Study the biological activity of some antibiotics and their complexes, with some metals

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Received11/11/2009 accepted15/2/2009

Abstract

The research is concerned with studying of activity of some antibiotics (Tetracycline, Gentamycine and streptomycine) and their complexes with some metal (Ca, Mg ,Fe) were been studied by electric conductivity, ultraviolet spectroscopy (UV) to compare the biological effects of these complexes with the physiochemical methods for determination of this complexes such as association constant K_A, equivalente conductance (Λ^o) at infinit dilution and the distance between ions in solution R at the best fit values of standard deviation ($\sigma\Lambda$) which are calculated for each antibiotic and their complexes with the absorbance of these complexes in neutral medium at room temperature.

دراسة الفعالية البايولوجية لبعض المضادات الحيوية ومعقداتها مع بعض الفلزات

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المستخلص

تم دراسة فعالية بعض المضادات الحيوية (تتراسايكلين، جينتامايسين وستربتومايسين) ومعقداتها مع بعض الفلزات (Mg ،Ca ،Fe) بطريقة التوصيلية الكهربائية وطيف الاشعة فوق البنفسجية لمقارنة التأثير البايولوجي لهذه المعقدات مع الطرق الفيزوكيميائية لتقدير هذه المعقدات من خلال بعض الثوابت التي تم الحصول اليها (KA) ثابت التجمع الايوني و Λ^o التوصيل المكافئ عند التخفيف الى ما لانهاية و (R) المسافة بين الايونات في المحلول عند احسن قيمة للانحراف القياسي (σ A) والتي حسبت لكل مضاد حيوي ومعقداته مع الامتصاصية للمعقدات في وسط مائي وفي درجة حرارة الغرفة.

Introduction

Physiochemical properties determine the process by which drugs reach and interact with sites of action, it is important to examine the extent to which any one property correlates with the observed biological activity. the possible importance of such properties as dissociation at the pH of the body fluid, interatomic distances between functional groups redox potential by hydrogen bonding, dimensional factor chelation and the special configuration of the molecule are worthy of consideration[1], A classic electroanalytical technique that finds application in a variety of chemical and biochemical studies is ameasurements of solution conductivity to provide basic determination of ionic strengths of solutions and thermodynamic data of other electrolytic solution[2].Many studies made for the complication like protenation and complication forming of divalent complexes of Quinotone with antibiotics by potentiometric titration and spectrophotometric method and this shown that the activity of antibacterial for these drugs depends on pH and the concentration of metal positive ion in the solution [3].Preparation and synthesis of ampiciline complexes with many metal ions by reaction with sodium ampicilinate were made by (Bravo) and (Anacona) at room temperature .The complexes were been studied by conductivity measurements, magnetic susptibility and spectrophctometric method and the study confirms that these complexes are octahedral[4] The electrical conductivities of 5-(P-substituted) phenylazo barbituric acid compounds and their complexes were measured, the results illustrate faint semi conductivity beheaviour for these system, the conductivities were found to depend on the structure of the compounds. The metal ion forms a bridge between the ligands to facilitate the transfer of current carriers with some degree of delocalization in the excited state [5].

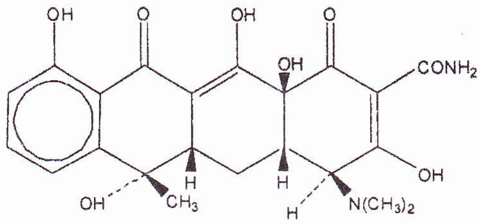
Experimental

Purification of solvent:

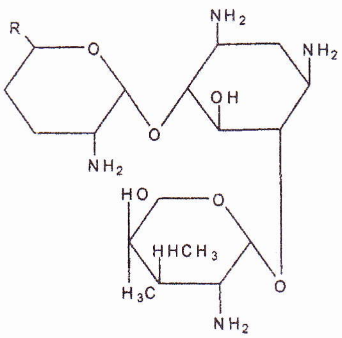
Conductivity water was prepared by redistilliny water three times with the addition of a little amount of potassium permangenate and small plettets of (KOH)[6], the specific conductance of water was less than 1.2×10^{-6} (S. cm^{-1}).

Preparation of solutions:

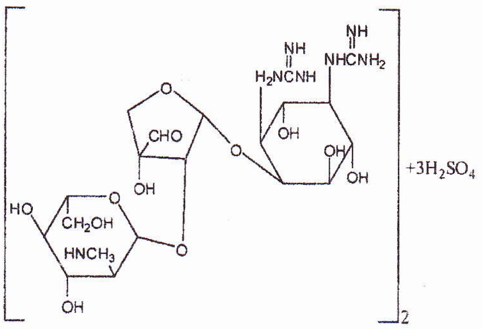
A solution of 10^{-3}M of each drug (streptomycine, Gentamycine, Tetracycline) were prepared from standard drugs from SDI by weighting a known amount of each drug in deionized water. The salts of metal ions prepared (1×10^{-3}M of each of $FeCl_2$, $MgCl_2$, $CaCl_2$) by the same way in deionized water also.



Tetracycline



Gentamycin



Streptomycin

General procedure:

All stock solutions were prepared freshly by weighting and using freshly prepared solvent, conductivity measurements were made using Conductivity water. The cell constant of the conductivity cell was measured by using the method of Jones and Bradshow[7], a standard addition method has been used for measuring the conductance of electrolyte solutions. The conductivity cell was washed, dried, and then weighted empty and kept at (25 C°) ± 0.1 C° using a water-circulating ultra thermostat. A certain amount of solution was injected in to the conductivity cell and the conductivity of the solution was measured Electronic conductivity, cell constant compens is 1.05, No. 911 F0013, Another known amount of the solution was added and the measurement was repeated as before. Generally (15) additions have been made through out each run. For the mixed solutions the addition was made by kept the concentration of the drug and changing the concentration of the salt added.

Results and Discussion

Lee and Wheaton derived a conductance equation based on a new model for ions in solution. The equation describes transport in solutions containing any number of ionic species, of any valency type, and hence is suitable for use with symmetrical, unsymmetrical or mixed electrolytes[8]. For symmetrical electrolytes where the cospheres of a pair of ions M+ and X- overlap, the pair is taken to be associated and thus takes no part in charge transport. Hence for free M+ and X- ions the charge density P(r) for r<R is by definition zero, using this model the conductance equation for symmetrical electrolyte has obtained having the general form Λeq. = f(Λ°, R, KA) where Λ° the equivalent conductance at infinite dilution, R is the mean distance between ions in solution and KA the pairwise ion association constant.

$$M_{aq}^{+} + X_{aq}^{-} \square (M_{aq}^{+} X^{-})^{0}$$

Where may be zero, and R the distance parameter which is defined as the distance between anion and cation.

The simplest form of L-W equation for symmetrical electrolyte (1:1) is:

$$\Lambda = \Lambda^{\circ}$$

$$[1 + C_1(KR)(\varepsilon K) + C_2(KR)(\varepsilon K)^2 + C_3(KR)(\varepsilon K)^3] - \frac{\rho K}{1+KR}\{1 + C_4 4(KR)(\varepsilon K) + C_5(KR)(\varepsilon K)^2 + KR/12\}$$

Where the concentration – dependent terms are both the "plasma parameter" (εK) and (KR). The concentration – coefficient are functions of these parameters.

$$\rho = \frac{F\delta|z|}{3\pi y}$$

Where F=Faraday constant (9.64867×104C, Mol^{-1}) and δ is a conversion factor (volt → e.s.u) = $\frac{1}{299.7925}$

The term C1 → C5 have been defined previously.(8)

For unsymmetrical electrolyte for example MX2 two possible association equilibrium can be noted:

$$M^{2+} + X^{K} \overset{A^{1}}{\rightarrow} MX^{+}$$

and

$$MX^{+} + X^{-} \overset{KA^{2}}{\rightarrow} MX_2$$

Where a new charge – carrying species MX^+ is created. Therefore there are three kinds of ions M^{2+}, MX^+ and X^- where are all conducting species.

$$\square\ \Lambda_{equiv.} = \sum_{i=1}^{s} |Zi| m_i \lambda_i |\Sigma C_i$$

Where s is the number of ionic species, Ci is the stoichrometric equivalent concentration, λi, mi and Zi are the ionic equivalent conductance, molar free ion concentration, and charge of the species, respectively. for electrolytes of a type (2:1) where ion association occurs:

$$\Lambda equiv.=f(\lambda^{\circ}M^{2+}, \lambda^{\circ}MX^{+}, K_A^{(1)}, K_A^{(2)}, R)$$

The general form of (L-W) equation of unsymmetrical electrolyte is

$$\lambda_j = \lambda_j^{0}\left\{1 + Z_j \sum_{p=2}^{s} X_j^{p} \sum_{v=1}^{s} tv X_v^{p}\left[A_v^{p}(t)(BK) + \sum \frac{|z|^2 e^2}{DKT}, K^2 = \frac{8\pi N^2 e|z|^2 C}{1000DKT} + B_v^{p}(t)(BK)^2 + C_v^{p}(t)(BK)^3\right]\right\} - \frac{|Z_j|(K_z}{2(1+t)}$$

$$\left\{1+V_j^{(1)}(t)(BK)+V_j^{(2)}(t)(BK)^2+\pi_j^{(5)}t/6\right\}$$

Where the plasma coefficient A_v^p, B_v^p ----- etc. are function of KR and q_p. The terms X_j^p and q_p are functions of the limiting mobilities, of the solution. The terms C_v^p and $V_v^{(2)}$ are in complete, and are usually set equal to zero. However, Pethybridge finds[9]. $V_j^{(2)}$ must be included in order to obtain reasonable $\lambda^{0}_{Ma^+}$ values for MCl_2 in water (M=alkaline earth metal).A multiparameter least square curve sitting procedure is used to give the lowest value of curve fitting parameter σ(Λ) between the experimental and calculated points. An iterative numerical method which was found to be very successful has been used to find the minimum σ(Λ)

$$\sigma\Lambda = \left\{\sum_{n=1}^{NP}(\Lambda_{calc.}-\Lambda_{exp.})^2/NP\right\}^{1/2}$$

The antibiotics (Gentamycine, , Streptomycine and Tetracycline) are used as a ligand from its reaction with some metal salts as ($FeCl_2$, $MgCl_2$, on $CuCl_2$) to form complexes which identified spectrophoto metrically to know the metallic bonding with ligand and their equilibrium constant under standard condition by using molar percent method which gives values of formation constant between (10^7 -10^{14}) and to know more information about Gibbs free energy which confirm with the high stability of the complex [10]. The following are the results of typical conductivity Λ as shown in table (1) and figur (1) for antibiotics only (Gentamycine, streptomycine and tetracycline) in aqueous medium.

Table (1): The equivalent conductivity (S. cm2. equiv-1) with M concentration of the antibiotics in water

Conc.10^{-4}	Λ Genta.	Λ Strep.	Λ Tetra.	$\sqrt{2}^{-2}_{10}$
0.40	11.042	26.2.11	26.9.19	0.63
1.20	10.3.83	20.8.75	26.4.62	1.09
2.00	6.0.90	20.4.81	23.0.54	1.41
2.80	5.2.76	18.5.95	21.6.91	1.67
4.00	4.7.54	16.4.23	17.2.42	2.00
5.20	4.0.15	15.2.23	15.7.74	2.28
6.40	3.9.21	15.0.15	15.6.32	2.52
7.60	3.6.11	14.9.15	14.5.24	2.75
9.20	3.3.32	14.6.51	14.2.94	3.03
10.00	2.1.05	14.4.13	13.8.17	3.16
12.00	2.9.11	14.2.10	13.5.20	3.46
14.00	2.5.78	14.0.05	13.0.15	3.74

From Table and Figure (1) it is clear that the behavior of antibiotics were very weak electrolyte. The molar conductivity follow the sequence:Λ Gentamycine < Λ streptemycine < Λ Tetracycline. This may be attributed to the structure effect toward water and to the increasing of molecular weight in the same manner of Λ°. Table (2) show the analysis of the results of the antibiotics by using (L- W) equation for symmetrical electrolytes.

Table (2): values of KA (association constant) Λ° (equivalent conductivity at infinite dilution) and R (distance parameter and σ standard deviation) of the three antibiotics in water at room temperature.

Drugs	R A°	K_A	Λ°	σ
Gentamycine	1×10^{-8}	84	52.5	0.015
Streptomycine	1.3×10^{-8}	1709	259.0	0.081
Tetracycline	1.6×10^{-8}	20025	275.5	0.074

From table (2) the data show also the same sequence of Λ°: Λ° Gentomycine < Λ° Streptomycine < Λ° Tetracycline, the association constant (KA) follow the sequence:

KA Gentomycine < KA Streptomycine < KA Tetracycline

This behavior is obey Bjeerum theory and the theoretical expression of KA is due to formation of CIP (contact ion pair)

Where: $KA = \frac{4\pi N}{3000}$ a3 $eb, \frac{\beta}{R}$ b =

Where β is Bjeerum constant and equal to the ratio between the electrostatic forces ei ej/Da for the ions 1, j which have a distance a between them, is the long energy forces, and when SSIP (solvent separated ion pair) formed so (R = a + ds) but when (CIP) (R=a) so the value of $e^{B/R}$ for (CIP) > SSIP and the value of KA (SSIP) less than KA(CIP) and (SSIP) and this as shown from the value of KA and R. The valuo of standard deviation (σ) are very small for the drugs which indicate that L-W) equation is applicable for each drug in solution.

Complexation of drugs with metal ions:

Different spectra were measured using shimadzu UV-1650 PC) with two microcuvetes operating in the UV-visible region with full scale explanation of 0.0-2 units for absorbance spectra. One microcuive was filled with distilled water, other measurements are for the drug and metal ion (1×10^{-4}) with distilled water. The results of complexation of different metal ions with drugs by conductivity method is shown in table 3 (A-C) and fig 2: (A-C)

Table (3-A): The equivalent conductivities (S. cm^2. equiv.-1) with concentration of complex:

Cone 10^{-5} mol/L	$\sqrt{C}$ $\times 10^{-3}$	Λ Strep.+$FeCl_2$	Λ Strept.+$CaCl_2$	Λ Strept.+$MgCl_2$
4.0	6.3	217	210	238
8.0	8.9	112	124	124
12.0	10.9	86	87	87
16.0	12.6	65	72	78
20.0	14.1	52	63	63
24.0	15.4	48	52	52
28.0	16.7	45	45	48
32.0	17.8	39	42	45
36.0	18.9	37	40	43
40.0	20.0	36	38	42

Table (3-B): The equivelent conductivities S. cm^2. equiv^{-1} With concentration of complex

Cone 10^{-5} mol/L	$\sqrt{C}\times 10^{-3}$	ΛTetra+ $FeCl_2$	ΛTetra+ $CaCl_2$	ΛTetra+ $MgCl_2$
4.0	6.3	175.8	210.6	199
8.0	8.9	105.0	112.0	119
12.0	10.9	76.1	76.0	87
16.0	12.6	62.3	65.0	72
20.0	14.1	52.5	52.5	63
24.0	15.4	48.1	48.1	52
28.0	16.7	45.0	46.7	48
32.0	17.8	42.6	42.6	45
36.0	18.9	37.6	40.0	43
40.0	20.0	36.7	36.0	42

Table (3-C) The equivalent conductivities (S . cm^2 .equiq.$^{-1}$)with concentration of complex

Cone 10^{-5} mol/L	$\sqrt{C}\times 10^{-3}$	ΛGenta.+ $FeCl_2$	ΛGenta.+ $CaCl_2$	ΛGenta.+ $MgCl_2$
4.0	6.3	215.25	233.0	228.0
8.0	8.9	118.12	124.0	123.0
12.0	10.9	81.33	87.0	87.0
16.0	12.6	65.62	72.0	72.0
20.0	14.1	57.75	63.0	63.0
24.0	15.4	48.12	52.0	56.0
28.0	16.7	48.75	48.0	48.0
32.0	17.8	42.65	45.0	45.0
36.0	18.9	40.85	43.0	43.0
40.0	20.0	34.37	39.6	41.0

The behavior of complexes were weak electrolytes and obeys Kolorash equation for weak electrolytes. The equivalent conductivity of the complexes solutions against the square root of concentration are shown before. Table (4) shows the results of analysis of the complexes of each antibiotic with each metal ion using Lee-Wheaton equation for unsymmetrical electrolytes by conductivity method.

Table (4): Values of KA association constant λm^{2+} (ionic conductivity) and R (distance parameter) for complex solution in water.

		K_A	λ_m^{2+}	R(A°)	σA
streptomycine	$+FeCl_2$	20500	200	33.5	0.0220
	$+CaCl_2$	11500	210	28.5	0.0201
Tetacycline	$+MgCl_2$	11750	249	31.5	0.020
	$+FeCl_2$	12126	136	20.5	0.0182
	$+CaCl_2$	11312	164	30.0	0.031
Gentamycine	$+MgCl_2$	11510	190	29.5	0.025
	$+FeCl_2$	6325	120	14.0	0.0285
	$+CaCl_2$	6160	130	16.0	0.0290
	$+MgCl_2$	6420	140	18.0	0.0310

The association constant for the complexes follow the series:KA Strepto. Complexes > KA Tetra. Complexes > KA Genta. Complexes as shown in table (4), since streptomycine contain (7) atoms of nitrogen and (12) atoms oxygen so it is more polar than Tetracycline which contain (2) atoms N and (8) atoms oxygen, Gentamycine complexes have (5) atoms nitrogen and (7) atoms oxygen and has less KA values than Tetracycline complexes because of steric effect and hydrogen bonding

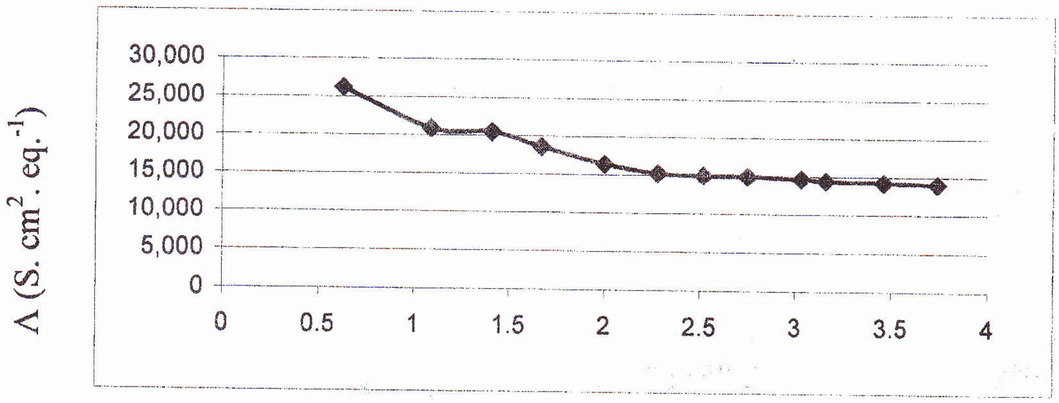


Fig (2-A): The plot of equivalent conductivities against square root of concentration for streptomycin with $FeCl_2$, $CaCl_2$, $MgCl_2$ in water

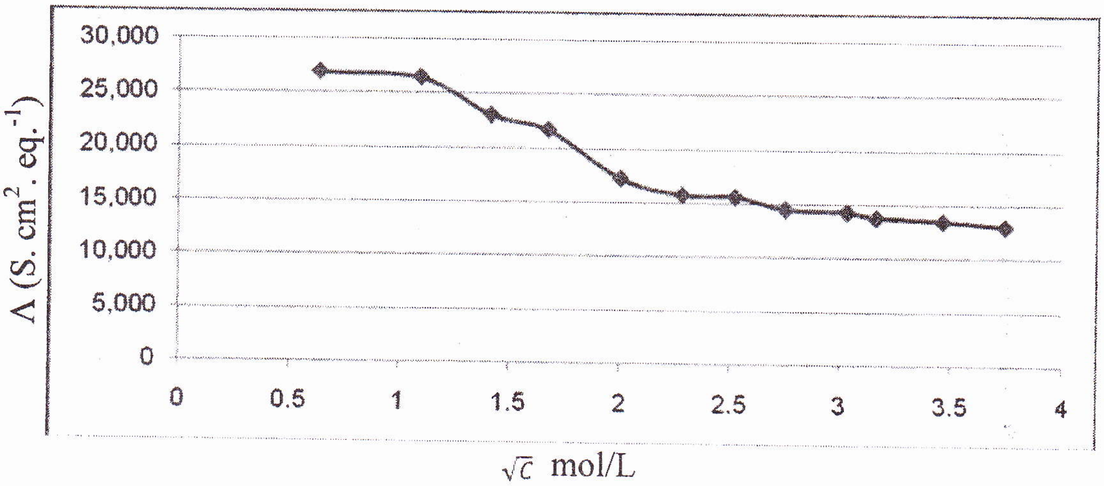


Fig (2-B): the plot of equivalent conductivities against square root of concentration for Tetracycline with $FeCl_2$, $CaCl_2$, $MgCl_2$ in water.

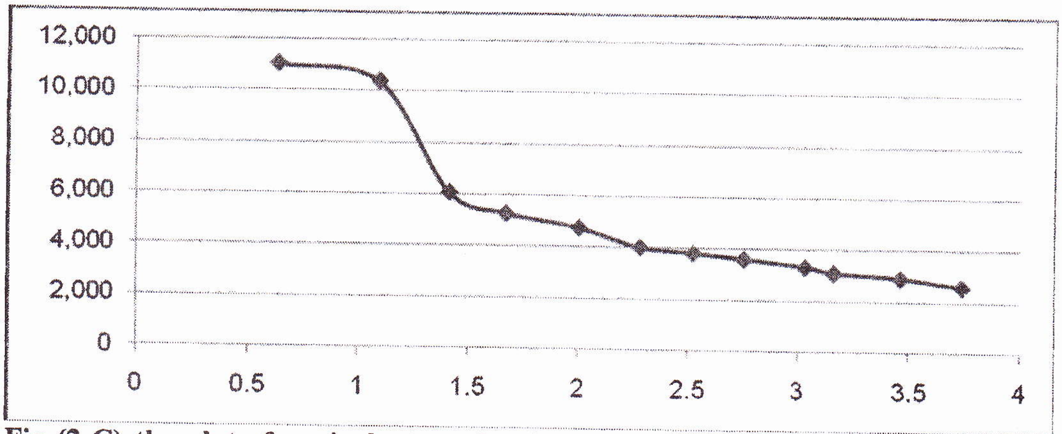


Fig (2-C) the plot of equivalent conductivities against square root of concentration for Gentamycine with $FeCl_2$, $CaCl_2$, $MgCl_2$ in water.

The ionic equivalent conductivity (λM^{2+}) of the complexes are as follows λM^{2+} Strept. Complexes > λM^{2+} Tetra. Complexes > λM^{2+} Genta. Complexes. This can be attributed to the structural effect(11) The association constant for the metal drug complexes follow the sequence KA Fe complexes > Mg complexes > Ca complexes And the ionic equivalent conductivity decreases as follows λM^{2+} Fe Complexes < λM^{2+} Ca Complexes < λM^{2+} Mg Complexes this is because of the periodic properties of the metals and the ionization energies of them. The study of spectral properties of complexes provides much information which usually sheds considerable light on structure and bonding concerned with the difference between the ground state and the excited state of molecules. Direct evidence of orbital energy levels can be obtained from electronic spectra.

Table (5) the value of the wave length and absorbance of the antibiotics only and their complexes with ($FeCl_2$, $CaCl_2$ and $MgCl_2$)

drugs	metal	Wave length	absorbance
streptomycine		272	0.407
streptomycine	$+FeCl_2$	344	0.650
streptomycine	$+CaCl_2$	274	0.621
streptomycine	$+MgCl_2$	272	0.678
Tetacycline		234	0.4
Tetacycline	$+FeCl_2$	266	0.65
Tetacycline	$+CaCl_2$	270	0.615
Tetacycline	$+MgCl_2$	232	0.670
Gentamycine		298	0.215
Gentamycine	$+FeCl_2$	308	0.252
Gentamycine	$+CaCl_2$	266	0.104
Gentamycine	$+MgCl_2$	288	0.298

Table (5) show the absorbance of the antibiotics only and the complexes with the wave lengths.The electronic spectra for the studied complexes have been measured to give new bands and the bands mentioned in table (5) for ligand are shifted to longer or shorter wave length in the complex spectra which indicate the formation of complexes. The association constant decrease with increasing the ionic radius as can be seen before and also the absorbance decrease in the order. K_A Ca (complexes) < K_A Fe (complexes) < K_A Mg (complexes) This is because Ca^{+2} have a tendency to react with oxygen and Mg^{+2} have more covalent character. The biological activity of the complexes show that gentamycine complexes was very sensitive toward many kinds of bacteria (Bacillus subfilis, Ps. aeruginos, KL pneumonia, E-coli, ---- ets). So there is a relationship between the ion association KA and ionic equivalent conductance λ^{2+} with the biological activity of the complexes of antibiotics. Gentamycine complexes have low values of KA and λ^{2+} so they have more active sits in the association form to increasing sensitivity toward many kinds of bacteria This mean that the motion of ions in the solution λ^{2+} is very slow and more effective towards the bacteria. This mean that as the value of K_A small for all complexes the activity increase toward the bacteria as show in table (6)(12).

Table (6) : Show the biological activity of the complexes toward many kinds of bacteria.

E-coli			Proteus			Kleb			Pseud			Complexes	
R	Ms	S	R	Ms	S	R	Ms	S	R	Ms	S		
		+			+			-			+	S + $Fecl_2$	Low
		+			-			+			-	S + $Fecl_2$	High
		+			-			-			-	S + $Mgcl_2$	Low
		+			-			-			-	S + $Mgcl_2$	High
		+			-			+			-	S + $Cacl_2$	Low
		+			-			+			-	S + $Cacl_2$	High
+					-			-			-	T + $Fecl_2$	Low
	+				-			-			-	T + $Fecl_2$	High
					-			+			-	T + $Mgcl_2$	Low
					-			-			-	T + $Mgcl_2$	High
					+			-			-	T + $Cacl_2$	Low
					-			-			-	T + $Cacl_2$	High
	+				-			-			+	G + $Fecl_2$	Low
		+			+			+			+	G + $Fecl_2$	High
		+			+			-			+	G + $Mgcl_2$	Low
		+			+			-			+	G + $Mgcl_2$	High
		+			+			-			+	G + $Cacl_2$	Low
		+			+			+			-	G + $Cacl_2$	High

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