
3.33	13.32	0.779	0.566
Cal.	**ε_- = 0.0578**	**ε_+ = 0.0443**	

Table (**22**) Absorbance-concentration change of ***Strept. -BZ. /A***

at (λ_{max} **= 277 ± 5 nm**), solvent; **methanol.**

C (g. L^{-1})	CL (g. L^{-1}.cm)	A
0.120	0.480	2.273
0.012	0.048	0.601
0.008	0.032	0.431
0.006	0.024	0.328
Cal.	ε_{max} **= 4.1005**	

Table (**23**) Absorbance-concentration change of ***Strept. -BZ. /B***

at (λ_{max} **= 296 ± 3 nm**), solvent; **methanol.**

C (g. L^{-1})	CL (g. L^{-1}.cm)	A
0.1395	0.5580	2.643
0.1116	0.4464	2.556
0.0670	0.2680	2.523
0.0480	0.1910	2.477
Cal.	ε_{max} **= 0.4137**	

Table (**24**) Absorbance-concentration change of ***Strept. -BZ.S. /A*** at (λ_{max} **= 270 nm**), solvent; **methanol.**

C (g. L^{-1})	**CL (g. L^{-1}.cm)**	**A**
0.120	0.480	0.457
0.024	0.096	0.358
0.012	0.048	0.303
0.008	0.032	0.283
Cal.	**ε_{max} = 0.3514**	

Table (**25**) Absorbance-concentration change of ***Strept. -BZ.S. /B*** at (λ_{max} **= 275 ± 1nm**), solvent; **methanol.**

C (g. L^{-1})	**CL (g. L^{-1}.cm)**	**A**
0.0650	0.2600	2.120
0.0560	0.2240	1.842
0.0278	0.1120	1.684
0.0186	0.0744	1.598
0.0139	0.0570	1.512
Cal.	**ε_{max} = 2.5034**	

Table (**26**) Absorbance-concentration change of ***Strept. -T.P.S. /A*** at (λ_{max} = **277 ± 3 nm**), solvent; **methunol.**

C (g. L^{-1})	**CL (g. L^{-1}.cm)**	**A**
0.120	0.480	2.193
0.012	0.048	1.860
0.006	0.024	1.645
0.004	0.016	1.453
Cal.	**ε_{max} = 1.2376**	

Table (**27**) Absorbance-concentration change of ***Strept. -T.P.S./B*** at (λ_{max} = **277 ± 3 nm**), solvent; **methanol.**

C (g. L^{-1})	**CL (g. L^{-1}.cm)**	**A**
0.11900	0.47600	2.180
0.01190	0.04760	1.866
0.00595	0.02380	1.692
0.00397	0.01588	1.528
0.00298	0.01190	1.451
Cal.	**ε_{max} = 1.256**	

Table **(28)** Absorbance-concentration change of ***Strept. -Ph.A /B***

at (λ_{max} **= 277 ± 3 nm**), solvent; **methanol**.

C (g. L^{-1})	CL (g. L^{-1}.cm)	A
0.120	0.480	2.302
0.012	0.048	1.990
0.006	0.024	1.474
0.004	0.016	1.580
0.003	0.012	0.824
Cal.	**ε_{max} = 2.1965**	

Table **(29)** possible number of reagent molecules substituted on antibiotic sample to form derivative calculated according to u.v- data.

Derivatives	***Reag.***	***u.v-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 B)	0.778
Strept. -BZ.S. /A	1.084	0.3514	0.12	0.457	0.1054	0.878(0.87)	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 (8)	3.76
Strept.-T.P.S. /B	0.611	1.2256	0.119	2.18	0.892	7.5 (8)	3.76
Strept.-Ph.A /B	1.048	2.1965	0.12	2.302	0.54914	4.57 (4)	3.03
Strept. /average = (0.0578 + 0.0443) / 2 = 0.05105					ε_C - 0.05105		

Table (**30**) methods validity test for small molecule ***D-glucose*** .

Methods / Derivatives	Titration		u.v at $\lambda_{max.}$ = 282, 275 nm, EtOH						
	OH Groups	Occupation	ε_R	ε_C	C_C	A_C	$C_{C\text{-}R}$	n_C	n_E
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.**

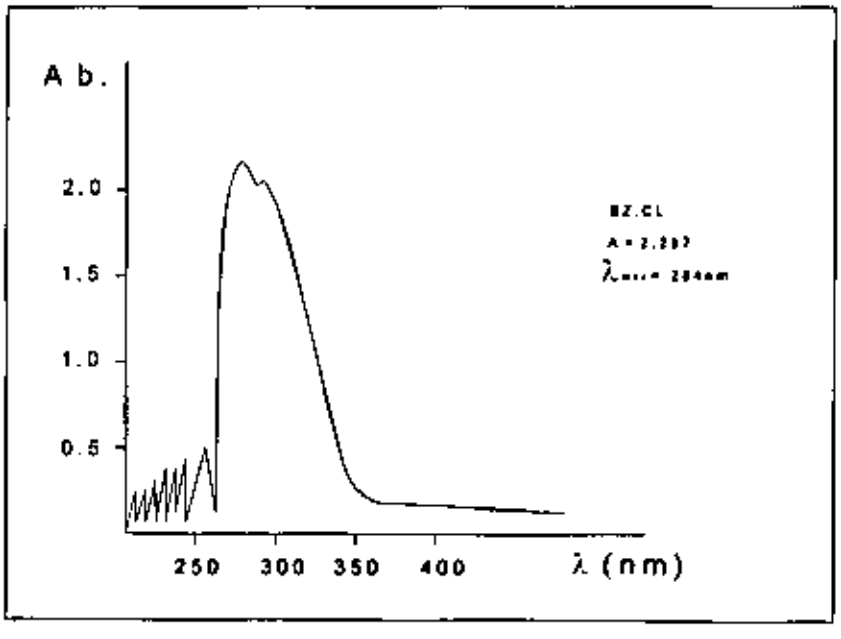


Fig. (1) uv spectrum of *BZ.Cl.*

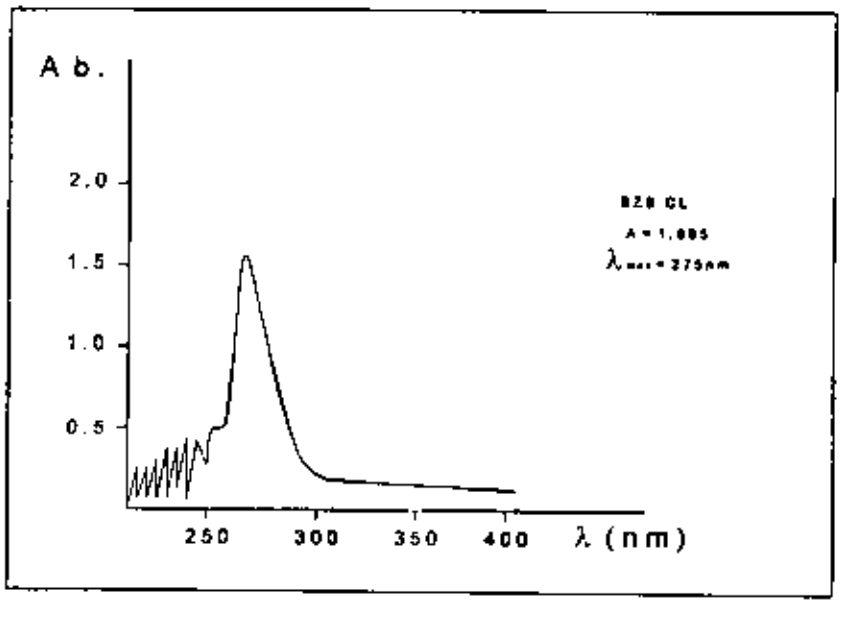


Fig. (2) uv spectrum of *BZ.S.Cl.*

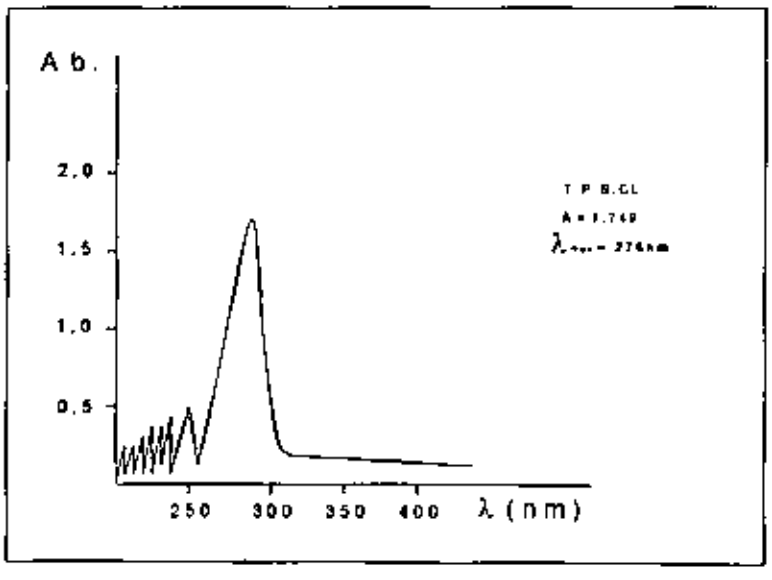


Fig. (3) uv spectrum of *T.P.S.Cl.*

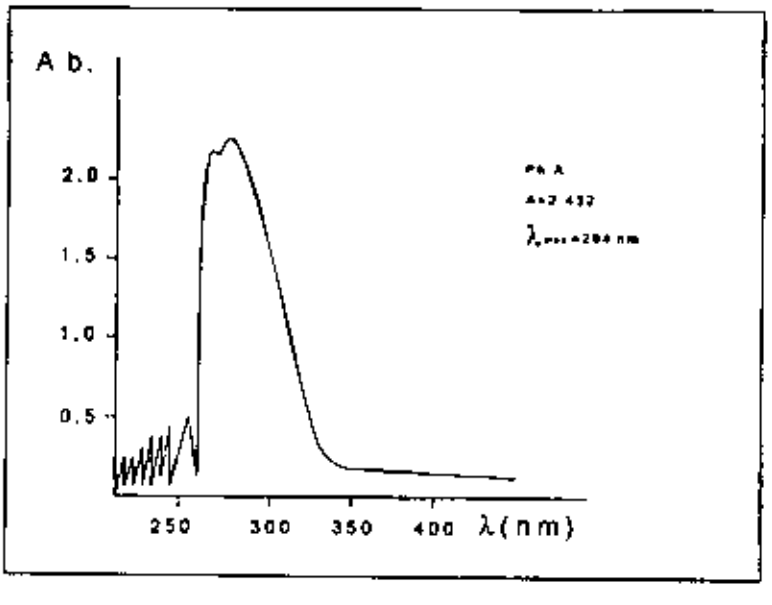


Fig. (4) uv spectrum of *Ph.A* .

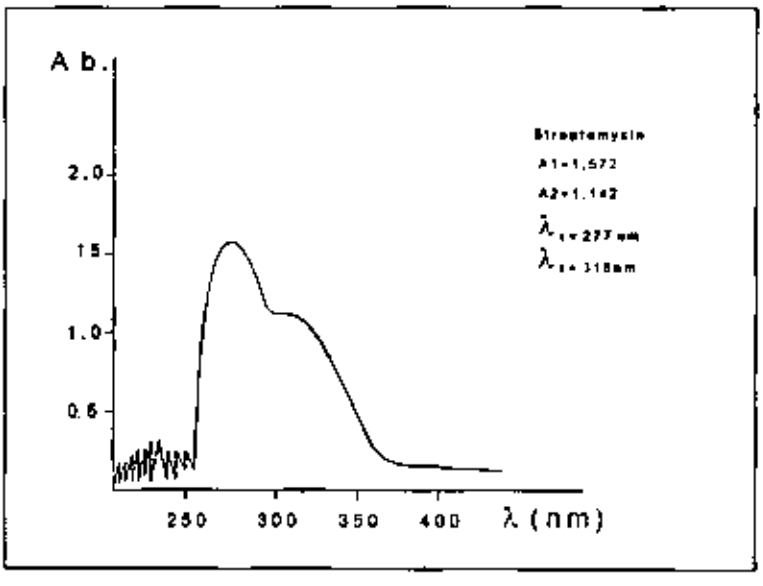


Fig. (5) uv spectrum of *streptomycin*.

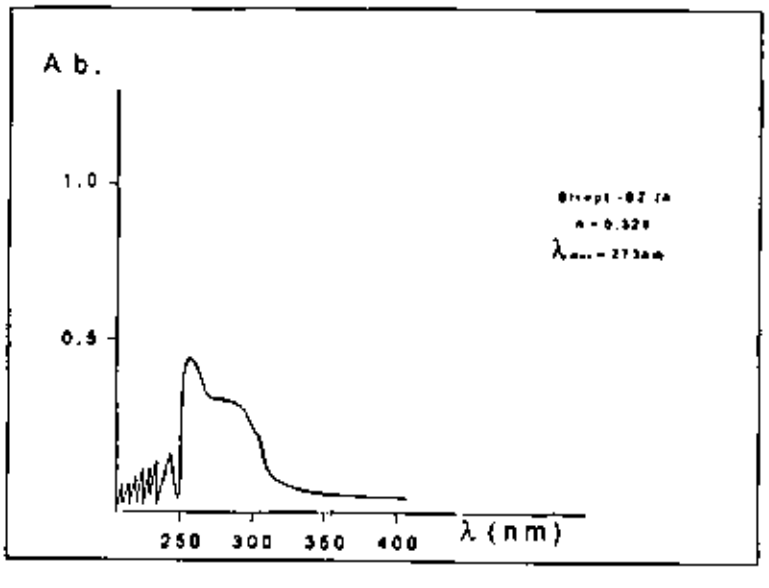


Fig. (6) uv spectrum of *strept. -BZ. /A*.

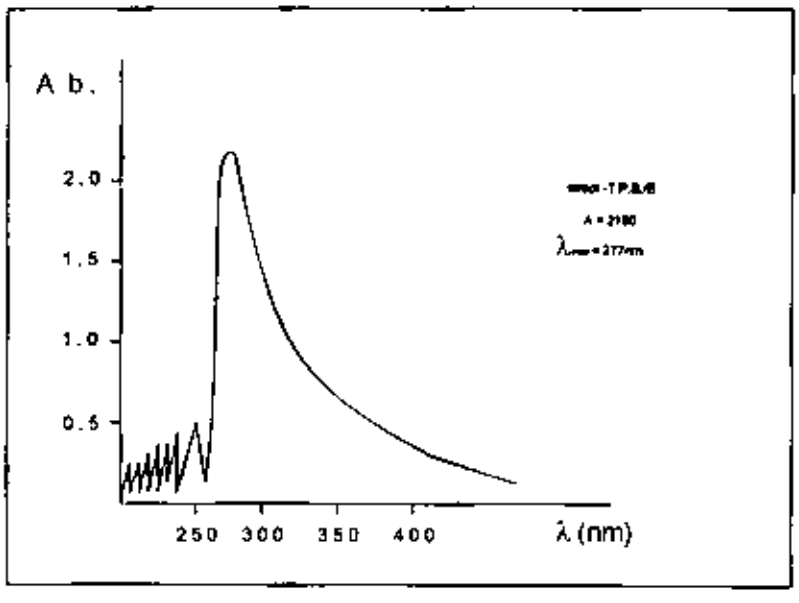


Fig. (7) uv spectrum of *Strept. –T.P.S./B.*

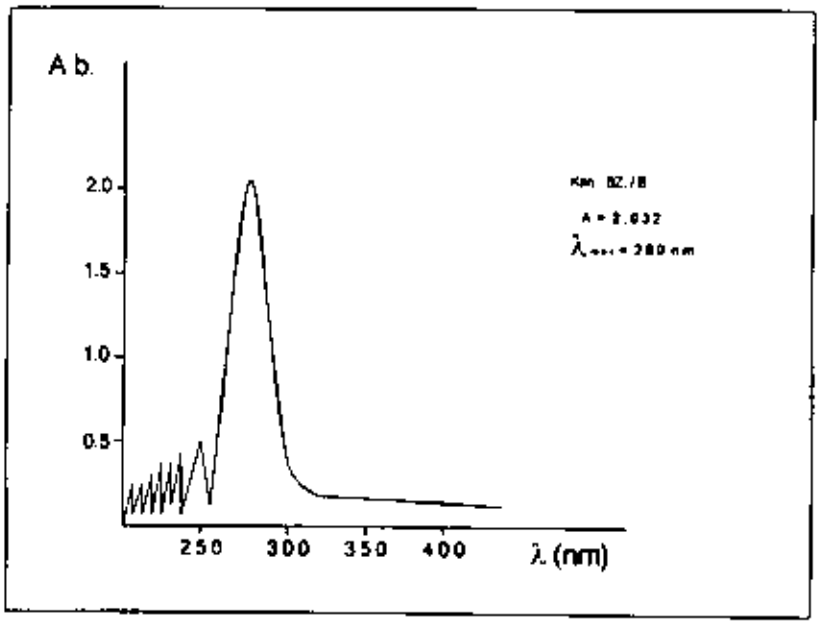


Fig. (8) uv spectrum of *Kan.-BZ. /B.*

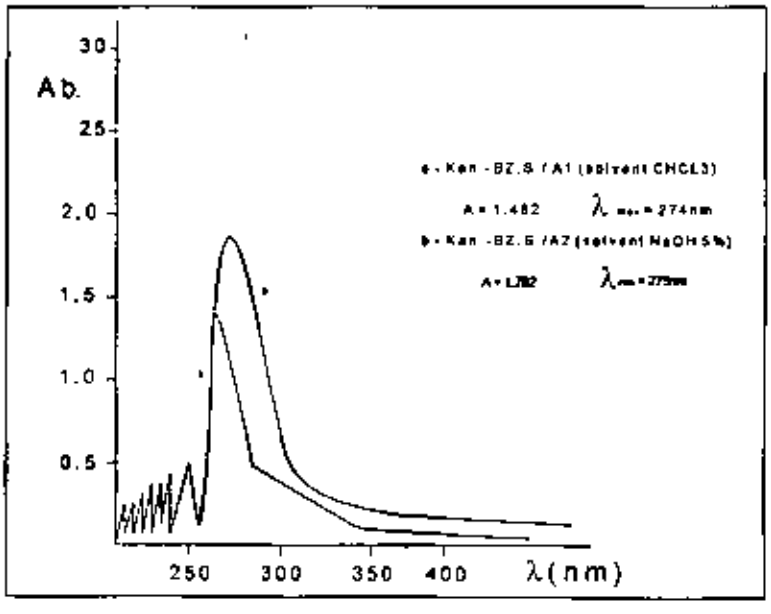


Fig. (9) uv spectrum of *Kan.-BZ.S. /A.*

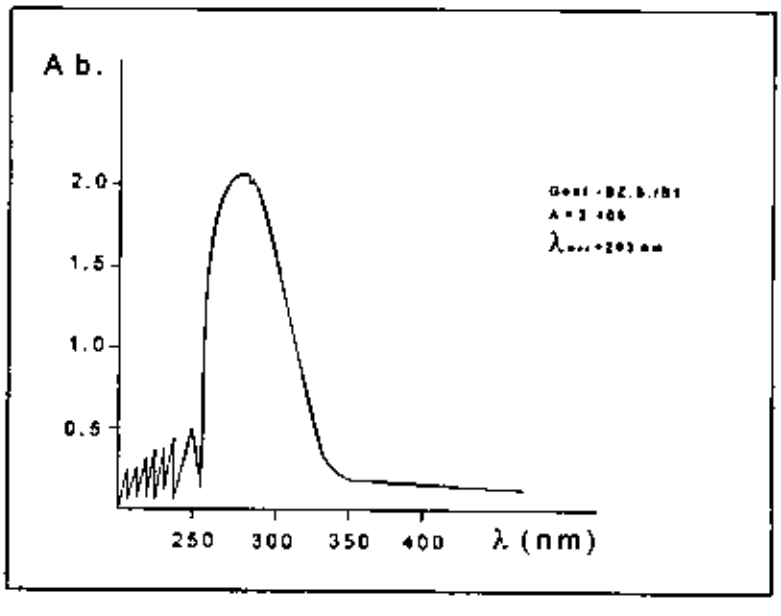


Fig. (10) uv spectrum of *Gent. -BZ.S. /B1.*

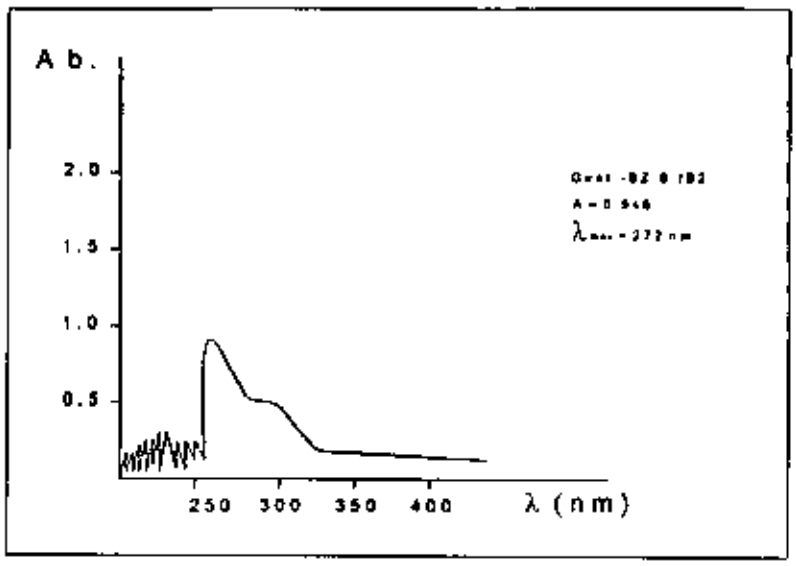


Fig. (11) uv spectrum of *Gent. -BZ.S. /B2*

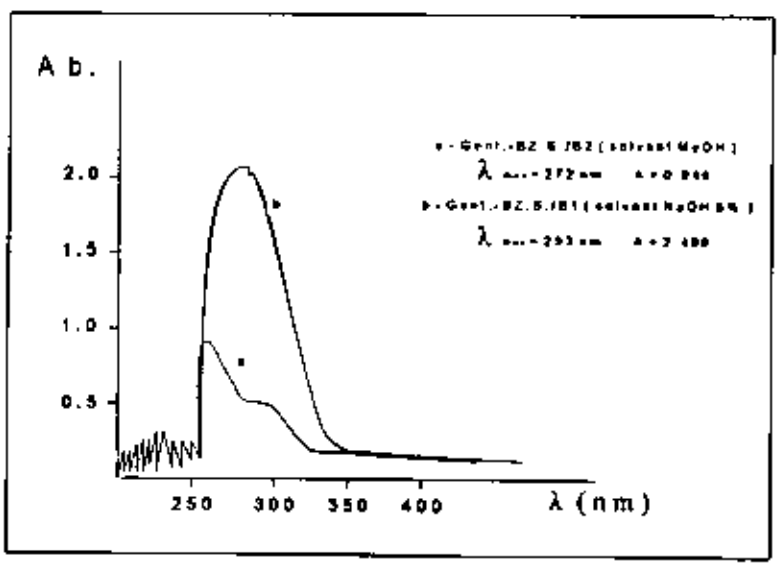


Fig. (12) uv spectrum of *Gent. -BZ.S/B.*

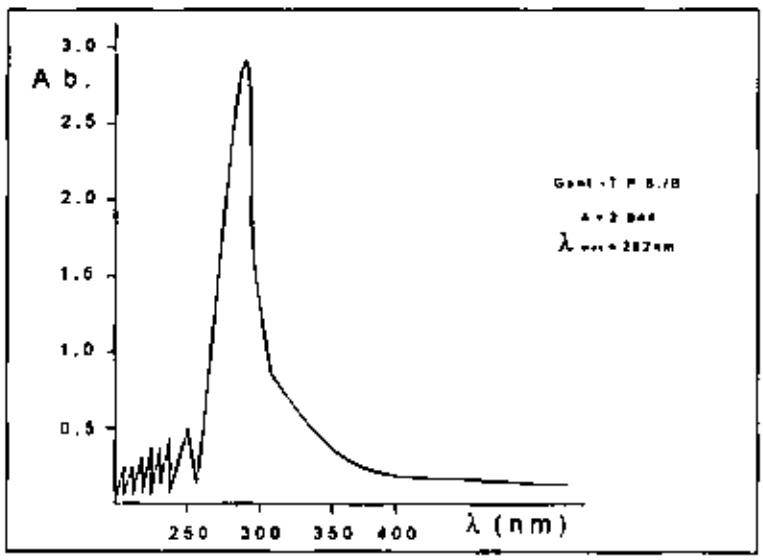
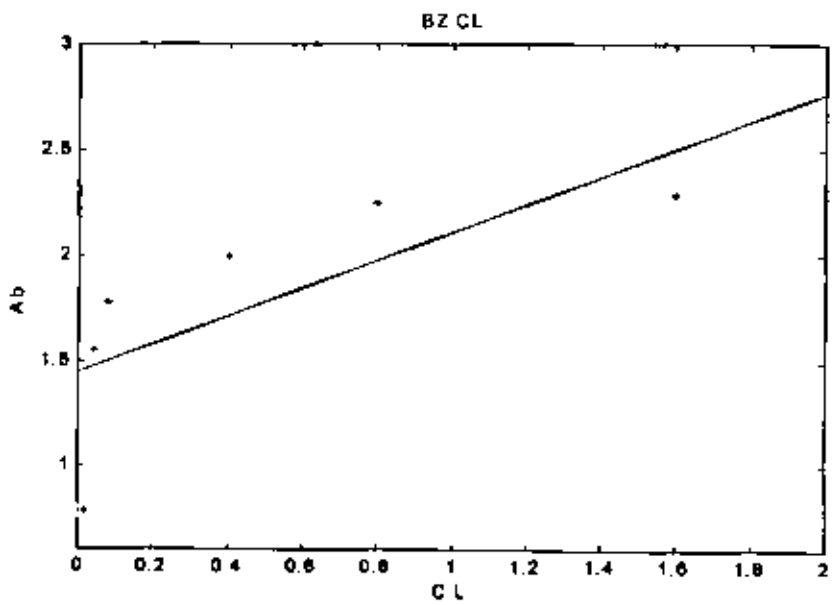
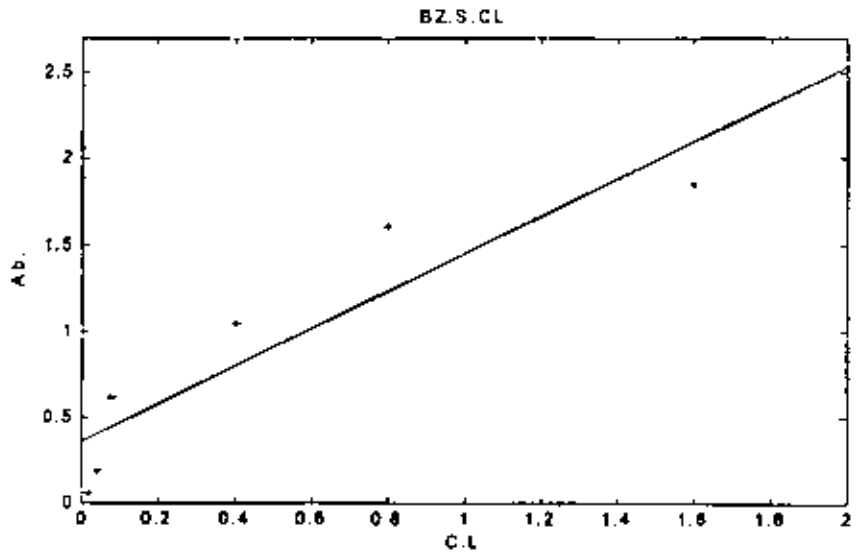


Fig. (13) uv spectrum of *Gent. -T.P.S./B.*



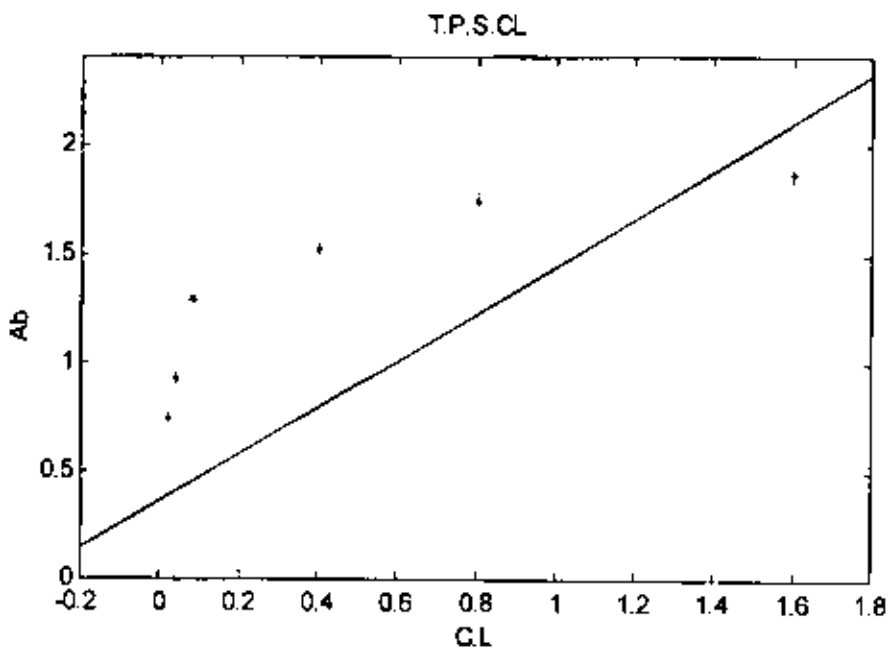
Slope = 0.6551 b = 1.455

Fig. (**14**) Variation of Absorbance with concentration for ***BZ.CL*** at ($\lambda_{max.}$= **280 ± 4 nm**).



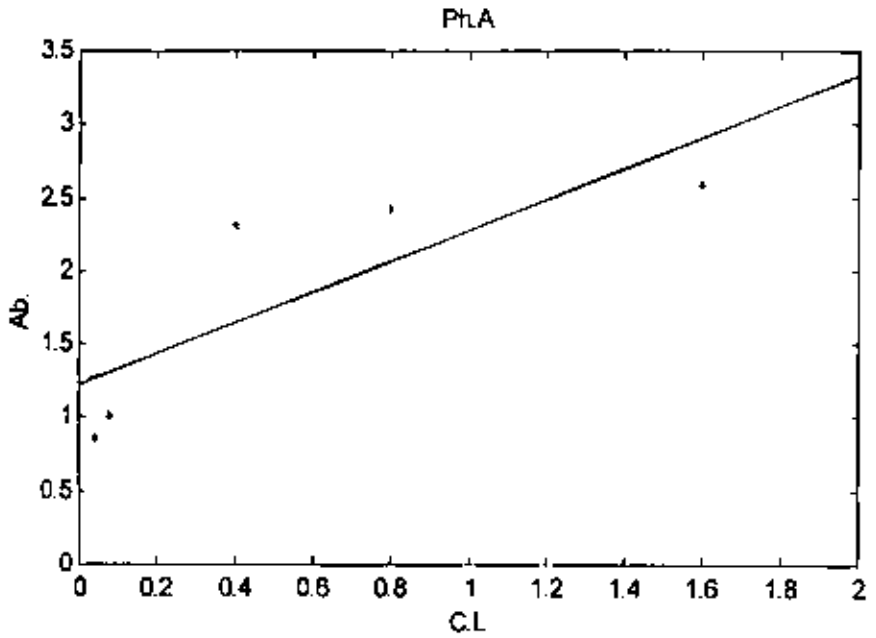
Slope = 1.0835 **b = 0.3652**

Fig. (**15**) Variation of Absorbance with concentration for ***BZ.S.CL*** at (λ_{max} **= 273 ± 2 nm**).



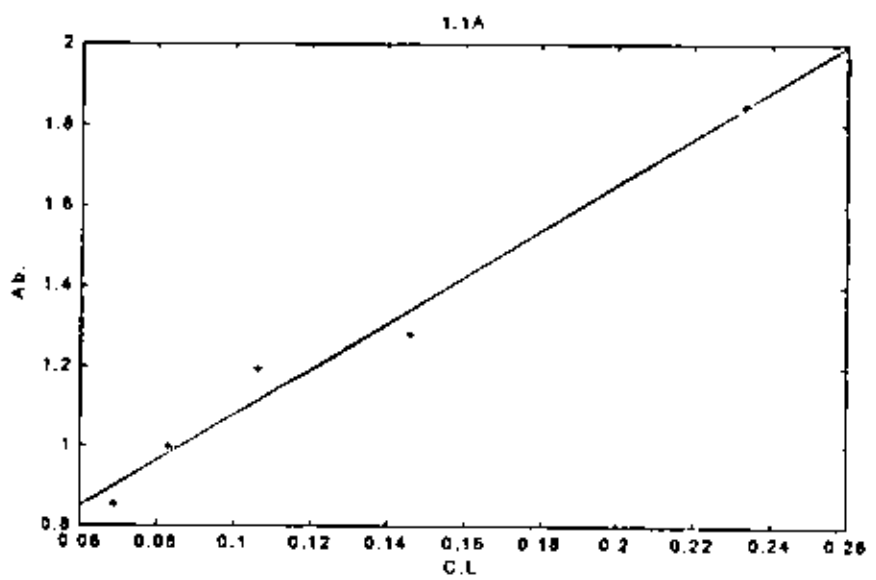
Slope = 0.6111 **b = 1.0509**

Fig. (**16**) Variation of Absorbance with concentration for ***T.P.S.CL*** at ($\lambda_{max.}$ **= 276 ± 2 nm**) .



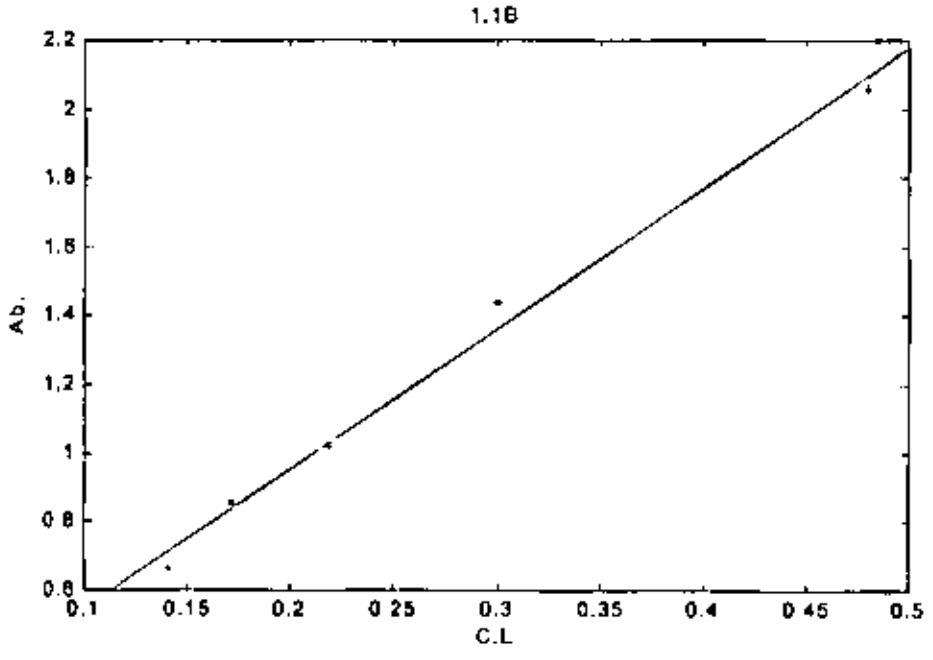
Slope = 1.0482 b = 1.2317

Fig. (17) Variation of Absorbance with concentration for ***Ph.A*** at ($\lambda_{max.}$ = 287 ± 7 nm).



Slope = 5.7281 b = 0.5078

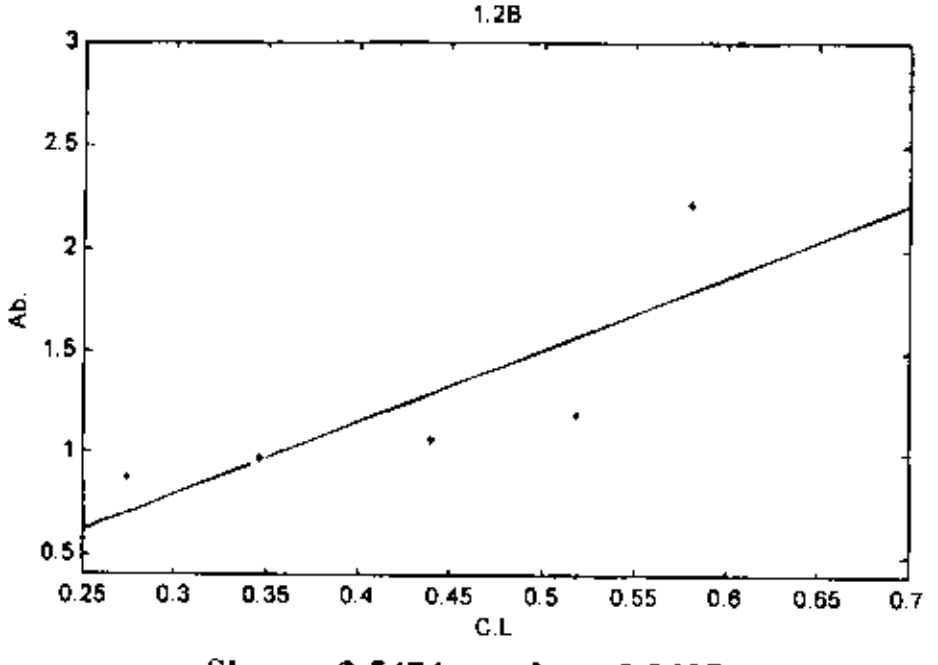
Fig. (**18**) Variation of Absorbance with concentration for ***Kan.-BZ. /A*** at (λ_{max} = 276 ± 3 nm).



Slope = 3.6224 b = 0.1858

Fig. (**19**) Variation of Absorbance with concentration for ***Kan. -BZ. /B***

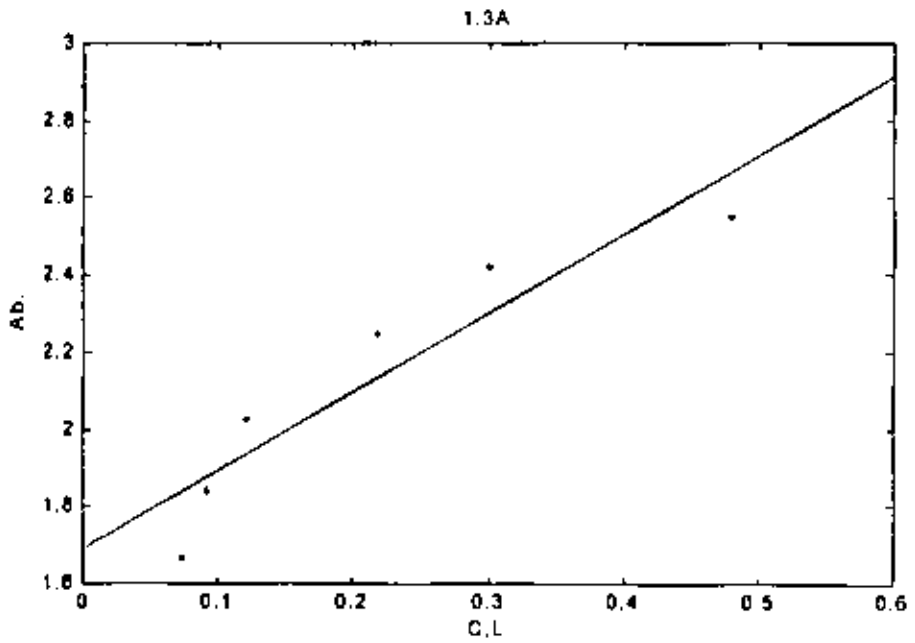
at (λ_{max} **= 276 ± 4 nm**).



Slope = 3.5474 b = - 0.2637

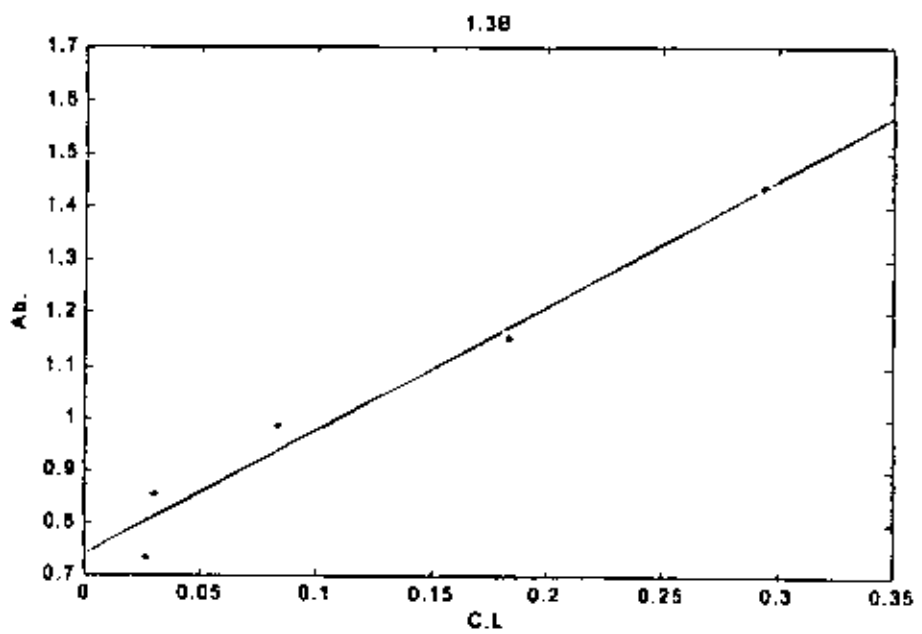
Fig. (**20**) Variation of Absorbance with concentration for ***Kan.-BZ.S. /B***

at ($\lambda_{max.}$ **= 276 ± 5 nm**).



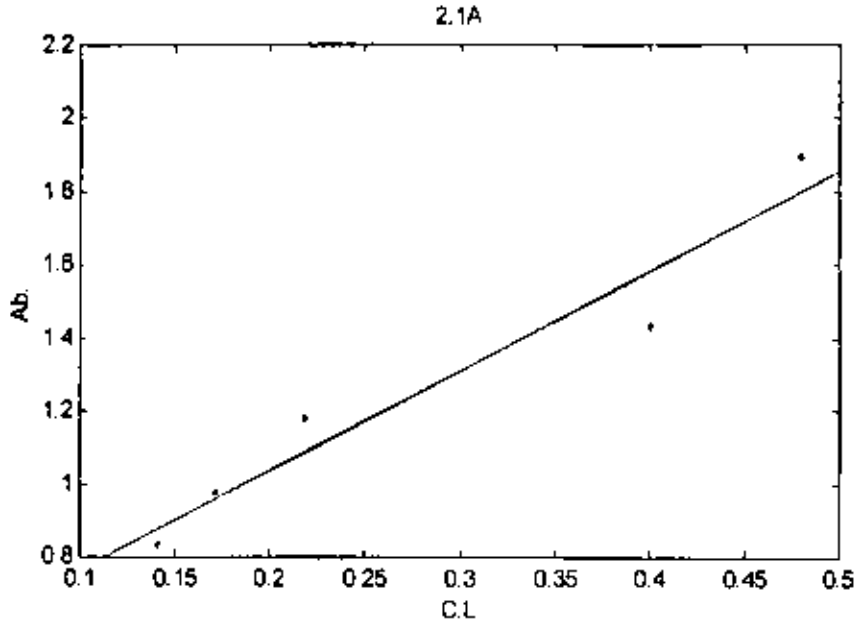
Slope = 2.0322 b = 1.6927

Fig. (**21**) Variation of Absorbance with concentration for ***Kan.-T.P.S./A*** at ($\lambda_{max.}$ **= 280 ± 4 nm**).



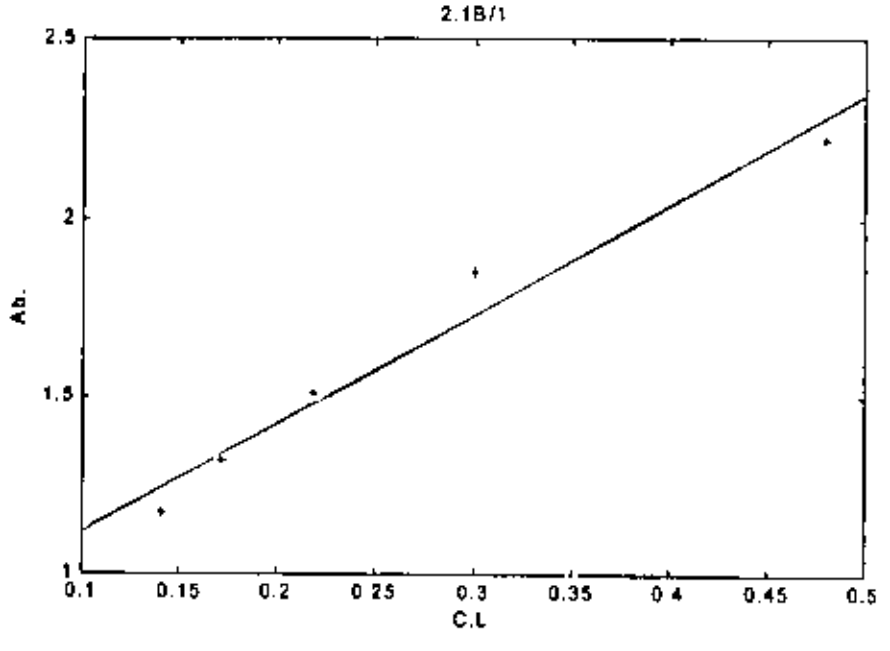
Slope = 2.3653 b = 0.7420

Fig. (**22**) Variation of Absorbance with concentration for ***Kan.-T.P.S./B*** at ($\lambda_{max.}$ **= 273 ± 1 nm**).



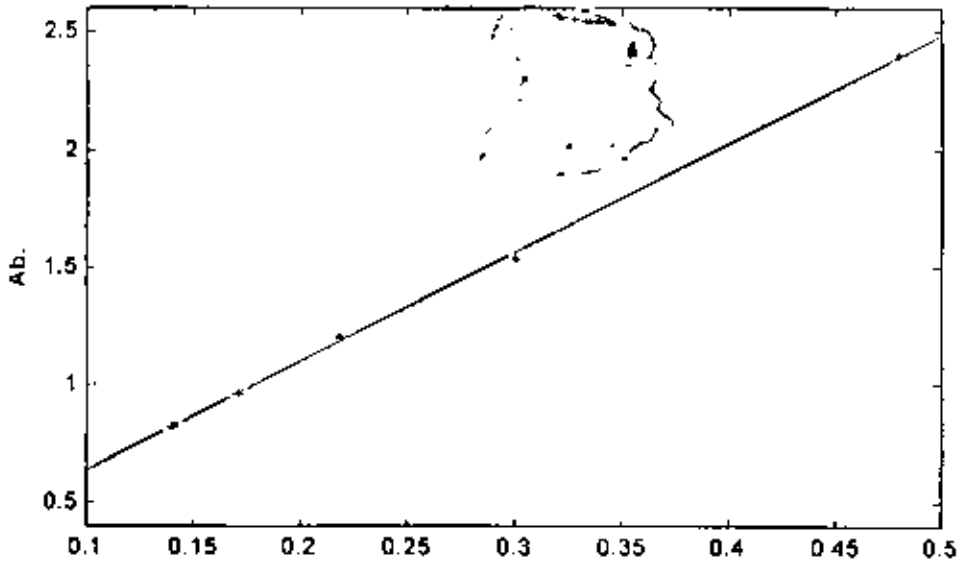
Slope = 2.7174 b = 0.4981

Fig. (**23**) Variation of Absorbance with concentration for ***Neo. -BZ. /A*** at ($\lambda_{max.}$ **= 276 ± 3 nm**).



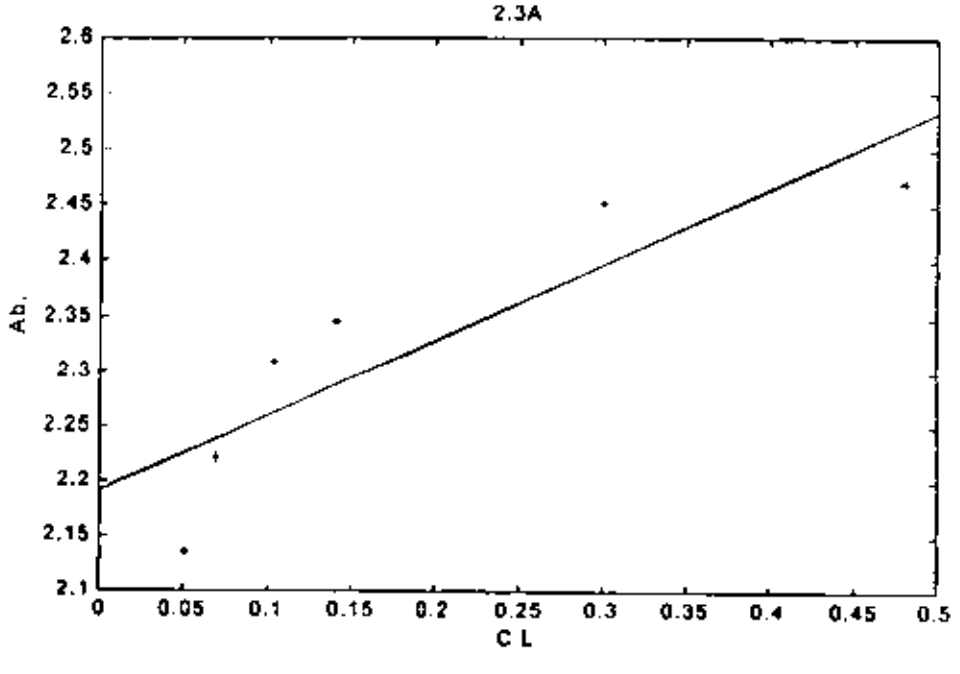
Slope = 3.0559 b = 0.8166

Fig. (**24**) Variation of Absorbance with concentration for ***Neo. -BZ. /B*** at ($\lambda_{max.}$ **= 277 ± 3 nm**).



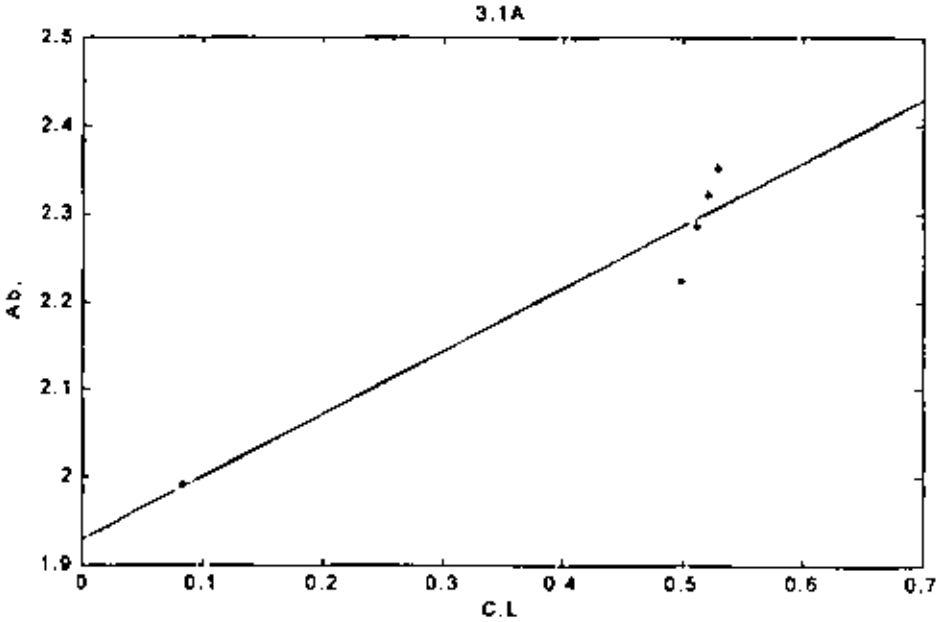
Slope = 4.6093 b = 0.1816

Fig. (**25**) Variation of Absorbance with concentration for ***Neo. -BZ. /B*** at ($\lambda_{max.}$ **= 278 ± 4 nm**).



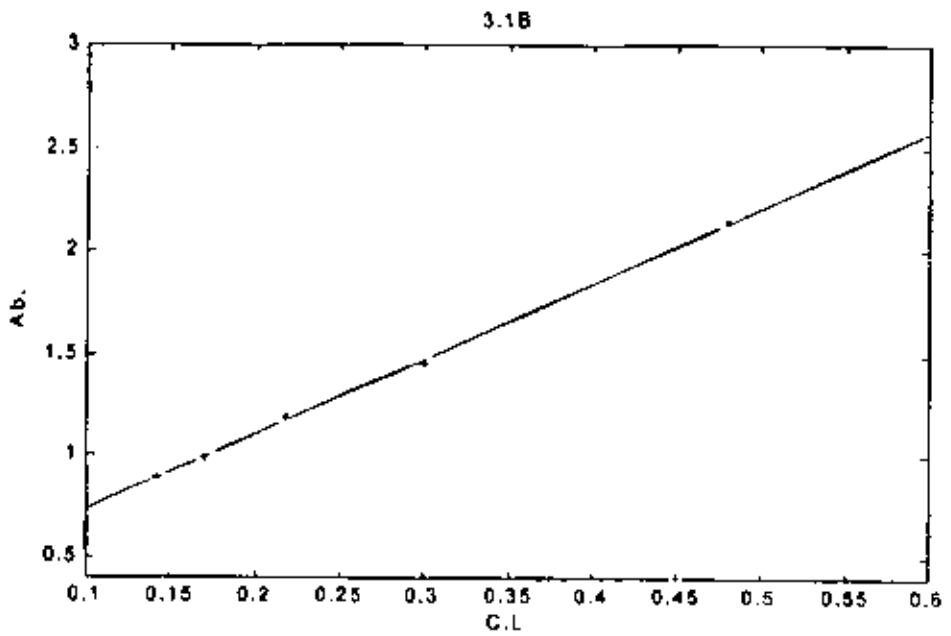
Slope = 0.6841 b = 2.1923

Fig. (**26**) Variation of Absorbance with concentration for ***Neo. -T.P.S./A*** at ($\lambda_{max.}$ **= 282 ± 2 nm**).



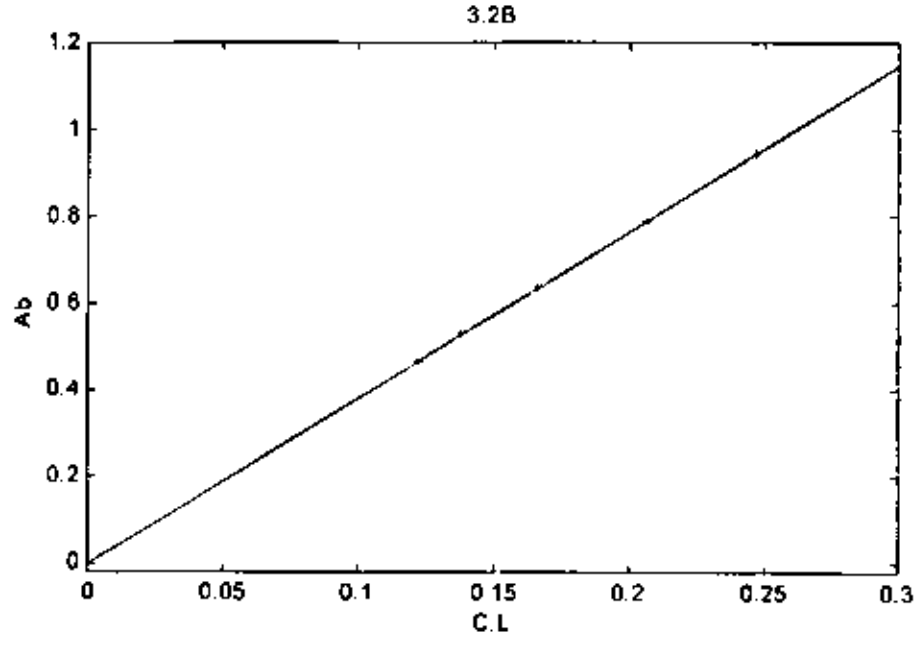
Slope = 0.714 b =1.9298

Fig. (**27**) Variation of Absorbance with concentration for ***Gent. -BZ. /A*** at ($\lambda_{max.}$ **= 282 ± 2 nm**).



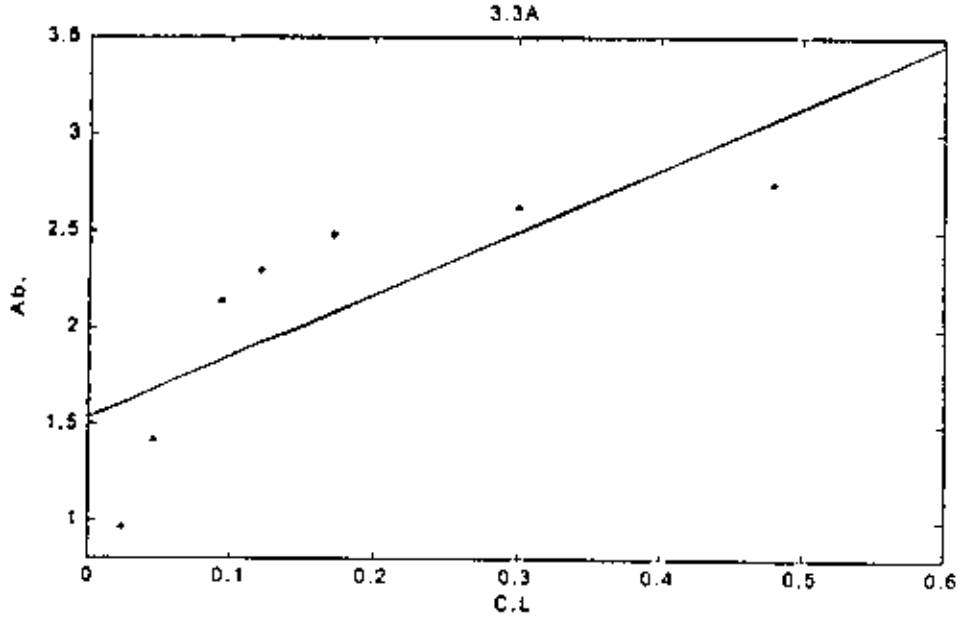
Slope = 3.6737 b = 0.3753

Fig. (**28**) Variation of Absorbance with concentration for ***Gent. -BZ. /B*** at ($\lambda_{max.}$ **= 277 ± 3 nm**).



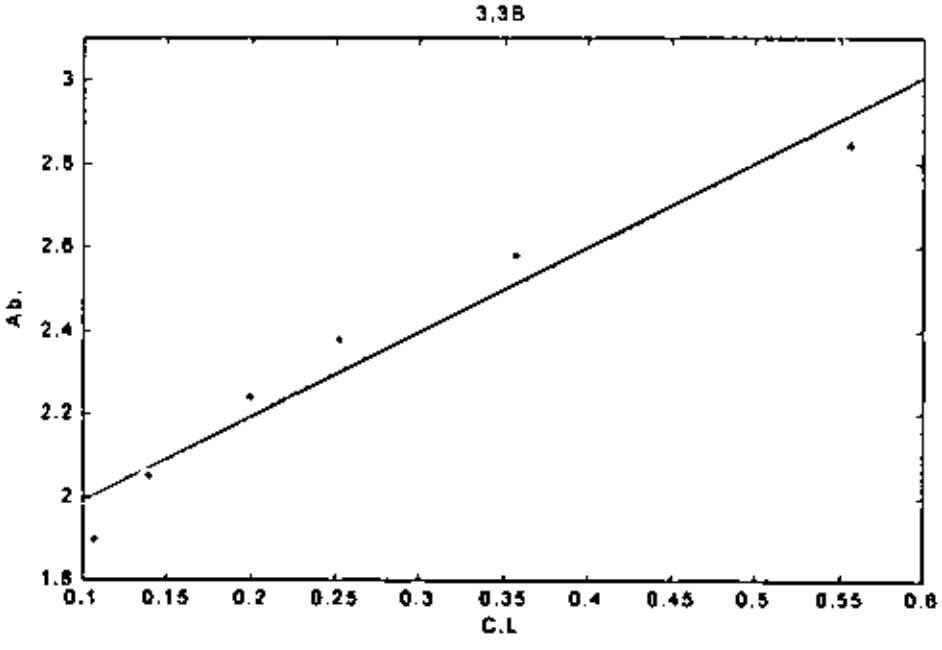
Slope = 3.8285 b = - 0.0002

Fig. (**29**) Variation of Absorbance with concentration for ***Gent. -BZ.S. /B*** at ($\lambda_{max.}$ = **271 ± 1 nm**).



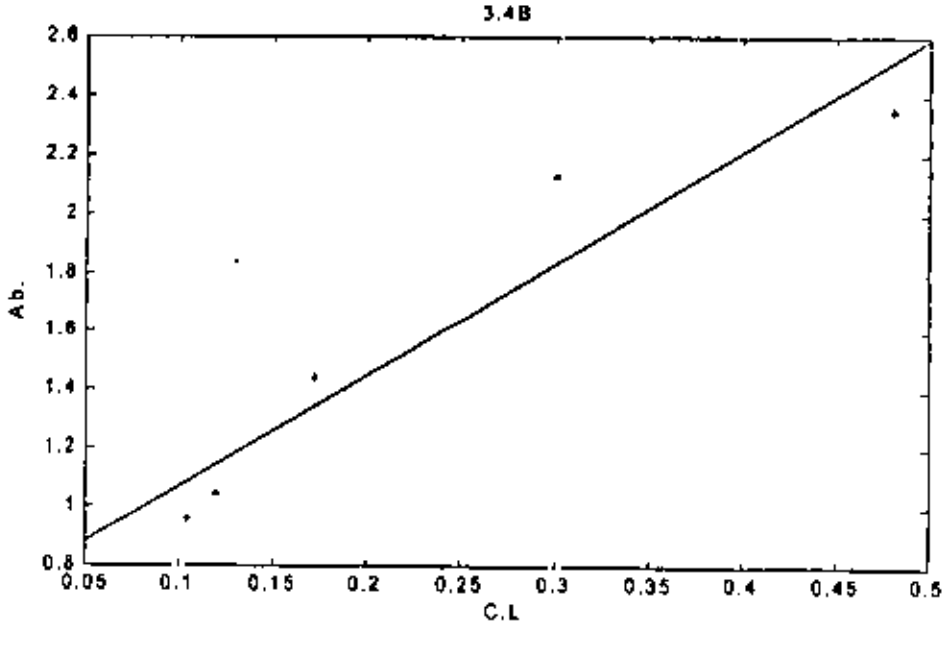
Slope = 3.2107 b = 1.5357

Fig. (**30**) Variation of Absorbance with concentration for ***Gent. -T.P.S./A*** at ($\lambda_{max.}$ = **280 ± 5 nm**).



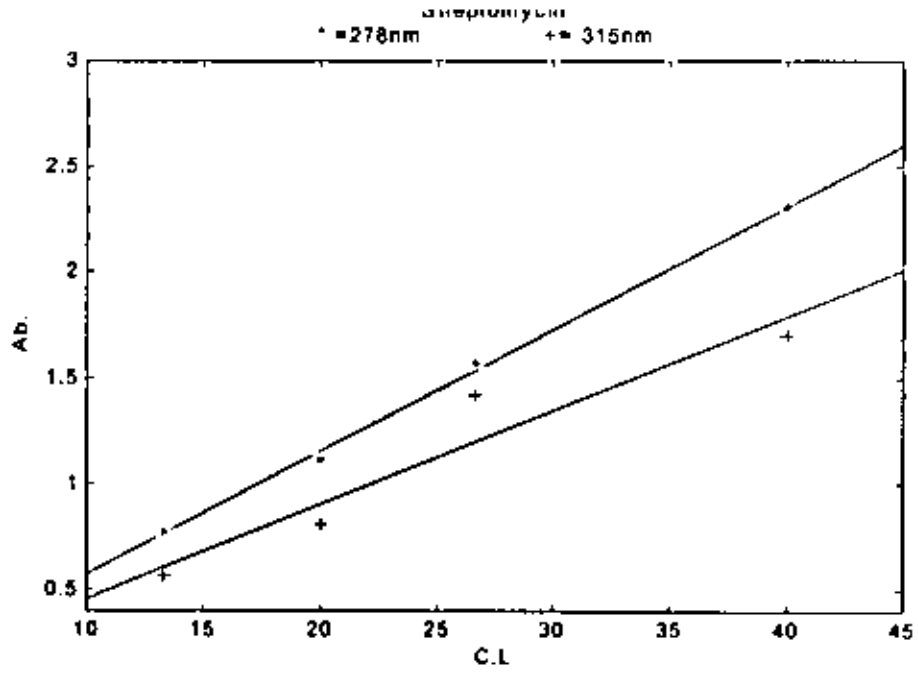
Slope = 2.0268 b = 1.7888

Fig. (**31**) Variation of Absorbance with concentration for ***Gent. -T.P.S./B*** at ($\lambda_{max.}$ **= 279 ± 3 nm**).



Slope = 3.7957 b = 0.66953

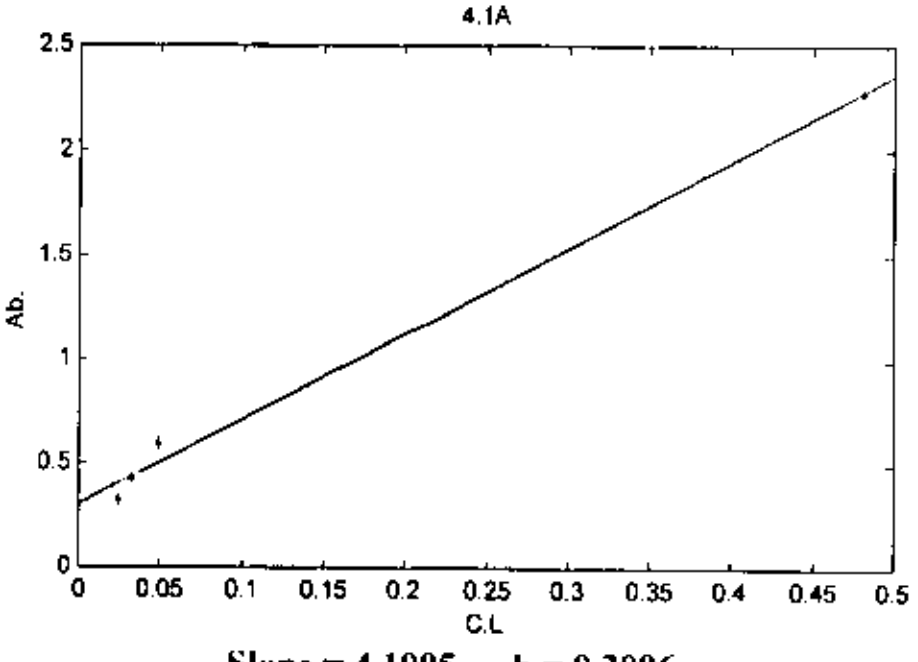
Fig. (**32**) Variation of Absorbance with concentration for ***Gent. -Ph.A /B*** at ($\lambda_{max.}$ **= 283 ± 1 nm**).



*** Slope = 0.0578 b = - 0.0015**

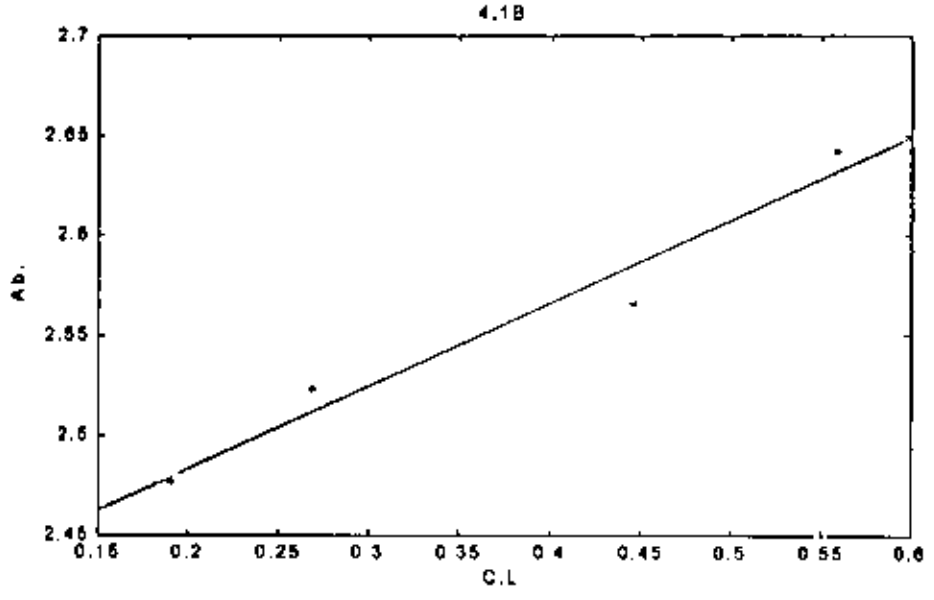
+ Slope = 0.0443 b = 0.0176

Fig. (33) Variation of Absorbance with concentration for ***Streptomycin*** **(λ_1 = 278 nm) and (λ_2 = 315 nm).**



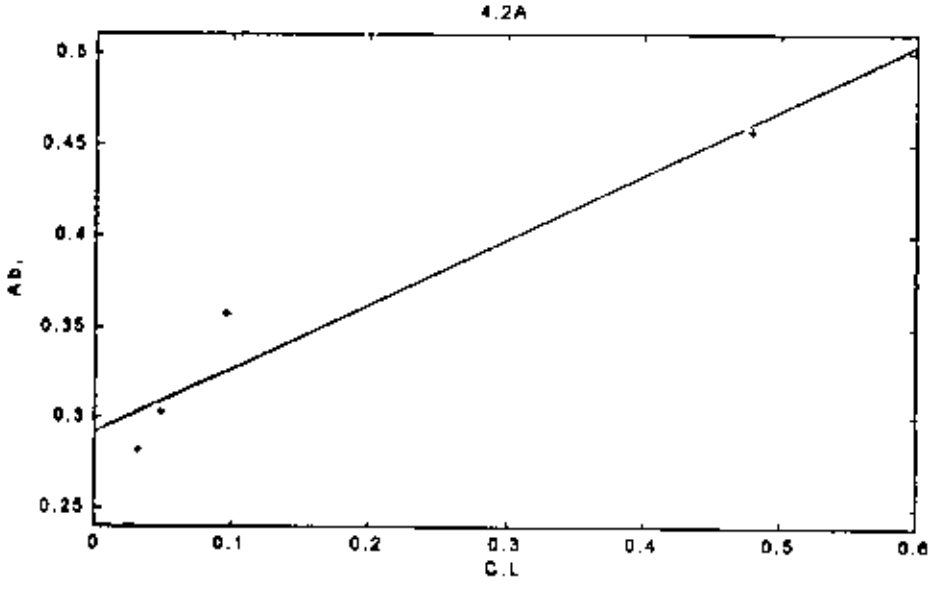
Slope = 4.1005 b = 0.3096

Fig. (34) Variation of Absorbance with concentration for ***Strept. -BZ. /A*** **at ($\lambda_{max.}$ = 277 ± 5 nm).**



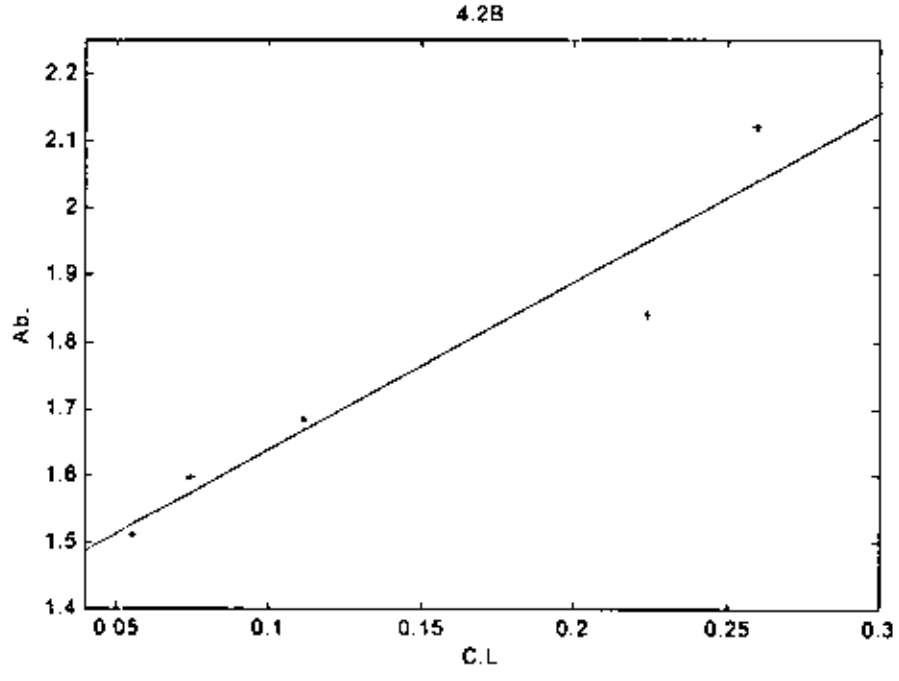
Slope = 0.4137 **b = 2.4009**

Fig. (**35**) Variation of Absorbance with concentration for ***Strept. -BZ. /B*** at ($\lambda_{max.}$ **= 296 ± 3 nm**).



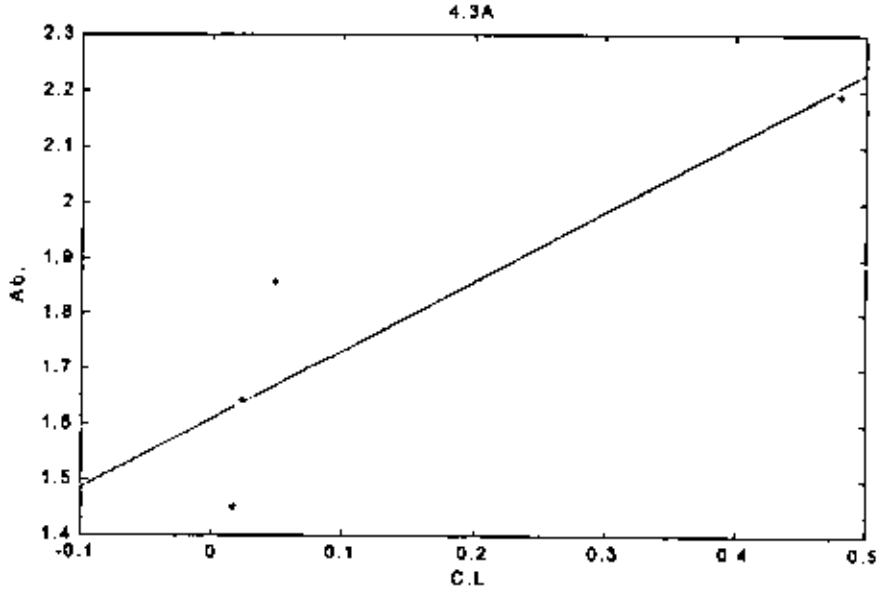
Slope = 0.3514 **b = 0.2926**

Fig. (**36**) Variation of Absorbance with concentration for ***Strept. -BZ.S. /A*** at ($\lambda_{max.}$ **= 270 nm**).



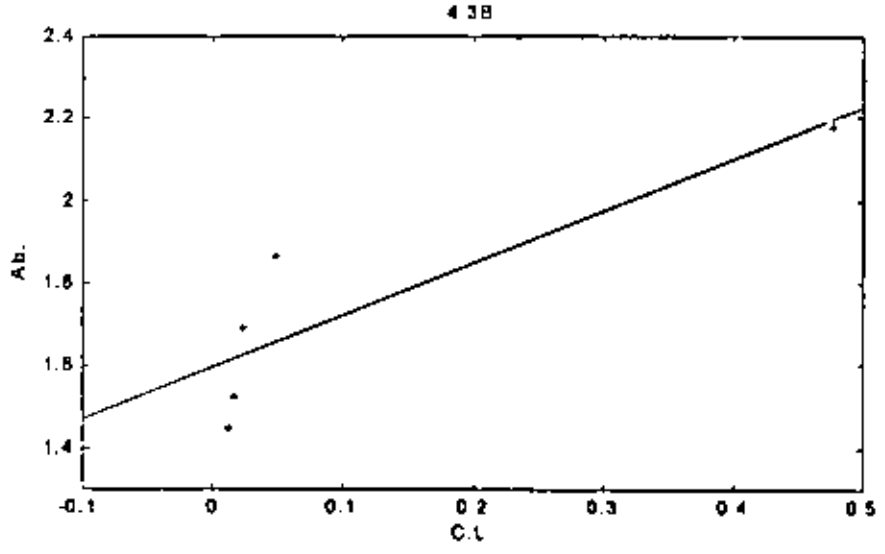
Slope = 2.5034 b = 1.3881

Fig. (37) Variation of Absorbance with concentration for ***Strept. -BZ.S. /B*** at ($\lambda_{max.}$ = 275 ± 1 nm).



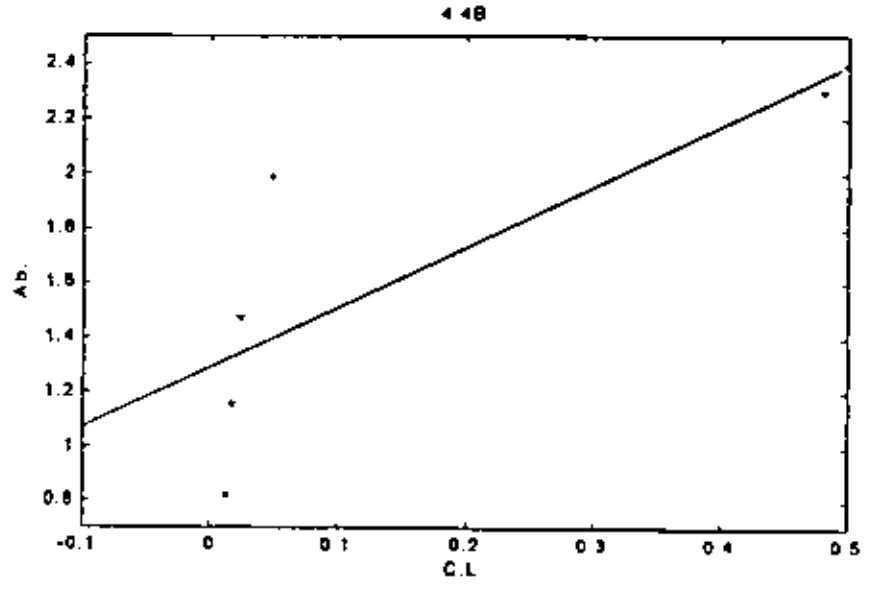
Slope = 1.2376 b = 1.6120

Fig. (38) Variation of Absorbance with concentration for ***Strept. -T.P.S./A*** at ($\lambda_{max.}$ = 277 ± 3 nm).



Slope = 1.256 b = 1.5989

Fig. (**39**) Variation of Absorbance with concentration for ***Strept. -T.P.S./B*** at ($\lambda_{max.}$ = **277 ± 3 nm**).



Slope = 2.1965 b = 1.2948

Fig. (**40**) Variation of Absorbance with concentration for ***Strept.-Ph.A/B*** at ($\lambda_{max.}$ = **277 ± 3 nm**)

3.2.2. Antibiotic – derivatives preparation and its uv - absorption discussion.

3.2.2.1. General.

The most antibiotics does not show uv-absorption above 215 nm so we need to introduce suitable chromophore (reagents) to them to yield products in order to get uv-absorption to simplify separation and quantitation[6, 35].

Electronic band shift depends on solvent polarity, pH and the relative orientation of neighboring chromophores[54]. 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 chromophores 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[55]. The uv-technique and their high sensitivity, accuracy, reliability and precision make uv- absorption analyzers suited to many process stream analysis problem[52].

In solution many molecules form diemer or higher polymers as the concentration increase. Another fraction at high concentration is aggregation this lead to increase or decrease wavelength of uv-absorption due to electronic interaction[54].

The derivatization under takes of the two types catalytic condition, sodium hydroxide and / or pyridine. Amine react so rapidly in pyridine, usually complete after refluxing for 10-15 minutes[15].

The yield for benzylation of hydroxy group by pyridine is the higher than the yield by sodium hydroxide [58].

3.2.2.2. uv-absorption of antibiotic-derivatives discussion.

Table (1) shows the antibiotic – derivatives also the reaction conditions which are used for derivatiztion as it found in the literature [15]. The physical properties recorded for the first time in this study.

The antibiotic – ph.A derivative shows the melting point (164 –165 C°) and white – powder in color and shape. This indicates that the derivative is proceed in the same way. The melting point for Kan-BZ., Gent.BZ. and Neo-BZ. in pyridine medium show 105 C°, 75 C° and 105 C° respectively.

Also the melting point of the derivative, Kan.-BZ.S., Gent.-BZ.S. and Strept.-BZ.S. in NaOH medium are 150 C°, 165 C° and 190 C° respectively, and gives different color and shape solid product. The Kan.-T.P.S., Gent.T.P.S., Neo.-T.P.S. and Strept.-P.T.S. derivatives in NaOH medium having 58 C°, 95 C°, 135 C°,and 95 C° respectively.

In general alcohol functional groups when it react with the reagents in used give low melting ester derivative[59]. Some of these antibiotic – derivatives give oily products. As well some of them gives liquid crystals phase properties[45].

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 , tables **(2 – 28)** and the curves represent those are in Fig. **(14 – 40)**.

The possible number of substituted reagents were calculated in two ways (n_ε and n_c) summarized in table **(29)** .

The comparism between these values (n_ε and n_c) for each antibiotic – derivatives show relatively no much different except for Strept.-derivatives, Neo.-T.P.S. (n_c =8.43 and n_ε = 2.13) , Gent.-BZ. (n_c = 6.8 and n_ε = 1.53) and and Gent.-BZ.S./B (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 [59] . 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 **(30)** which is similar to subunit of antibiotics under investigation .

The G-BZ. Derivative is give, n_c = 5.56 which nearly equal to experimental result penta – benzoate .

G – ph.A derivatives according OH – groups determination method [19] give the value (5.052), occupation method[50] the value is (4.04) while uv-data calculation gives (n_c = 3.7) and (n_ε = 5.23) .

The uv- spectrum for reagents Fig.**(1 - 4)**, Fig.**(5)** represent the uv-spectrum of streptomycin and Fig.**(6)** is for strept.BZ.The similarity between uv-spectrum Fig.**(1, 5 ,6)** specially those peaks at λ_{max} = 284, 277 and 273 nm respectively,

indicate that the uv-spectrum Fig **(6)** is look like the hyperidized spectrum of that in Fig.**(1)** and Fig. **(5)** . The reagent chromophore absorbance are at λ_{max} (287±7) for Ph.A, (280 ± 4) (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} (276 ± 3 nm) for Kan.-BZ., $\lambda_{max.}$ (276 ± 5) for Kan.-BZ.S. and λ_{max} (280 ± 4) for Kan.-T.P.S.. The reagent that gives the higher absorpitivity (ε) is the best for the detection limits for example phthalic acid anhydride reagent in methanol $\lambda_{max.}$274 (ε =1290). While in benzoylchloride reagent at $\lambda_{max.}$271 nm (ε =1000).

The effect of solvent clearly appear in uv-spectrum Fig.**(9)** Kan. -BZ-S. in $CHCl_3$, λ_{max} = 274 nm (a) and Fig. **(9)** Kan.-BZ.S. in 5 % NaOH, λ_{max} = 275 nm (b) .

While uv-spectrum Fig. **(12)** show solvent effect as well as a antibiotic - derivatives solubility effect according to the 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 wave length from λ_{max} = 272 nm (a) to higher wave length , λ_{max} = 293 nm (b) which clearly appear the pH of the solvent affecting the absorption of a chromophore[54] .

3.3.1. Conductivity measurement results.

Tables (**31– 34**) and Fig.s (**41 – 44**) show dissociation constant (K_a) at several concentration for reagents (BZCl, BZ.S.Cl, T.P.S.Cl, and PhA) while the tables (**35 – 41, 43 – 46**) and Fig.s (**45 –51**) gives the antibiotic-derivatives results.

Table (**42**) and Fig. (**52**) represent the dissociation constant (K_a) at several concentration for streptomycin . Tables (**48 – 56**) and Fig.s (**85 – 66**) used to determine the rate constant (k) of antibiotic- derivatives . And the table (**57**) and Fig. (**67**) explain the relation between logarithm of rate constant (log k) and dissociation constant (Pk_a) of antibiotic- derivatives.

Fig (**67A**) for (Kan.-BZ., Neo.-BZ. and Gent.-BZ.), Fig. (**67B**) for (Gent.-BZ. and Gent.-BZ.S.), and Fig.(**67C**) for (Strept.-T.P.S. and Strept.-BZ.S.) .

Table (31) 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	98.50000	0.001015	9.850
0.065	6.97	107.2310	0.009330	6.970
0.048	5.68	118.3330	0.008450	5.679
0.038	4.72	124.2110	0.008051	4.720
0.032	4.27	133.4375	0.007490	4.270
Cal.	**Λ_{∞} = 176.923**		**K_a = 0.0661**	

Table (32) 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_a = 0.2423**	

Table (33) 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.	**$\Lambda_{(o)}$= 109.61**		**K_a = 0.0643**	

Table (34) Dissociation constant of ***Ph.A*** 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
0.100	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

Table (**35**) Dissociation constant of ***Kan.BZ. /A*** 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	0.0028180	11.0
0.002000	7.90	3950.000	0.0002532	7.90
0.001490	6.10	4093.960	0.0002443	6.10
0.001185	5.30	4472.574	0.0002236	5.30
0.000980	4.50	4577.820	0.0002184	4.50
Cal.	**$\Lambda_{(z)}$ = 357.73**		**K_a = 0.0154**	

Table (**36**) Dissociation constant of ***Kan.BZ.S. /B*** 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
0.039	58.0	1487.179	0.0006724	58.0
0.025	42.0	1680.000	0.0005950	42.0
0.019	32.0	1684.200	0.0009400	32.0
0.015	25.4	1693.400	0.0005910	52.4
0.012	22.4	1866.700	0.0005360	22.4
Cal.	**$\Lambda_{(s)}$ = 2076.85**		**K_a = 0.063**	

Table (**37**) Dissociation constant of ***Neo.BZ. /A*** 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
0.02600	5.4	207.692	0.00481	5.4
0.01700	3.9	229.410	0.00436	3.9
0.01200	3.1	258.340	0.00387	3.1
0.00980	2.9	295.920	0.00338	2.9
0.00813	2.6	325.000	0.00308	2.6
Cal.	**Λ_∞ = 652.13**		**K_a = 0.00353**	

Table (**38**) Dissociation constant of ***Gent.BZ. /A*** 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
0.02880	7.5	260.417	0.00384	7.5
0.01872	5.7	304.487	0.00328	5.7
0.01390	4.6	330.935	0.00302	4.6
0.01100	4.2	381.818	0.00262	4.2
0.00900	3.6	400.000	0.00250	3.6
Cal.	**Λ_∞ = 1222.67**		**K_a = 0.0015**	

Table (**39**) Dissociation constant of ***Gent.BZ.S. /B*** 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
0.025	8.5	340.00	0.002940	8.5
0.019	7.1	373.68	0.002676	7.1
0.015	6.2	314.34	0.002450	6.2
0.013	5.8	446.15	0.002240	5.8
0.011	5.4	490.91	0.002040	5.4
Cal.	**Λ_{∞} = 1601**		**K_a = 0.0014**	

Table (**40**) Dissociation constant of ***Gent.T.P.S. /A*** 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	64.0	927.54	0.001100	64.0
0.045	49.0	1088.9	0.000920	49.0
0.033	37.5	136.40	0.000880	37.5
0.027	29.3	1085.2	0.000920	29.3
0.022	25.2	1145.5	0.000873	25.2
Cal.	**Λ_{∞} = 1456.7**		**K_a = 0.06**	

Table (**41**) Dissociation constant of ***Gent.Ph.A /B*** 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**
0.045	5.5	122.22	0.00810	5.5
0.030	4.0	133.33	0.00750	4.0
0.022	3.3	150.00	0.00667	3.3
0.018	2.9	161.11	0.00621	2.9
0.015	2.6	173.33	0.00577	2.6
Cal.	**Λ_{∞} = 273.67**		**K_a = 0.0153**	

Table (**42**) 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**
0.0100	2.89	289.00	0.003460	2.89
0.0087	2.48	285.10	0.003508	2.48
0.0070	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 (**43**) Dissociation constant of ***Strept.BZ. /A*** 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
0.018	2.4	133.33	0.00750	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	2.0	250.00	0.00400	2.0
Cal.	**Λ_∞ = 66.49**		**K_a = 0.024**	

Table (**44**) Dissociation constant of ***Strept.BZ.S. /A*** at several concentration from conductivity measurements (**Solvent MeOH**).

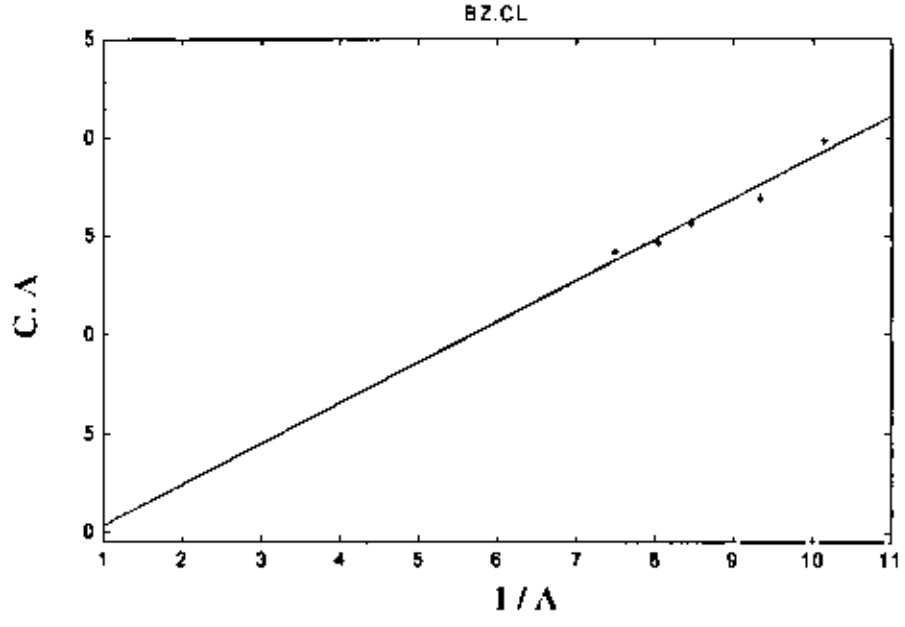
C (g. L^{-1})	k ($\mu s.cm^{-1}$)	Λ ($\mu S.cm^2.mol^{-1}$)	1 / Λ	C. Λ
0.026	9.2	353.85	0.00283	9.2
0.020	7.9	395.00	0.00253	7.9
0.016	6.9	431.25	0.00232	6.9
0.013	6.2	476.92	0.00210	6.2
0.011	5.7	518.18	0.00190	5.7
Cal.	**Λ_∞ = 2125.056**		**K_a = 0.000485**	

Table (**45**) Dissociation constant of ***Strept.T.P.S. /A*** 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**
0.021	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	488.890	0.00205	4.4
Cal.	**Λ_∞ = 556.1**		**K_a = 0.0065**	

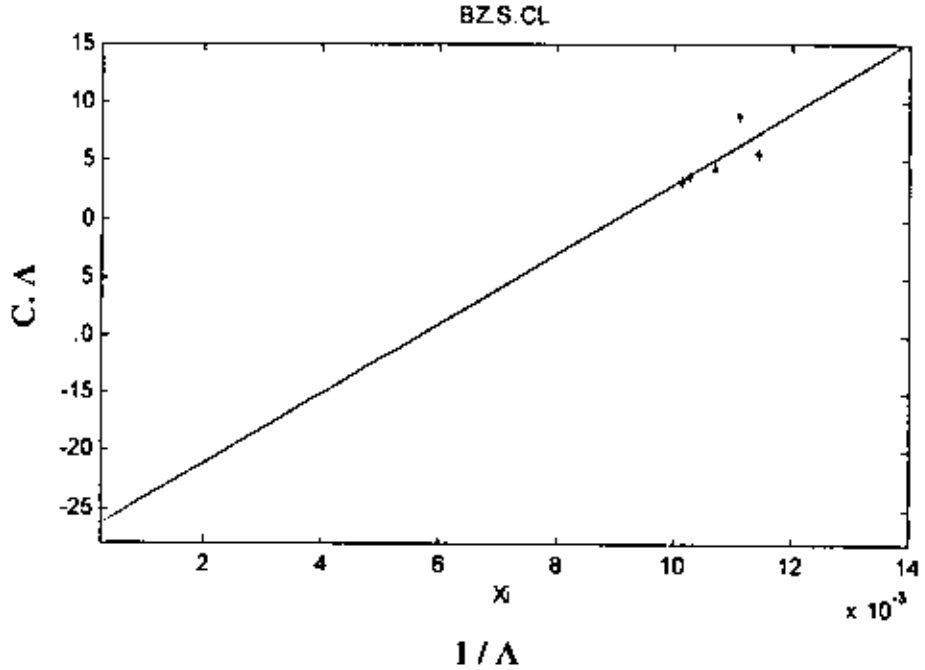
Table (**46**) Dissociation constant of ***Strept.Ph.A /B*** at several concentration from conductivity measurements (**Solvent MeOH**).

C (g. L^{-1})	**k ($\mu s.cm^{-1}$)**	**Λ ($\mu S.cm^2.mol^{-1}$)**	**1 / Λ**	**C. Λ**
0.029	2.8	96.550	0.01036	2.8
0.023	2.6	113.04	0.00885	2.6
0.018	2.5	138.89	0.00720	2.5
0.015	2.4	160.00	0.00625	2.4
0.012	2.4	200.00	0.00500	2.4
Cal.	**Λ_∞ = 38.42**		**K_a = 0.051**	



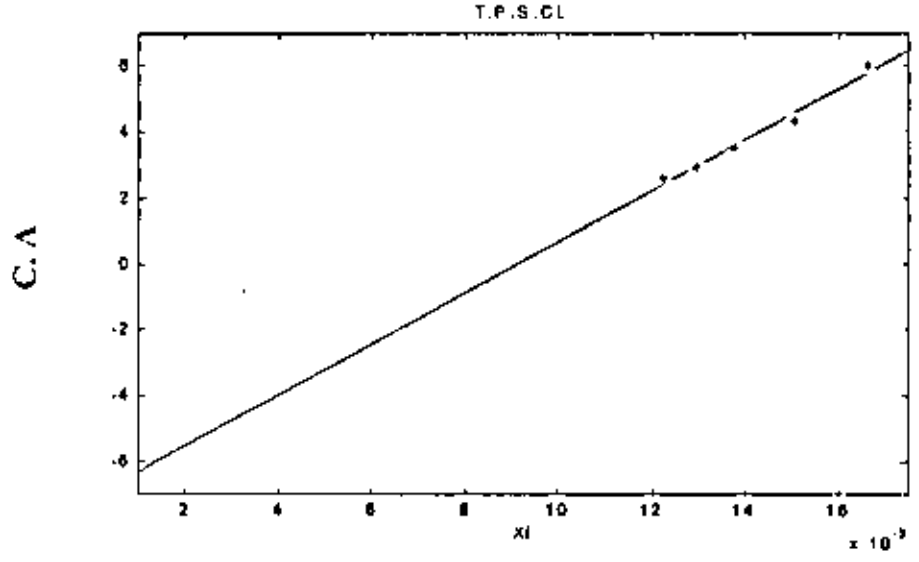
Slope = 2070.0 **b = - 11.70**

Fig. (**41**) determination of dissociation constant (K_a) from plotting (**1/Λ**) against (**C. Λ**) for ***BZ.CL.***



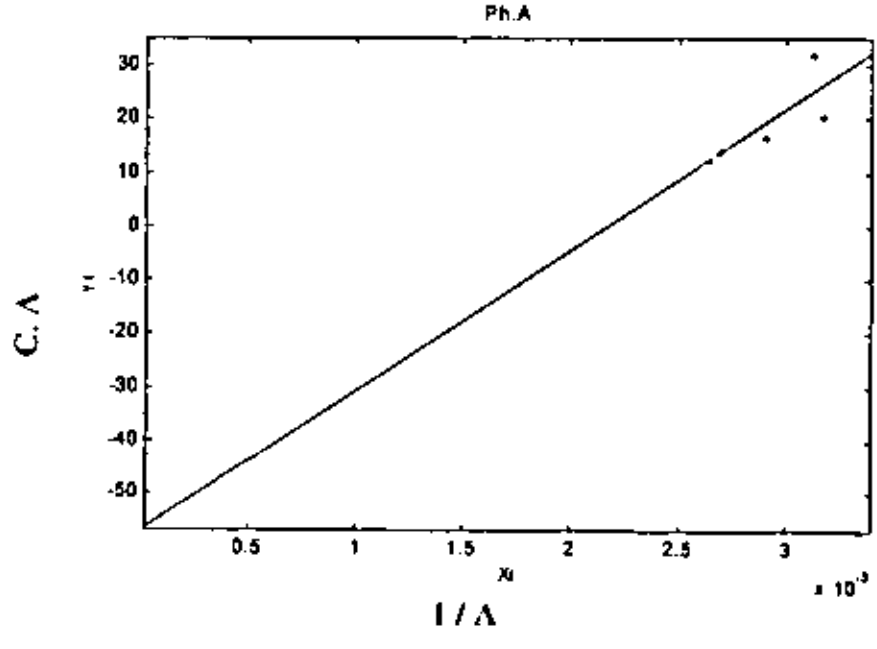
Slope = 3008.9 **b = - 27.0**

Fig. (**42**) determination of dissociation constant (K_a) from plotting (**1/Λ**) against (**C. Λ**) for ***BZ.S.CL.***



Slope = 772.778 b = -7.0504

Fig. (**43**) determination of dissociation constant (K_a) from plotting (**1/Λ**) against (**C. Λ**) for ***T.P.S.CL.***



Slope = 26295.0 b = -57.0

Fig. (**44**) determination of dissociation constant (K_a) from plotting (**1 / Λ**) against (**C. Λ**) for ***Ph.A.***

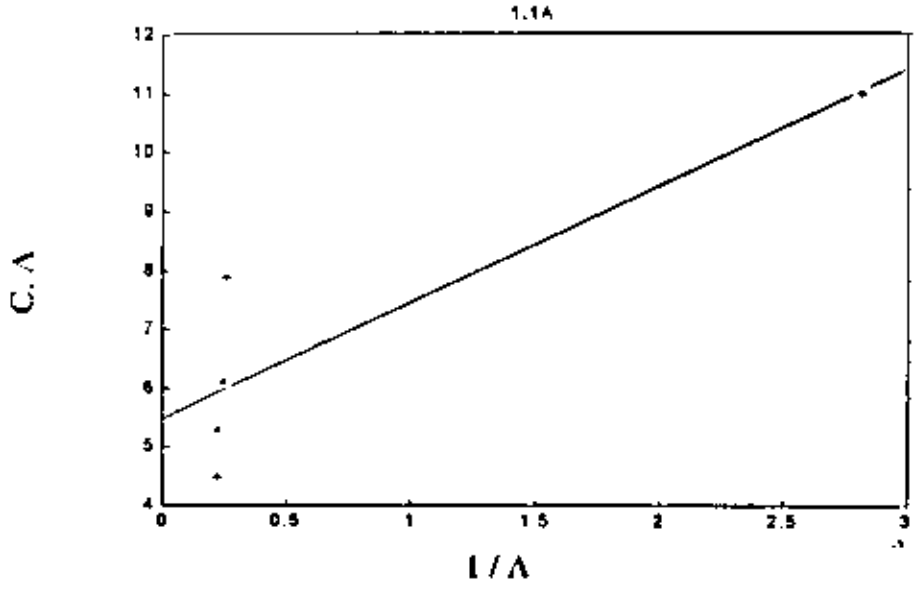


Fig. (**45**) determination of dissociation constant ($\mathbf{K_a}$) for ***Kan.BZ. /A*** from plotting (**1 / Λ**) against (**C. Λ**).

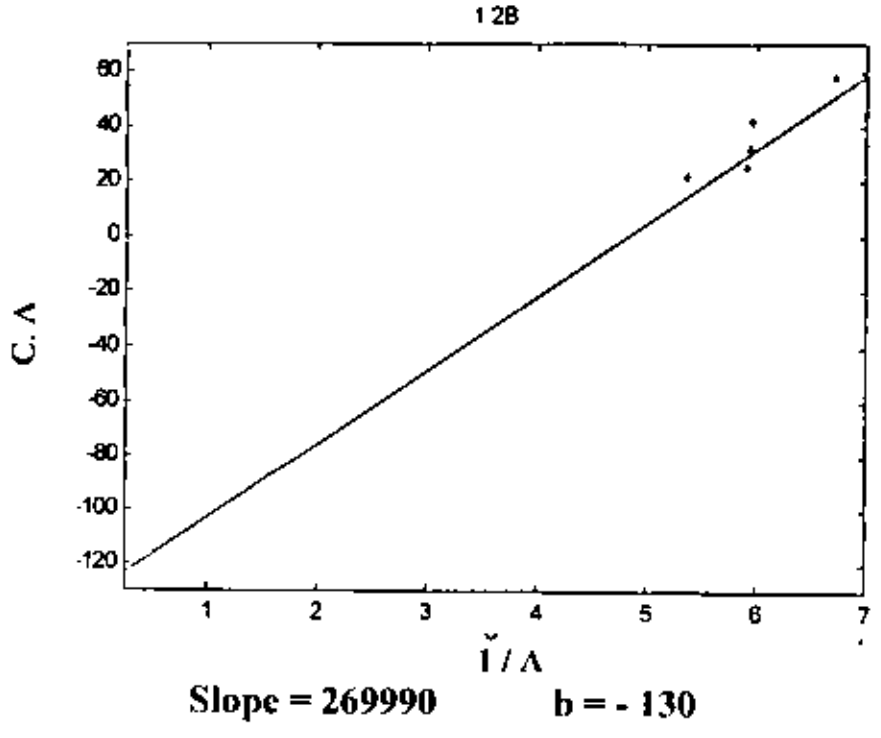
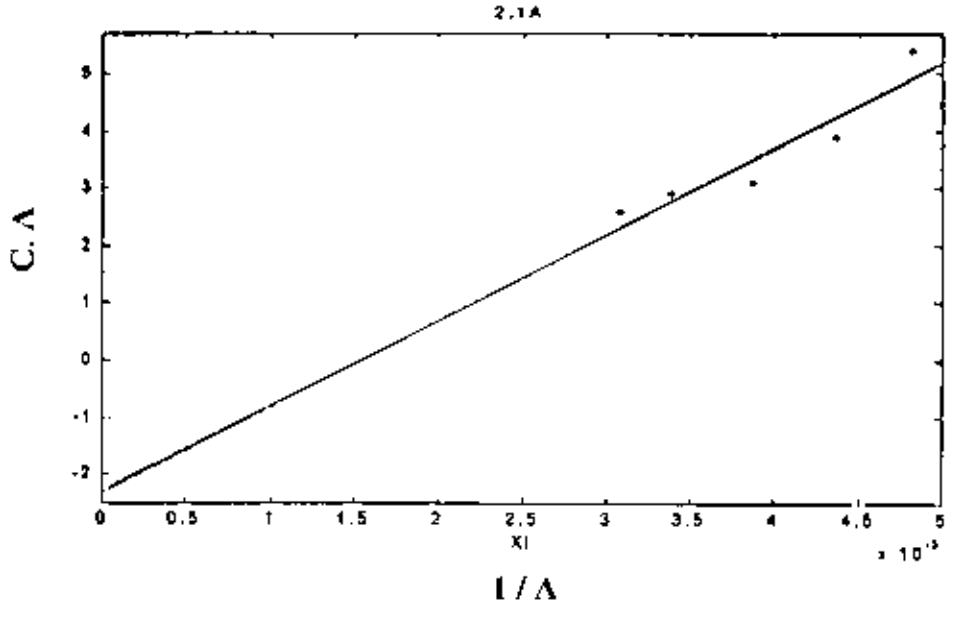
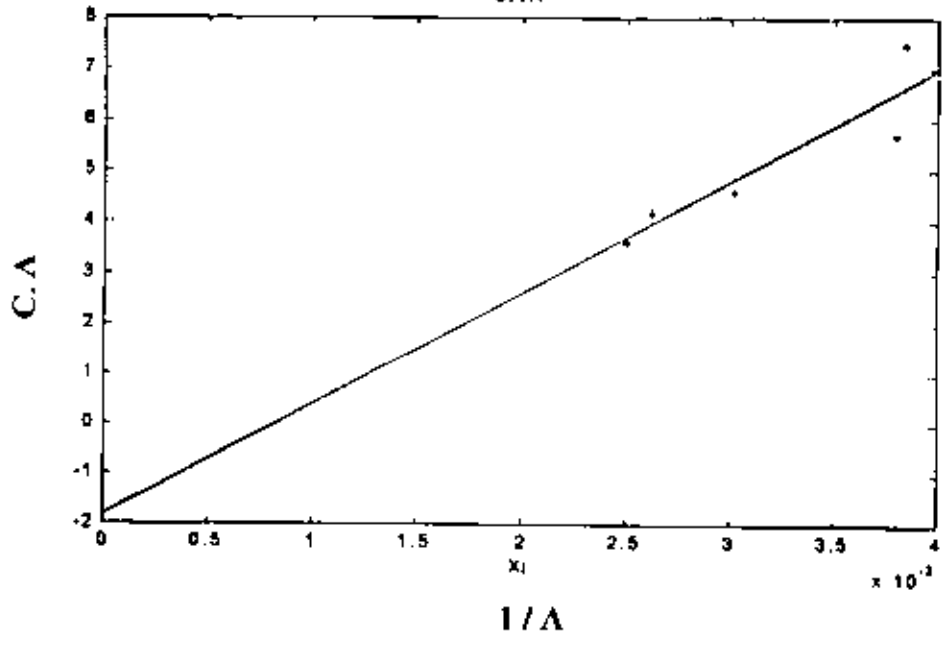


Fig. (**46**) determination of dissociation constant ($\mathbf{K_a}$) for ***Kan.BZ.S. /B*** from plotting (**1 / Λ**) against (**C. Λ**).



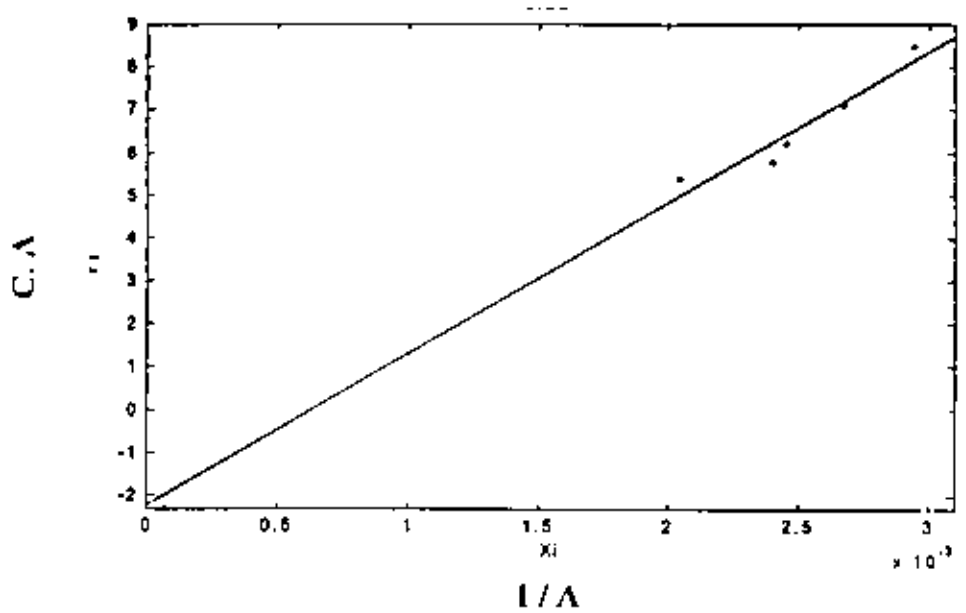
Slope = 1499.9 **b = -2.3**

Fig. (**47**) determination of dissociation constant (K_a) for ***Neo.BZ. /A*** from plotting (**1 / Λ**) against (**C. Λ**).



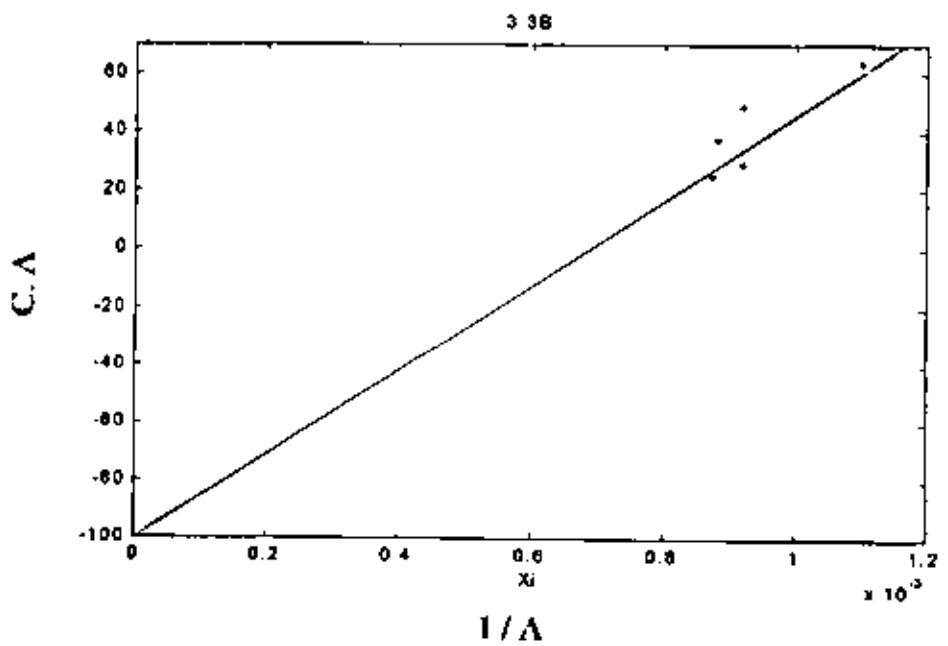
Slope = 2200.8 **b = - 1.8**

Fig. (**48**) determination of dissociation constant (K_a) for ***Gent.BZ. /A*** from plotting (**1 / Λ**) against (**C. Λ**).



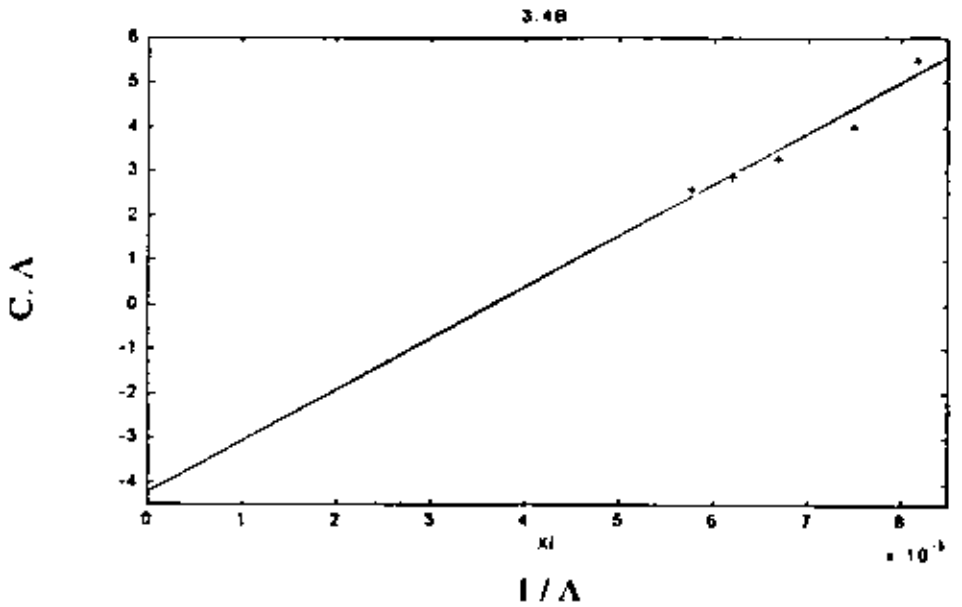
Slope = 3522.2 **b = - 2.2**

Fig. (**49**) determination of dissociation constant (K_a) for ***Gent.BZ.S. /B*** from plotting (**1 / Λ**) against (**C. Λ**).



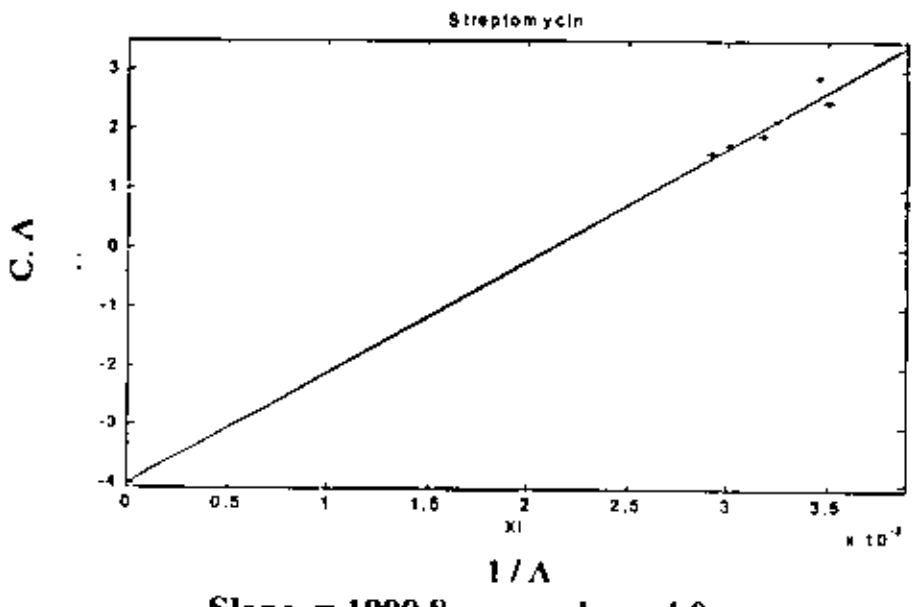
Slope = 145670 **b = - 100**

Fig. (**50**) determination of dissociation constant (K_a) for ***Gent.T.P.S. /B*** from plotting (**1 / Λ**) against (**C. Λ**).



Slope = 1149.4 b = - 4.2

Fig. (**51**) determination of dissociation constant (K_a) for ***Gent.Ph.A /B*** from plotting (**1 / Λ**) against (**C. Λ**).



Slope = 1900.8 b = - 4.0

Fig. (**52**) determination of dissociation constant (K_a) for ***Streptomycin*** from plotting (**1 / Λ**) against (**C. Λ**).

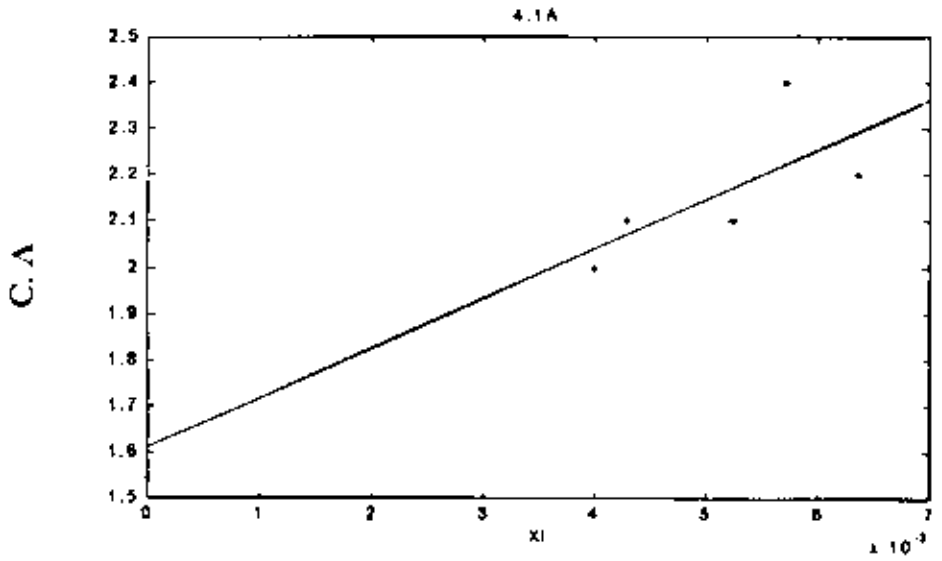


Fig. (**53**) determination of dissociation constant (K_a) for ***Strept.Ph.A /B*** from plotting (**1 / A**) against (**C. A**).

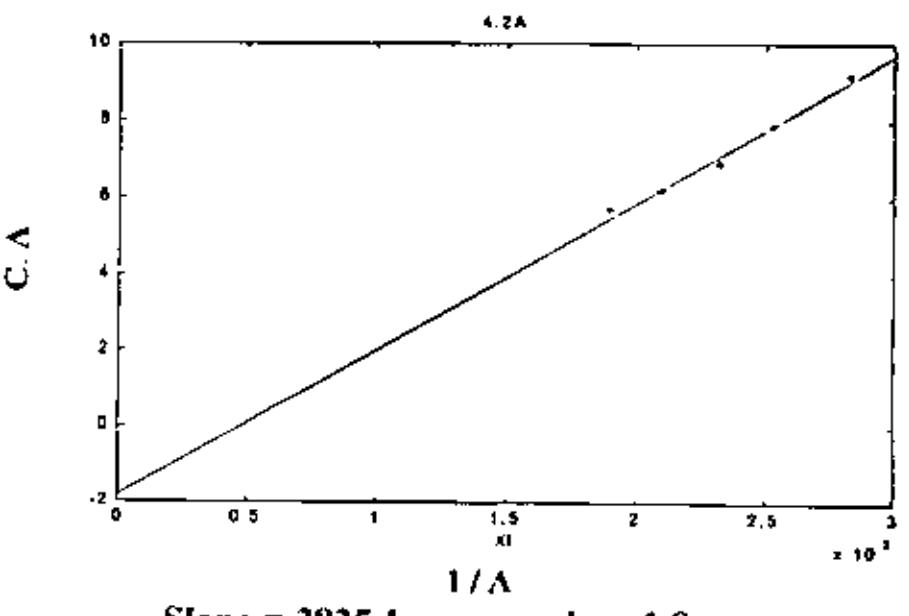
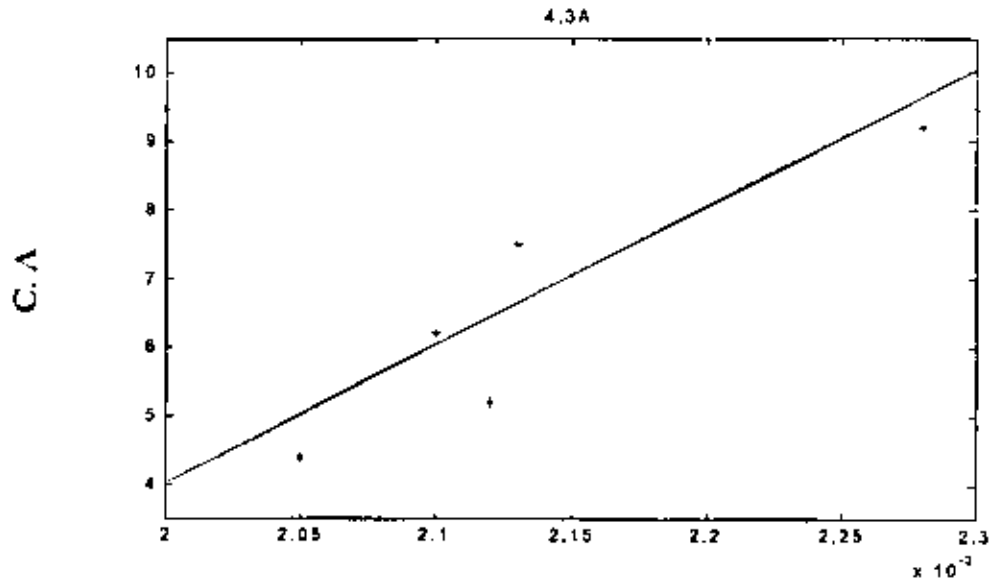
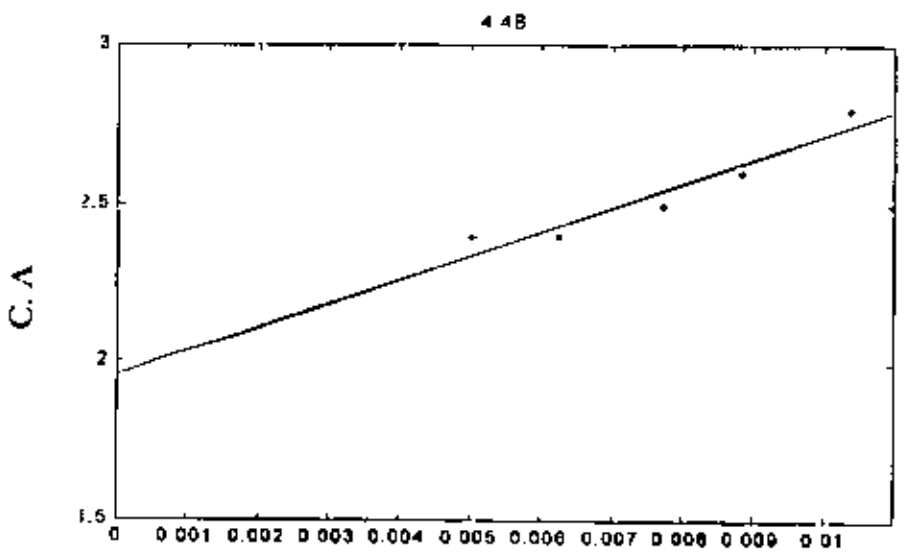


Fig. (**54**) determination of dissociation constant (K_a) for ***Strept.BZ.S. /A*** from plotting (**1 / A**) against (**C. A**).



Slope = 20020 **b = - 36.0**

Fig. (**55**) determination of dissociation constant ($\mathbf{K_a}$) for ***Strept.T.P.S. /A*** from plotting (**1 / Λ**) against (**C. Λ**).



Slope = 75.45 **b = 1.9639**

Fig. (**56**) determination of dissociation constant ($\mathbf{K_a}$) for ***Strept.T.P.S. /B*** from plotting (**1 / Λ**) against (**C. Λ**).

3.3.2. Conductivity measurements discussion.

3.3.2.1. Dissociation constant (k_a).

The conductivity measurements to calculate dissociation constant (K_a) through dilution for reagents as in tables **(31-34)** and for antibiotic-derivatives, tables**(34-46)**. The dissociation constant (K_a) for reagents follow the order T.P.S.Cl (0.0643) < BZ.Cl (0.0661) < Ph.A (0.124) < BZ.S.Cl(0.2423) .

The comparism between K_a-values for Kan.-BZ./A (0.0154) and Kan.-BZ.S./B (0.063) this give indication that either the substituted number of reagent in both cases are differ or the position of substituted reagent on the Kanamycin not the same . While in case of Gent.-BZ./A (0.0015) and the Gent.-BZ.S./B (0.0014) the situation seem to be not much differ this leads to thought that the type of antibiotics (Kan.,Gent.) may be the predominate effecting factor controlling derivatization process to give antibiotic-derivative product. In the Figs **(41 – 56)** the molar conductivity at infinite dilution, Λ_{∞} , is differ for antibiotic – derivatives which leads to give various values of dissociation degree . From table **(39)** the value of Λ_{∞}, for Gent.-BZ.S. is (1601 $\mu S.cm^2.mol^{-1}$) which give dissociation degree (α) is equal to (0.212) while Λ_{∞} for Gent.-T.P.S. from table **(40)** is equal to (1456.7 $\mu S.cm^2.mol^{-1}$) and the value of (α) is (0.786) , at concentration nearly equal for both .

Table (**47**) kinetic study of *D -glucose-phtalic 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

Table (**48**) determination of rate constant ***Kan.BZ. /B*** by conductivity measurement :-

t (min).	$(C_0 - C_t)/t$	C_t (μs.cm^{-1})	k (μs.cm^{-1}. min^{-1})
0	∞	69.3	-
2	22.95	23.4	706.15
4	11.45	23.5	327.14
6	7.670	23.3	255.56
8	5.673	23.2	209.55
10	4.610	23.2	167.64
15	3.080	23.1	123.20
20	2.310	23.1	92.40
42	1.124	22.1	-
Cal. Rate constant (k) = 1159.42			**Average = 268.85**

Table (**49**) determination of rate constant ***Kan.T.P.S./B*** by conductivity measurement:-

t (min)	$(C_0 - C_t)/t$	C_t (μs.cm^{-1})	k (μs.cm^{-1}. min^{-1})
0	∞	69.3	-
2	9.050	51.2	172.381
4	4.575	51.0	96.320
6	3.000	51.3	54.850
8	2.263	51.2	43.100
13	1.400	51.1	28.000
18	1.020	51.0	21.400
23	0.804	50.8	18.930
28	0.668	50.6	17.810
55	0.367	49.1	-
Cal. Rate constant (k) = 376.648			**Average = 56.6**

Table (**50**) determination of rate constant ***Neo.BZ. /A*** by conductivity measurement: -

t (min)	$(C_0 - C_t)/t$	C_t (μs.cm^{-1})	k (μs.cm^{-1}. min^{-1})
0	∞	69.3	-
2	20.85	27.6	926.670
4	10.40	27.7	416.000
6	6.920	27.8	251.520
8	5.175	27.9	172.500
13	3.185	27.9	106.154
18	2.300	27.9	76.852
34	0.990	26.7	-
Cal. Rate constant (k) = 4301.08			**Average = 350.59**

Table (**51**) determination of rate constant ***Neo.Ph.A/B*** by conductivity measurement: -

t (min)	$(C_0 - C_t)/t$	C_t (µs.cm^{-1})	k (µs.cm^{-1}. min^{-1})
0	∞	69.3	-
2	20.400	28.5	408.00
4	10.175	28.6	193.81
6	6.8700	28.1	171.67
8	5.1750	27.9	147.86
13	3.1900	27.8	98.225
18	2.3100	27.7	77.040
23	1.8200	27.5	72.700
58	0.7380	26.5	-
Cal.	**Rate constant (k) = 531.208**		**Average = 167.043**

Table (**52**) determination of rate constant ***Gent.BZ. /B*** by conductivity measurement: -

t (min).	$(C_0 - C_t)/t$	C_t (µs.cm^{-1})	k (µs.cm^{-1}. min^{-1})
0	∞	69.3	-
2	22.450	24.4	598.67
4	11.275	24.2	346.92
6	7.5200	24.2	231.28
8	5.6500	24.1	188.33
10	4.5500	23.8	101.11
15	3.0400	23.7	152.20
20	2.2900	23.5	152.57
25	1.8360	23.4	146.88
51	0.9100	22.9	-
Cal.	**Rate constant (k) = 725.953**		**Average = 239.76**

Table (**53**) determination of rate constant ***Gent.BZ.S. /B*** by conductivity measurement: -

t (min).	$(C_o - C_t)/t$	C_t (µs.cm^{-1})	k (µs.cm^{-1}. min^{-1})
0	∞	69.3	-
2	17.75	34.0	141.20
4	8.875	33.8	73.960
6	5.867	34.1	46.010
8	4.400	34.1	34.510
14	2.550	33.6	22.174
19	1.920	32.9	19.650
24	1.525	32.7	16.490
49	0.822	29.0	-
Cal.	**Rate constant (k) = 269.91**		**Average = 50.57**

Table (**54**) determination of rate constant ***Strept.BZ.S. /A*** by conductivity measurement: -

t (min)	$(C_o - C_t)/t$	C_t (µs.cm^{-1})	k (µs.cm^{-1}. min^{-1})
0	∞	69.3	-
4	-6.40	94.9	54.074
6	-4.58	96.8	21.695
8	-3.55	97.7	13.382
13	-2.20	98.3	9.730
18	-1.63	98.6	5.870
23	-1.27	98.5	4.200
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 (**57**) the relation between ***rate constant(k)***, and ***dissociation constant (K_d)*** for antibiotic-derivative.

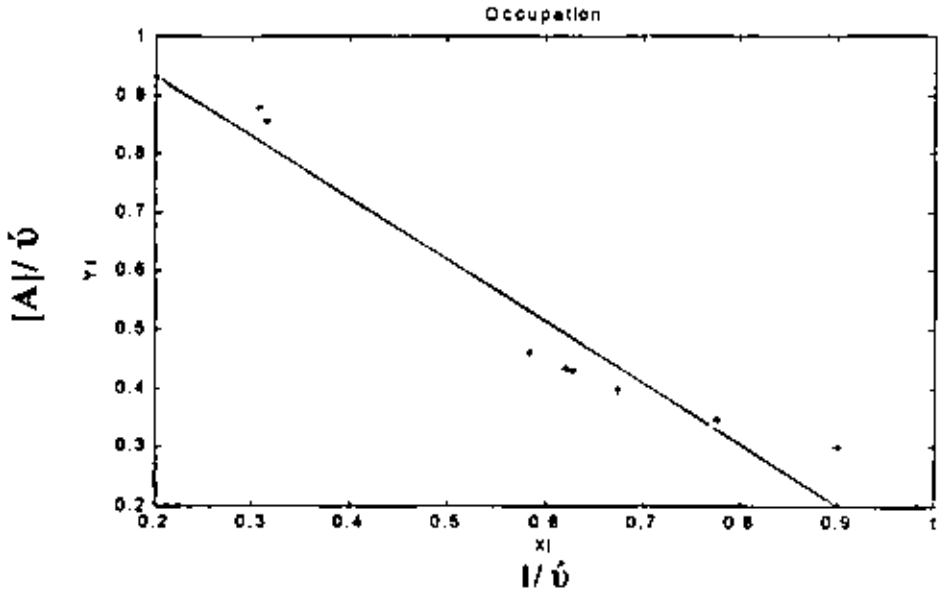
Set No.	Antibiotic-derivatives	Rate constant		Dissociation constant	
		$K_{aver.}$	Logk	K_d	pK_d
A	Neo.-BZ	350.59	2.500	0.003500	2.450
	Kan.-BZ	268.85	2.430	0.015000	1.820
	Gent.-BZ	239.76	2.380	0.001500	2.820
	Strept.-BZ	-	-	0.024000	1.620
B	Gent.-BZ	239.76	2.380	0.001500	2.800
	Gent.-BZ.S.	50.570	1.700	0.069000	2.850
C	Strept.-BZ	13.030	1.115	0.000485	3.314
	Strept.-T.P.S.	140.68	2.148	0.006500	2.187
	Strept.-ph.A	49.102	1.690	0.051000	1.292

Table (**55**) determination of rate constant ***Strept.T.P.S. /A*** by conductivity measurement: -

t (min).	$(C_o - C_t)/t$	C_t ($\mu s.cm^{-1}$)	k ($\mu s.cm^{-1}. min^{-1}$)
0	∞	69.3	-
2	17.90	33.5	397.780
4	8.975	33.4	211.176
6	5.950	33.6	125.263
8	4.463	33.6	93.9500
13	2.760	33.4	64.7960
18	2.000	33.3	50.0000
23	1.570	33.2	41.8600
50	0.752	31.7	-
Cal.	**Rate constant (k) = 863.93**		**Average = 140.689**

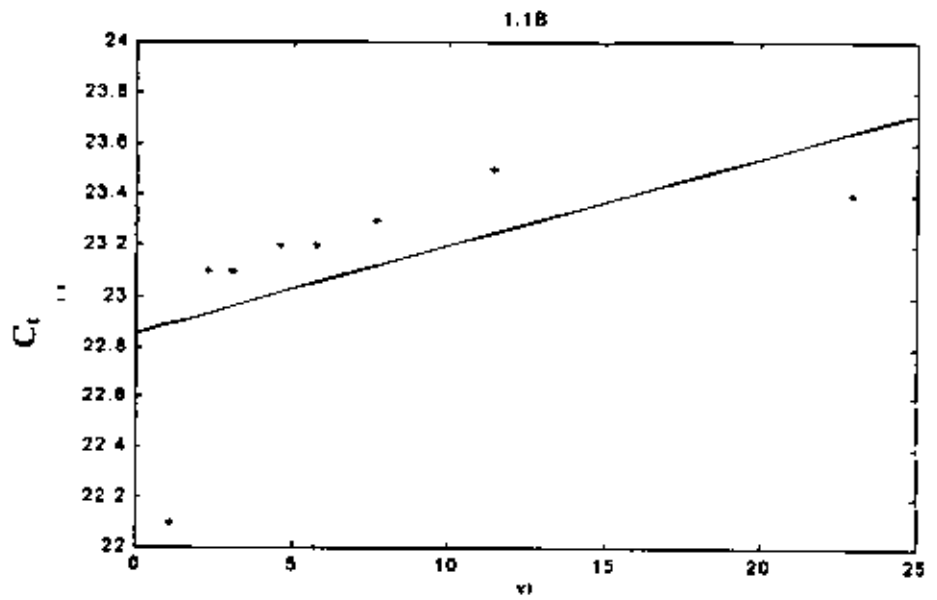
Table (**56**) determination of rate constant ***Strept.Ph.A/B*** by conductivity measurement : -

t (min).	$(C_o - C_t)/t$	C_t ($\mu s.cm^{-1}$)	k ($\mu s.cm^{-1}. min^{-1}$)
0	∞	69.3	-
2	9.700	49.9	125.16
4	4.830	50.0	60.313
6	3.220	50.0	40.210
8	2.440	49.8	32.500
13	1.500	49.7	20.796
18	1.090	49.6	15.635
27	0.834	46.8	-
Cal.	**Rate constant (k) = 255.9**		**Average = 49.102**



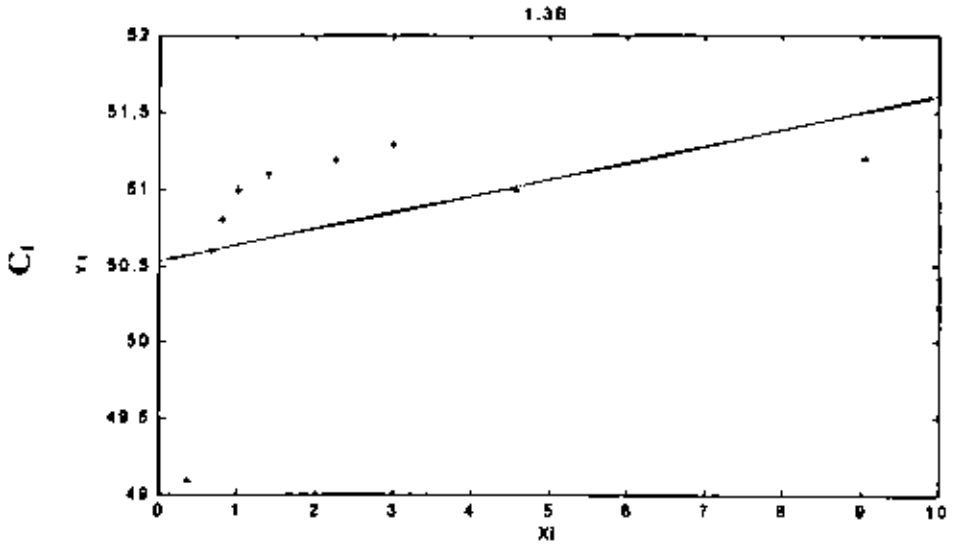
Slope = -1.0502 b = 1.1448

Fig. (**57**) The relation between (**1 / ύ**) and **|A|/ ύ.**



Slope = 0.0345 b = 22.858

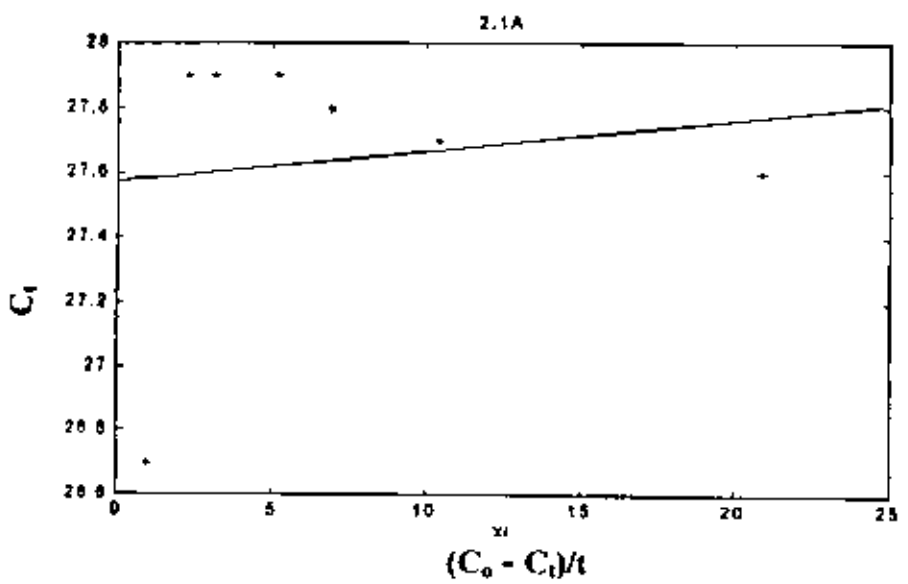
Fig. (**58**) Curve of (C_o - C_t) / t vs. C_t for ***Kan.BZ. /B.***



$(C_o - C_t)/t$

Slope = 0.1062 b = 50.5379

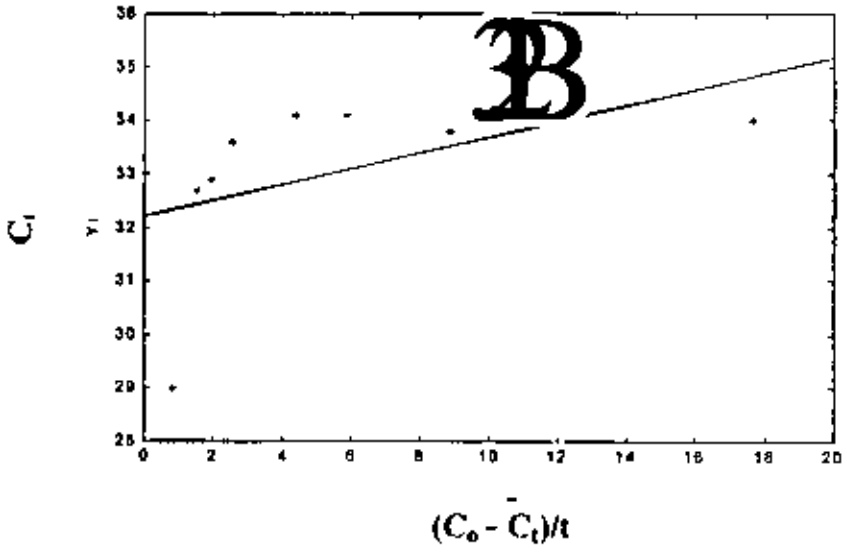
Fig. (**59**) Curve of $(C_o - C_t) / t$ vs. C_t for ***Kan.T.P.S. /B.***



$(C_o - C_t)/t$

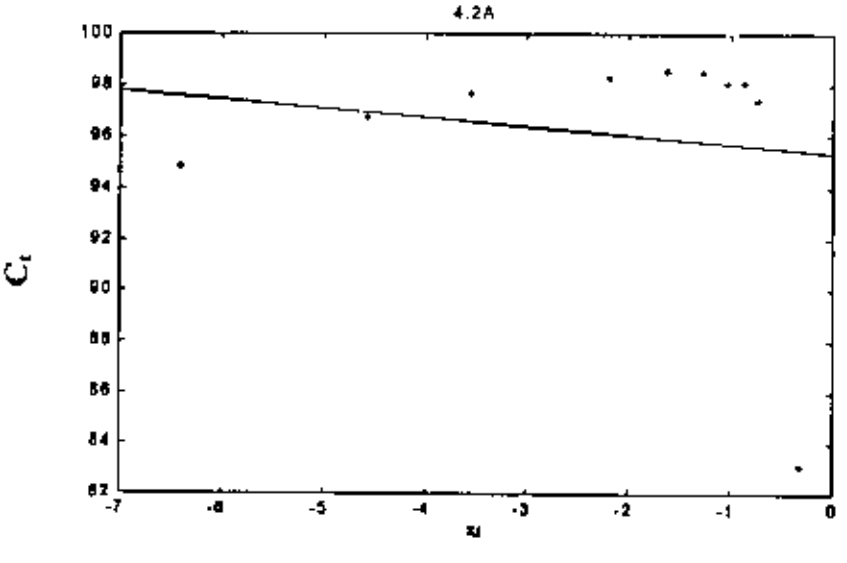
Slope = 0.0093 b = 27.5767

Fig. (**60**) Curve of $(C_o - C_t) / t$ vs. C_t for ***Neo.BZ. /A.***



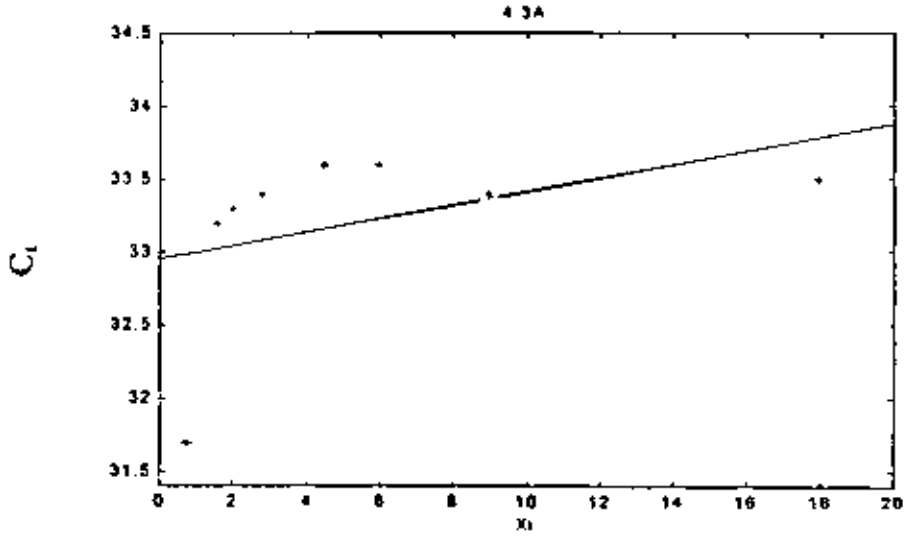
Slope = 0.1482 **b = 32.2169**

Fig (**63**) Curve of $(C_o - C_t) / t$ vs. C_t for ***Gent.BZ.S. /B.***



Slope = -0.3603 **b = 95.3361**

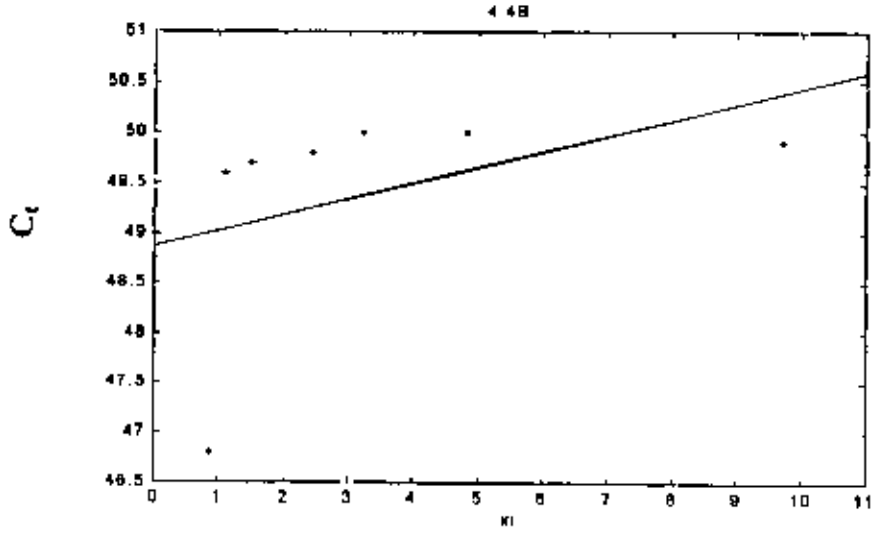
Fig. **(64)** Curve of $(C_o - C_t) / t$ vs. C_t for ***Strept.BZ.S. /A.***



$(C_o - C_t)/t$

Slope = 0.0463 **b = 32.956**

Fig. (**65**) Curve of $(C_o - C_t) / t$ vs. C_t for ***Strept.T.P.S. /A.***



$(C_o - C_t)/t$

Slope = 0.1563 **b = 48.8720**

Fig. (**66**) Curve of $(C_o - C_t) / t$ vs. C_t for ***Strept.Ph.A/B.***

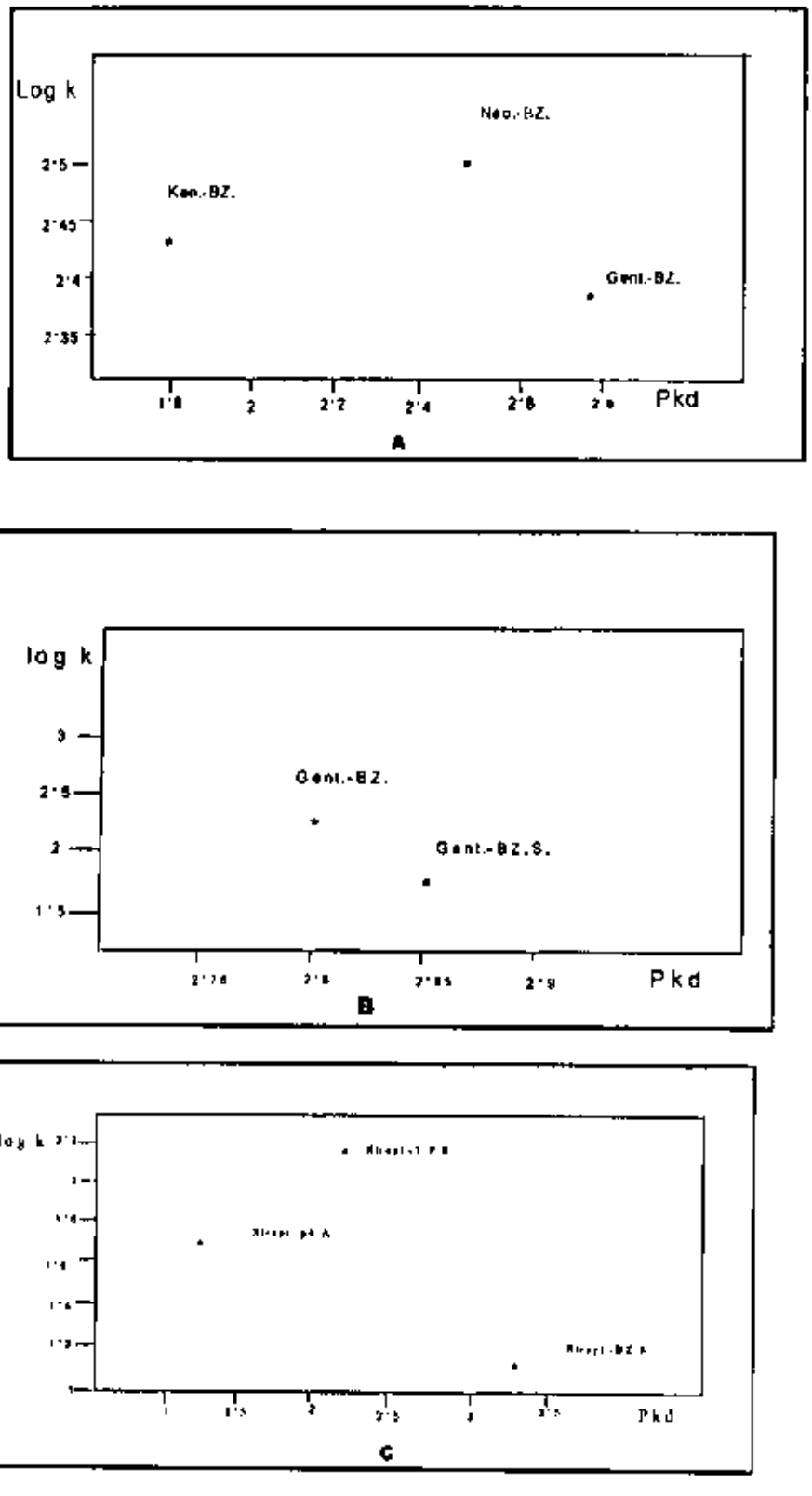
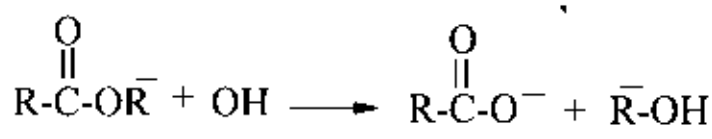


Fig. (**67**) ***A, B, C*** plots log k against pk_d.

3.3.2.2. Rate constant (k) .

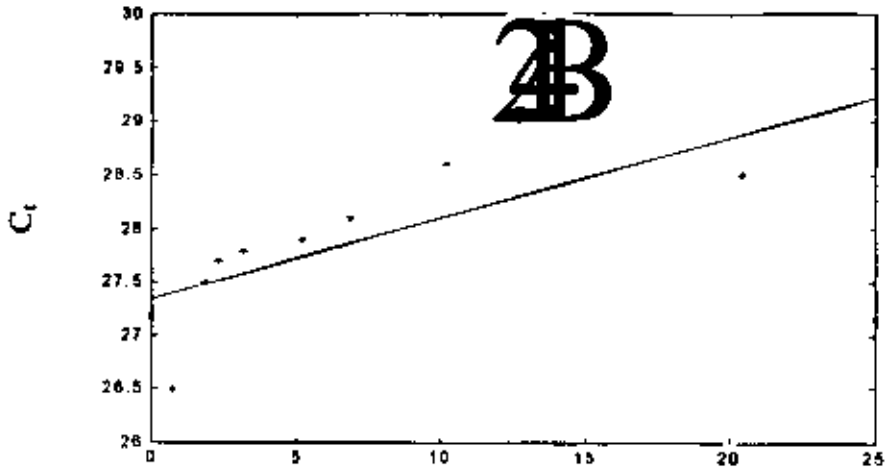
Determination the rate constant (k) of alkaline (NaOH) hydrolysis Of antibiotic-derivatives (as ester) , the formation of benzoate or sulfonate ion and alcohol , as in general :



The rate formation of acetate ion is proportional both to the concentration of ester and to the concentration of hydroxide ion[60].

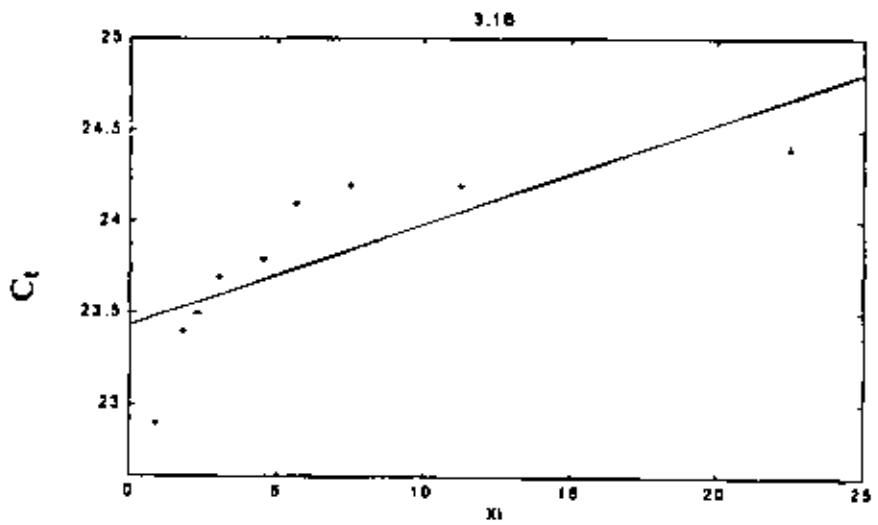
The rate constant represented in tables (**48 - 56**) for a antibiotic-derivatives, from theoretical view these types of reaction should be follow second – order reaction , but the experimental values (tables, **48 - 56**, and Figs. **58 - 66**) of derivatives not obey second – order rule Because it gives different values for rate constant (k) when integrated law rate law of second – order is use. 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 this type of reaction[61]. The variant values of rate constant of the antibiotic derivatives table **(57)** related to the fact that the amides hydrolysis more slowly than do esters[17].



Slope = 0.0753 **b = 27.3478**

Fig. (**61**) Curve of $(C_o - C_t) / t$ vs. C_t for ***Neo.Ph.A/B.***



Slope = 0.0551 **b = 23.4355**

Fig. (**62**) Curve of $(C_o - C_t) / t$ vs. C_t for ***Gent.BZ. /B.***

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 / or functional groups where differ[62].

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:

1- Electrophilic character of carbonyl carbon atom .

2- Steric hindrance, and 3 stabilization of the carbonyl group by conjugation.

The steric handrance 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 nucleophilic reagent[63] .

Fig. **(67-A)** , **(67-B)** and **(67-C)** show the relation between rate constant, log K and dissociation constant pK_d.

In Fig.**(67-A)** reagents for the derivatization are constant while 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. , Gent.-BZ. , and Neo.-BZ.) while in Fig.**(67-B)** and **(67-C)** the predominant factor control the hydrolysis (Gent.-BZ. and Gent.-BZ.S.) also (Strept.-BZ.S., Strept.-Ph.A and Strept.-T.P.S.) are reagent character[49 , 59].

CONCLUSIONS

Conclusions

According to the antibiotic – derivatives results we can conclude the following :-

1 -The reactivity in the formation of products and hydrolysis of antibiotic-derivatives esters and / or amides are related to the electrophilic and nucleophilic characteristics and the stability of transition state and products form.

The rate of SN^2 reaction is strongly dependent on the nature of nucleophilic reagent used, and it increases with nucleophilic strength of the incoming nucleophilie.

2 -The rate of reaction is dependent on the nature of the solvent. The rate of reaction increases with increase of solvent due to stabilized transition state of the reaction (formation of cation intermediate). The best solvent and / or catalyst used in preparation of antibiotic-derivatives is pyridine.

3 - uv- spectroscopy is very sensitive to distortion of the chromophore and the consequently the steric repulsions which oppose the co planarity of conjugated π- electrons system.

The pathochromic or hypsochrome shift occurs with antibiotic-derivatives belong to different substituted functional groups on (—OH or — NH2) to gives esters which absorbs at higher wavelength (relative to absorption of corresponding to reagent) while in case of amide the wavelength displaced to short wavelength. This result give the good indication in stability of the antibiotic- derivatives (amide more stable than ester). In contrast with his amide is slowly hydrolysis while ester readily fast hydrolysis.

4 - We presume that the crystalline form for some of these antibiotic-derivatives, does not melt

directly to a liquid phase but first passes through an intermediate stage (liquid crystal 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. Also liquid crystal polymer does not melt, it decomposed.

5 -The chemical kinetics of antibiotic-derivatives hydrolysis follow parallels type of reaction (different reactants amide and ester produce a common product).

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

The curvature of most curves of antibiotic-derivatives during the initial part of the reaction and after a sufficient length of time the curve becomes linear which means the reaction mechanisms are proceed through different reaction order.

6 -The chromatographic characters of pre - column antibiotic – derivatives for HPLC separation will improve through polarity change (possible number of substituted a reagent dependent) leading to reduced analyst time .

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SUMMARY IN ARABIC

الخـــــلاصـــة

تتضمن مركبات الأمينوكلايكوسيد (مضادات حيوية) مشـتقات متعـددة منها النيومايسين والستربيتومايسين والكاناماسين والجنتامايسين و هى مركبات دوائية لها أهمية فى معالجة العديد من الأمراض ولكي يتم فصلها وتقـديرها بشـكل دقيق تم تفاعلها مـع المركبـات التاليـة (البنزويـل كلوريـد والبنـزين باراسلفونيل كلوريد و التلوين بـارا سـلفونيل كلوريـد و الفثاليـك انهيدريـد) للحصول على معقدات يمكن دراستها باستخدام طيف الأشعة فوق البنفسـجية (uv spectrophotometer) وذلك بقيـاس الامتصاصـية (A) و عامـل الامتصاص المولاري (ε) لهذه النواتج .

و التي عن طريقهما تم حساب عدد جزيئات الكاشف التي ارتبطت بكل مركب وكذلك عينا ثابت التفكك ومعدل التحلل مـن خـلال دراسـة توصـيلية هـذه المعقدات ، وأيضا تم تحديد العوامل التي تتحكم في عملية تحللها.

الجماهيرية العربية الليبية
الشعبية الاشتراكية العظمى
شعبية سرت
جامعة التحدي

ان الدراسة ليست غاية في حد ذاتها
وانما هي خلق الانسان نموذجي الجديد

G.S.P.L.A.J.
AL - TAHDI UNIVERSITY

التاريخ : ....
الموافق : 2005.9.11

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كليــة العلــوم
قسم الكيمياء

عنــوان البحـــث

((الخواص الفيزيائية لمشتقات الأمينو كلايكو سايد)) ((المضادات الحيوية))

سـرت / ليبيا

مقدمـــة مـن الطـــالبة

عنه محمد أحمد أفحيمة

**لجنــة المناقشـــة

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(مشـرف الرســالـة)

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2005/9/6
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كلـــية العلـــوم

قسم الكيمياء

الخواص الفيزيائية لبعض مشتقات الأمينوكلايكوساید

(المضادات الحيوية)

بحث مقدم كجزء من متطلبات الحصول على درجة الماجستير في العلوم – فرع الكيمياء

للطالبة /

عند محمد أحمد افحيمة

تحت إشراف /

الدكتور : زيدان جاسم خلف

أستاذ مساعد بكلية العلوم قسم الكيمياء جامعة التحدي

جامعة التحدي

(2005 / 2004 م)