Conductometric, Spectrophotometric and *In vivo* Investigation of the Interaction of Ca(II) Ion with Oxytetracycline Hydrochloride

Elsayed T. Helmy[1, 2] and Esam A. Gomaa[2]
[1] Central Laboratory, National Institute of Oceanography and Fisheries, Ministry of Scientific Research, Alexandria, Egypt
[2] Chemistry Department, Faculty of Science, Mansoura University, 35516-Mansoura, Egypt
Email: stalaat41@yahoo.com

Elsayed M. Abou Elleef
Basic Science Department, Delta Higher Institute for Engineering & Technology, 35681-Dakhlia, Mansoura, Egypt

Amr Negm
Biochemistry Department, Faculty of Science, Mansoura University, 35516-Mansoura, Egypt

***Abstract*—The interaction of antibiotic oxytetracycline hydrochloride (OTC) with calcium chloride has been investigated by conductometric, spectrophotometric and in-vivo, methods under equilibrium conditions. The interaction of OTC with calcium ion in water and in methanol solvents has been found to form two complexes 1:1 and 2:1 (metal to ligand molar ratio) conductometricallyand the values of K_f has been calculated from conductivity measurements at 293.15, 298.15, 303.15 and 308.15 K, Gibbs free energy of formation, enthalpy and entropy change of complexation were determined from the temperature dependence of the formation constant. Also K_f calculated from the absorption spectra of $CaCl_2$ – OTC complexes in methanol solvent at 293.15 K indicates the formation of 2:1. In case of in-vivo studies there is no significant change was observed in the mean value of serum ionized calcium of the treated rats, this indicates that the values obtained from Conductometric and spectrophotometric studies are accepted because the stability constant values were found to be in biologically active range. All values are discussed.**

***Index Terms*—conductometric, spectrophotometric, in vivo, formation constant, thermodynamic parameters, oxytetracycline hydrochloride**

I. Introduction

A stability constant (formation constant, binding constant) is an equilibrium constant for the formation of a complex in solution. It is a measure of the strength of the interaction between the reagents that come together to form the complex. There are two main kinds of complex compounds formed by the interaction of a metal ion with a ligand and supramolecular complexes, such as host-guest complexes and complexes of anions. The stability constant provides the information required to calculate the concentration of the complex in solution [1].

Oxytetracycline hydrochloride has many areas of application in chemistry, biology and medicine. It's a broad-spectrum antibiotic, active against a wide variety of bacteria. However, some strains of bacteria have developed resistance to this antibiotic, which has reduced its effectiveness for treating some types of infections.

Calcium is the most abundant mineral element in the body with about 99% in the bones primarily as hydroxyapatite. The remaining calcium is distributed between the various tissues and the extracellular fluids where it performs a vital role for many life sustaining processes. Among the extra skeletal functions of calcium are involvement in blood coagulation, neuromuscular conduction, excitability of skeletal and cardiac muscle, enzyme activation, and the preservation of cell membrane integrity and permeability. Serum calcium levels and hence the body content are controlled by parathyroid hormone (PTH), calcitonin, and vitamin D. An imbalance in any of these modulators leads to alterations of the body and serum calcium levels.

Increases in serum PTH or vitamin D are usually associated with hypercalcemia. Increased serum calcium levels may also be observed in multiple myeloma and other neoplastic diseases. Hypocalcaemia may be observed e.g. in hyperparathyroidism, nephrosis, and pancreatitis [2]-[4]. In-vivo are those in which the effects of various biological entities are tested on whole living organisms usually animals including humans, and plants as opposed to a partial or dead organism. In vivo studies are necessary for medical, different fields and research purposes.

In the medical field the animal models can be used to test the safety of drugs before they are used on patients. However, in the research field it used to validate in vitro

Manuscript received July 1, 2015; revised August 14, 2015

doi: 10.18178/ijpmbs.4.3.197-203

findings in vertebrates closest to humans. The most used animal models are mice, rats, and other rodents. It is useful for the production of polyclonal antibodies applied in immunoassays and diagnostic immunology [5].

In our last work we did in-vivo investigation of some antibiotics to show its effect on ionized calcium [6]. In this study, we did more than one method to obtain and compare stability constant from different methods to more accurate and to compare it with *in vivo* studies.

II. Experimental

A. Apparatus

The specific conductance values were recorded using conductivity bridge HANNA, H1 8819Nwith a cell constant equal to 1 cm^{-1}. The conductometer was connected to the type Kottermann 4130 ultra thermostat. Electronic spectra of the ligand and its Ca^{+2} complexes in methanol solvent were recorded on a Unicam UV/VIS Spectrometer.

B. Reagents

Oxytetracycline hydrochloride (OTC) were purchased from sigma Aldrich and Calcium chloride from Merck Germany, Methanol from El Nasr Pharmaceutical Chemicals Co. and used directly without purification, Double-distilled water was used throughout this study.

C. Preparation of Reagents Solutions

1) *Conductometric studies*

The Conductometric titration of the $CaCl_2$ (1×10^{-3}) mol/L against OTC (1×10^{-4}) mol/L in water and in methanol was performed with 0.5 ml interval additions from $CaCl_2$ solution.

2) *Spectroscophotometric studies*

The stock solution of ligand (2.5×10^{-2}M) and metal solution (2.5×10^{-2}M) were prepared according to the requirement of Jobs method. In series of flasks the sum of the number of moles of ligand plus sum of the number of moles of metal were kept constant.

3) *In-vivo Studies*

All experiments were performed using adult male albino rats, with an average body weight of 100 to 120 g purchased from Theodore Bilharz Research Institute, Giza, Egypt. The rats were housed in steel mesh cage and were provided with commercial standard diet and tap water ad libitum. For the investigation of the effect of OTC on serum calcium level, the compound was orally administered one dose for one day. At the end of treatment period, the rats deprived of food and were sacrificed by decapitation. Blood samples were collected by sacrificing the rats by decapitation under ether anesthesia. The collected blood samples were placed in dry clean centrifuge tubes and allowed to clot at room temperature for 30 minutes. Serum samples were then obtained by centrifugation at 3000 rpm for 10 minutes. These samples were kept in clean well-stopped glass vials at -20°C performing serum calcium was analyzed usually within the same day.

III. Results and Discussion

A. Conductometric Studies

The specific conductance values (K_s) of different concentrations of $CaCl_2$ in water and methanol were measured experimentally in the presence of ligand at (293.15, 298.15, 303.15 and 308.15 K). The molar conductance ($\mathbf{\Lambda}_m$) values were calculated [7]-[9] using equation (1)

$$\Lambda_m = \frac{(K_s - K_{solv})K_{cell} \times 1000}{C} \quad (1)$$

Where K_s and K_{solv} are the specific conductance of the solution and the solvent, respectively; K_{cell} is the cell constant and C is the molar concentration of the $CaCl_2$ solution. The limiting molar conductance (Λ_o) at infinite dilutions was estimated for $CaCl_2$ in water and methanol in the presence of the ligand (OTC) by extrapolating the relation between Λ_m and $\sqrt{C_m}$ to zero concentration. By drawing the relation between molar conductance ($\mathbf{\Lambda}_{mb}$) and the molar ratio of metal to ligand (M/L) concentrations (Fig. 1 and Fig. 2), different lines are obtained with sharp breaks indicating the formation of 1:1 and 2:1 (M:L) stoichiometric complexes. The experimental data of ($\mathbf{\Lambda}_m$) and ($\mathbf{\Lambda}_o$) were analyzed for the determination of formation constants for each type of the stoichiometric complexes. The formation constants (K_f) for $CaCl_2$ complexes were calculated for each type of complexes (1:1) and (2:1) (M:L) by using equation (2) [10]-[13]

$$K_f = \frac{[ML]}{[M][L]} = \frac{\Lambda_M - \Lambda_{obs}}{(\Lambda_{obs} - \Lambda_{ML})[L]} \quad (2)$$

$$[L] = C_L - \left\{C_M + \frac{\Lambda_M - \Lambda_{obs}}{(\Lambda_M - \Lambda_{ML})}\right\}$$

where Λ_M is the limiting molar conductance of the $CaCl_2$ alone, Λ_{obs} is the molar conductance of solution during titration and Λ_{ML} is the molar conductance of the complex. The obtained values (K_f) for $CaCl_2$-ligand stoichiometric complexes are presented in Table I to Table IV.

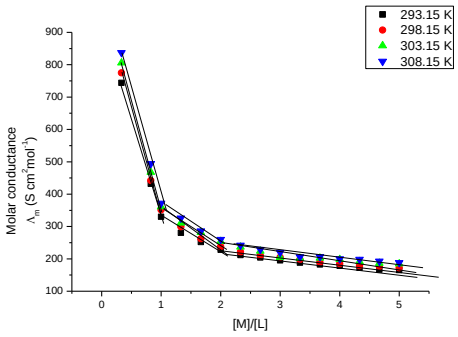

Figure 1. The relation between molar conductance (Λ_m) and the [M]/[L] molar ratio of $CaCl_2$ to oxytetracycline hydrochloride in water at different temperatures.

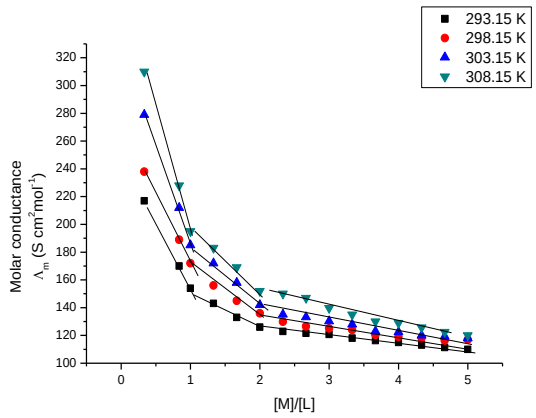

Figure 2. The relation between molar conductance (Λ_m) and the [M]/[L] molar ratio of $CaCl_2$ to oxytetracycline hydrochloride in methanol at different temperatures.

TABLE I. LIMITING MOLAR CONDUCTANCE (Λ_o), FORMATION CONSTANT (K_F), GIBBS FREE ENERGY CHANGE (ΔG_F) FOR $CaCl_2$ AND OXYTETRACYCLINE HYDROCHLORIDE COMPLEX (1:1 M/L) FORMATION IN WATER AT DIFFERENT TEMPERATURES

Temp. K	Λ_o (S cm^2.mol^{-1})	$\Lambda_{obs.}$ (S cm^2.mol^{-1})	K_f	ΔG_f (kJ mol^{-1})
293.15	1479.05	330.48	6.160E +04	-26.8792
298.15	1527.45	348.21	6.438E +04	-27.4469
303.15	1596.15	359.36	6.551E +04	-27.951
308.15	1760.21	366.64	6.837E +04	-28.5214

TABLE II. LIMITING MOLAR CONDUCTANCE (Λ_o), FORMATION CONSTANT (K_F), GIBBS FREE ENERGY CHANGE (ΔG_F) FOR $CaCl_2$ AND OXYTETRACYCLINE HYDROCHLORIDE COMPLEX (2:1 M/L) FORMATION IN WATER AT DIFFERENT TEMPERATURES

Temp. K	Λ_o (S cm^2.mol^{-1})	$\Lambda_{obs.}$ (S cm^2.mol^{-1})	K_f	ΔG_f (kJ mol^{-1})
293.15	1479.05	210.34	2.763E +04	-24.9249
298.15	1527.45	215.98	2.493E +04	-25.0948
303.15	1596.15	232.25	2.251E +04	-25.2587
308.15	1760.21	238.92	1.745E +04	-25.0232

TABLE III. LIMITING MOLAR CONDUCTANCE (ΛO), FORMATION CONSTANT (K_F), GIBBS FREE ENERGY CHANGE (ΔG_F) FOR $CaCl_2$ AND OXYTETRACYCLINE HYDROCHLORIDE COMPLEX (1:1 M/L) FORMATION IN METHANOL AT DIFFERENT TEMPERATURES

Temp. K	Λ_o (S cm^2.mol^{-1})	$\Lambda_{obs.}$ (S cm^2.mol^{-1})	K_f	ΔG_f (kJ mol^{-1})
293.15	387.15	152.42	2.600E+04	-24.7769
298.15	434.39	171.16	1.573E+04	-23.9534
303.15	509.65	184.72	2.144E+04	-25.1356
308.15	599.02	192.94	2.435E+04	-28.1671

TABLE IV. LIMITING MOLAR CONDUCTANCE (ΛO), FORMATION CONSTANT (K_F), GIBBS FREE ENERGY CHANGE (ΔG_F) FOR $CaCl_2$ AND OXYTETRACYCLINE HYDROCHLORIDE COMPLEX (2:1 M/L) FORMATION IN METHANOL AT DIFFERENT TEMPERATURES

Temp. K	Λ_o (S cm^2.mol^{-1})	$\Lambda_{obs.}$ (S cm^2.mol^{-1})	K_f	ΔG_f (kJ mol^{-1})
293.15	387.15	124.90	2.583E+04	-24.7605
298.15	434.39	133.49	2.116E+04	-24.6885
303.15	509.65	142.36	2.080E+04	-25.0599
308.15	599.02	152.00	1.733E+04	-25.0046

The data show that using water the 1:1 [M]/[L] complexation is more stable(favoured) at lower temperature but 2:1[M]/[L] is favoured at higher temperature, while hile the complex formation is favoured at the higher temperatures using methanol.

The Gibbs free energies of formation for each stoichiometric complexes were calculated [14]-[17] by using the equation (3)

$$\Delta G_f = - 2.303\, RT \log K_f \qquad (3)$$

The enthalpy change of formation (ΔH_f) for the metal-ligand was calculated from the formation constants by using van't Hoff equation 4 and equation 5 [18-20]

$$\frac{d\ln K}{dT} = \frac{\Delta H_f^0}{RT^2} \qquad (4)$$

$$\log K = -\frac{\Delta H}{2.303R}\left(\frac{1}{T}\right) + \text{constant} \qquad (5)$$

By drawing the relation between log K_A and 1/T, straight line with slope (-ΔH_A/2.303R) was given (Fig. 3 to Fig. 6), where R is the gas constant (8.341 J.mol^{-1} K^{-1}) and T is the absolute temperature.

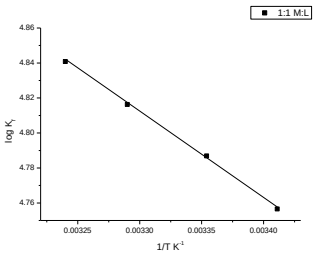

Figure 3. The relation between (log Kf) and (1/T) for (1:1) [M] to [L] stoichiometric complexes of $CaCl_2$ and oxytetracycline hydrochloride in water.

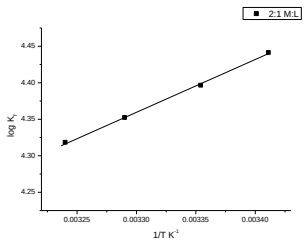

Figure 4. The relation between (log K_f) and (1/T) for (2:1) [M] to [L] stoichiometric complexes of $CaCl_2$ and oxytetracycline hydrochloride in water.

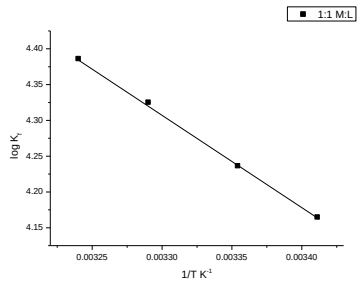

Figure 5. The relation between (log K_f) and (1/T) for (1:1) [M] to [L] stoichiometric complexes of $CaCl_2$ and oxytetracycline hydrochloride in methanol.

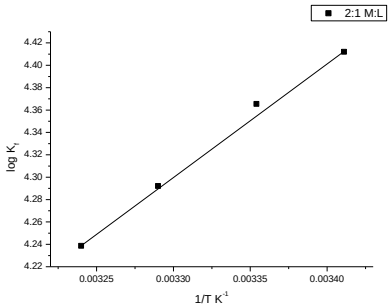

Figure 6. The relation between (log Kf) and (1/T) for (2:1) [M] to [L] stoichiometric complexes of $CaCl_2$ and oxytetracycline hydrochloride in methanol.

The entropies of formation (ΔS_f) for the metal-ligand were calculated by using Gibbs-Helmholtz equation (6) [21]

$$\Delta G_f = \Delta H_f - T\Delta S_f \quad (6)$$

The calculated values of (ΔH_A) and (ΔS_A) for $CaCl_2$ are presented in Table V to Table VIII).

TABLE V. ENTHALPY CHANGE (ΔH_F) AND ENTROPY CHANGE (ΔS_F) FOR $CaCL_2$ AND OXYTETRACYCLINE HYDROCHLORIDE COMPLEX (1:1 M/L) FORMATION IN WATER AT DIFFERENT TEMPERATURES

Temperature	ΔH_f (kJ mol^{-1})	$T\Delta S_f$ (kJ mol^{-1})	ΔS_f (kJ mol^{-1}K^{-1})
293.15 K	8.986175	35.86533	1.223E-01
298.15 K	8.986175	36.43306	1.222E-01
303.15 K	8.986175	36.93719	1.218E-01
308.15 K	8.986175	37.50755	1.217E-01

TABLE VI. ENTHALPY CHANGE (ΔH_F) AND ENTROPY CHANGE (ΔS_F) FOR $CaCL_2$ AND OXYTETRACYCLINE HYDROCHLORIDE COMPLEX (2:1 M/L) FORMATION IN METHANOL AT DIFFERENT TEMPERATURES

Temp. K	ΔH_f (kJ mol^{-1})	$T\Delta S_f$ (kJ mol^{-1})	ΔS_f (kJ mol^{-1}K^{-1})
293.15	-14.191	10.73386	3.662E-02
298.15	-14.191	10.90373	3.657E-02
303.15	-14.191	11.0677	3.651E-02
308.15	-14.191	10.83223	3.515E-02

TABLE VII. ENTHALPY CHANGE (ΔH_F) AND ENTROPY CHANGE (ΔS_F) FOR $CaCL_2$ AND OXYTETRACYCLINE HYDROCHLORIDE COMPLEX (1:1 M/L) FORMATION IN METHANOL AT DIFFERENT TEMPERATURES

Temperature	ΔH_f (kJ mol^{-1})	$T\Delta S_f$ (kJ mol^{-1})	ΔS_f (kJ mol^{-1}K^{-1})
293.15 K	25.5244	50.30133	1.716E-01
298.15 K	25.5244	49.47779	1.659E-01
303.15 K	25.5244	50.65998	1.671E-01
308.15 K	25.5244	51.40041	1.668E-01

TABLE VIII. ENTHALPY CHANGE (ΔH_F) AND ENTROPY CHANGE (ΔS_F) FOR $CaCL_2$ AND OXYTETRACYCLINE HYDROCHLORIDE COMPLEX (2:1 M/L) FORMATION IN METHANOL AT DIFFERENT TEMPERATURES

Temp. K	ΔH_f (kJ mol^{-1})	$T\Delta S_f$ (kJ mol^{-1})	ΔS_f (kJ mol^{-1}K^{-1})
293.15	-20.7365	4.023935	1.373E-02
298.15	-20.7365	3.951999	1.326E-02
303.15	-20.7365	4.323353	1.426E-02
308.15	-20.7365	4.268098	1.385E-02

Thermodynamics is a branch of science dealing with the affiliation between heat and work. The advent of thermodynamics helped in understanding many systems, which in turn had a great impact in chemical engineering. Kinetic and thermodynamic aspects became primary criteria in design of reactors, mass and heat transfer equipment. Till 1950, thermodynamics was not prominent area in biotechnology. The probable reason is the lack of data with respect to biomolecular properties, thermodynamic equilibrium positions, energy efficiency relations and the complexity of biological systems [22]-[33].

As observed, the Λ_o values increase with increase in temperature indicating less solvation or higher mobility of ions. This is due to the fact that the increased thermal energy results in greater bond breaking and variation in vibrational, rotational, and translational energy of molecules that leads to higher frequency and hence higher mobility of ions. Also the negative value of ΔG_f indicates that the reaction is spontaneous [11].

A decrease in metal ligand stability constant (K_f) with increase in temperature and negative values the negative values of enthalpy change (ΔH_f) for complexation suggests that the complexation reaction are exothermic [35]. Favorable at lower temperature and the metal ligand binding constant are enthalpy driven and metal-ligand bonds are fairly strong [36].

B. Spectrophotometric Determination of Stability Constants

The Job's method of continuous variation is especially well suited for the study of complexes not sufficiently stable to permit their isolation from solution. Work of this type has been done by Job who developed the method of continuous variation [40]. This Method makes use of any measurable additive property of two species [37], [38].

Any complex formed by the two species must give a value for the separate species. The simple application of the method involves equilibrium of type:

$$A + nB = AB_n$$

where A represents a metal, B a coordinating group and ABn a complex. Solutions are prepared in which the mole fraction of the compounds are varied and the total number of moles of both, is kept constant. If there is no complexing, the plot of extinction coefficient or absorbance against mole fraction or concentration is a straight line but if a complex is formed, the plot deviates from linearity. The deviation is the maximum at mole fraction corresponding to the composition of the complex. When the deviation is plotted against mole fraction, the maximum point gives the desired composition. The conclusion may be verified by repeating the process at other wavelengths. Since the position of the maximum is independent of wavelength.

A Complex ion is formed in solution by general formula

$$M^{n+} + L \longrightarrow [ML]^{n+}$$

This equilibrium can be represented as equation (7):

$$K_f = \frac{a_{ML}}{(a_{M^{n+}})(a_L)} \quad (7)$$

where "*a*" is activity of each species present at equilibrium.

The activity of any compound A is the product of its concentration and an activity coefficient $\gamma\pm$ as:

$$[\alpha] = [A]\ \gamma\pm$$

For dilute solutions the above equilibrium will reduce to:

$$K_f = \frac{[ML]}{[M^{n+}]\,[L]} \quad (8)$$

If a number of ligand co-ordinate with a metal ion in successive steps are as follows equation 9 and equation 10:

$$M^{n+} + L \longrightarrow [ML^{n+}]$$

$$K_1 = \frac{[ML^{n+}]}{[M^{n+}]\,[L]} \quad (9)$$

$$ML^{n+} + L \qquad [ML_2{}^{n+}]$$

$$K_2 = \frac{[ML_2{}^{n+}]}{[ML^{n+}]\,[L]} \quad (10)$$

And so on, the stepwise equilibrium constants K_1, K_2, K_n are known as stability constants.

The overall equilibrium is as follows:

$$M^{n+} + XL \qquad [ML_x{}^{n+}]$$

$$B_x = \frac{[ML_x{}^{n+}]}{[M^{n+}][L]^x} \quad (11)$$

where B_x is known as the overall stability constant.

The stoichiometry of various complexes was determined by plotting absorbance against various 'concentration of the metal to ligand. This graph was used to determine the stability constants of various complexes by using the following formula

$$K_f = \frac{\frac{A}{A_m}}{n^n C^n \left(1-\frac{A}{A_m}\right) C^{n+1}} \quad (12)$$

where A is absorbance at break point, A_m is theoretical absorbance n is number of coordinating ligand and C is concentration of metal or ligand.

Spectrophotometric studies of complex between (OTC) and calcium chloride in methanol solution was studied at pH=2.6 and λ_{max}= 360. Absorbance of this complex was measured and is listed in Table IX. It is apparent from Fig. 7 that formation of 1:2 M:L molar ratio of calcium and OTC complex takes place and K_f value equal 88.88×10^4.

TABLE IX. SPECTROPHOTOMETRIC STUDIES OF COMPLEX IN SOLUTION (OTC) AND CALCIUM CHLORIDE

Sr. No.	Metal ion(1×10^{-2}M) Ml	ligand(1×10^{-2}M) ml	Absorbance At pH=2.6
1	10	0	1.096
2	9	1	1.098
3	8	2	1.103
4	7	3	1.109
5	6	4	1.113
6	5	5	1.118
7	4	6	1.115
8	3	7	1.110
9	2	8	1.105
10	1	9	1.100
11	0	10	1.090

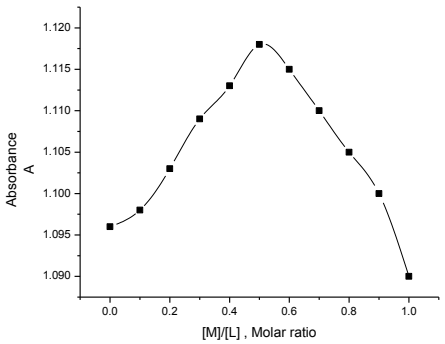

Figure 7. Absorbance spectra of OTC and calcium chloride solution in methanol at λmax= 360.

C. *In-vivo studies*

In order to test effect of OTC on serum level of ionized calcium, eight rats were used and the treated groups were classified as follows:

1) *Control group:* Healthy rats treated with 1 ml saline using an intragastric tube.

2) *Oxytetracycline treated group*: The rats were orally adminstrated with oxytetracycline hydrochloride (1.4 mg/kg body weight) using an intragastric tube.

Serum ionized calcium was estimated in serum (Table X) by employing the method of Mclean and Hastings,

1935 [40] as adopted by Beeler and Catrou, 1983 [41] using the following equation (13)

$$IC(mg\backslash dl) = \frac{Sca(mg\backslash dl)x6 - Spr(g\backslash dl)x0.33}{Spr(g\backslash dl)+6} \quad (13)$$

where IC = Ionized calcium, SCa = Serum calcium and SPr = Serum protein. Table IV shows the statistical analysis of the mean serum levels of ionized calcium in normal and compounds-treated groups after oral administration of dose (1.4 mg/Kg) for OTC.

TABLE X. MEAN VALUES OF IONIZED CALCIUM FOR CONTROL AND TREATED RAT GROUPS WITH OXYTETRACYCLINE HYDROCHLORIDE

Group	Mean ± SD
Control	1.09±0.07 N.S
Oxytetracycline hydrochloride	1.07±0.06 N.S

*N.S Not Significant compared to control group

Table X illustrates that, there were no significant change observed in the mean value of serum ionized calcium of the treated rats compared with the corresponding levels of control untreated rat group upon treatment with 1.4 mg/kg body weight. This is an interesting result because calcium forms a complex ion in aqueous solutions with OTC, but the same investigated compounds doesn't show significant decrease in serum ionized calcium due to the ability of the compounds to form complexes with calcium at physiological conditions i.e. (inside living organisms). So administration of those compounds has no side effect on serum ionized calcium on developing any hypocalcaemia related diseases.

Moreover Stability constant values are used as an eminent tool by biochemists because it helps to determine the properties of metal-ligand reactions in aqueous medium over and above the actual biological system. Extremely low stability constant values (log K_f) (ranging from negative to 1) indicate that the metal-ligand complex is not only soluble in water but also readily dissociates into metal ion and ligand. For stability constants values above 6, less metal ions are released .and these compounds are not significant in biological systems as they consume more stomach acids to dissociate the metal ion from the complex. For various drugs to remain in biologically active form, the stability constants values should be in the range of 3 to 5 [42].The stability constant values were found to be in biologically active range (log K_f less than 6). These stability constant values could be quite informative for a biochemist during drug-design or drug discovery, is from the major implication of present study.

REFERENCES

[1] G. Arena, A. Contino, E. Longo, D. Sciotto, and G. Spoto, "Selective complexation of soft Pb^{2+} and Hg^{2+} by a novel allyl functionalized thioamide calix[4]arene in 1, 3-alternate conformation: A UV-visible and H-1 NMR spectroscopic investigation," *J. Chem. Soc. Perkin Trans.*, vol. 2, no. 12, pp. 2287-2291, 2001.

[2] Institute of Medicine, *Dietary Reference Intakes: Calcium, Phosphorus, Magnesium, Vitamin D, and Fluroide Washington*, DC: The National Academies Press, 1997.

[3] J. E. Kerstetter, K. O. O'Brien, and K. L. Insogna, "Dietary protein affects calcium absorption," *The American Journal of Clinical Nutrition*, vol. 68, pp. 859-865, 1998.

[4] J. E. Kerstetter, M. E. Mitnick, C. M. Gundberg, D. M. Caseria, A. F. Ellison, T. O. Carpenter, *et al*., "Changes in bone turnover in young women consuming different levels of dietary protein," *The Journal of Clinical Endocrinology & Metabolism*, vol. 84, pp. 1052-1055, 1999.

[5] H. B. Roman, L. L. Hirschberger, J. K. Alessandro V. V. Kozich, and M. H. Stipanuk, "The cysteine dioxgenase knockout mouse: Altered cysteine metabolism in Nonhepatic tissues leads to excess H2S/HS- production and evidence of pancreatic and lung toxicity science," *Antioxidants & Redox Signaling*, vol. 19, no. 12, pp. 1321-1336, 2013.

[6] E. A. Gomaa, A. Negm, and E. T. Helmy, "*In vivo* Investigation of the Interaction of Ampicillin (AMP), Oxytetracycline (OTC) and 2-Mercaptoimidazol (MI) with calcium chloride salt," *AASCIT Communications*, vol. 2, no. 4, pp. 109-114, 2015.

[7] T. Kiss, H. Kozlowski, G. Micera, and L. S. Erre, "Cupric ion binding by dihydroxybenzoic acids," *Polyhedron*, vol. 8, no. 5, pp. 647-651, 1989.

[8] W. Gryzybkowski and R. Pastewski, "Conductometric studies of Co (II), Ni (II), and Cu (II) nitrates in dimethyl sulfoxide solutions at 25 ℃," *Electrochimica Acta*, vol. 25, p. 279, 1980.

[9] A. A. El-Khouly, E. A. Gomaa, and S. E. Salem, "Conductometric study of complex formation between 2, 3-pyrazinedicar-boxylic acid and some transition metal ions in methanol, South," *Braz. J. Chem.*, vol. 20, pp. 43-50, 2012.

[10] G. Rounaghi, Z. Eshaghi, and E. Ghiamati, "Thermodynamic study of complex formation between 18-crown-6 and potassium ion in some binary non-aqueous solvents using a conductometric method," *Talanta*, vol. 44, pp. 275-282, 1997.

[11] M. Rahimi-Nasrabadi, F. Ahmadi, S. M. Pourmortazavi, M. R. Ganjali, and K. Alizadeh, "Conductometric study of complex formations between some substituted pyrimidines and some metal ions in acetonitrile and the determination of thermodynamic parameters," *Journal of Molecular Liquids*, vol. 144, pp. 97-101, 2009.

[12] Y. Takeda, "Thermodynamic study for Dibenzo-24-crown-8 complexes with alkali metal Ions in non-aqueous solvents," *Bull. Chem. Soc. Jpn.*, vol. 56, pp. 3600-3602, 1983.

[13] D. P. Zollinger, E. Bulten, A. Christenhuse, M. Bos, and W. E. Van Der Linden, "Computerized conductometric determination of stability constants of complexes of crown ethers with alkali metal salts and with neutral molecules in polar solvents," *Anal. Chim. Acta*., vol. 198, pp. 207-222, 1987.

[14] O. Popuych, A. Gibofsky, and D. H. Berne, "Medium effects for single ions in acetonitrile and ethanol-water solvents based on reference-electrolyte assumption," *Analytic. Chem.*, vol. 44, p. 811, 1972.

[15] M. S. Isabel, L. Lampreia, and L. A. V. Ferreira, "Thermodynamic study of the ternary system NaCl–H_2O–Et_3N at 25 ℃, Part 3.—Solubilities," *J. Chem. Soc. Faraday. Trans.*, vol. 92, pp. 1487-1491, 1996.

[16] E. A. Gomaa, E. M. Abouelleef, E. T. Helmy, "Solvation of oxytetracycline hydrochloride in ethanol-water mixed solvents," *Reseach and Review J. of Chem.*, vol. 3, no. 2, 2014.

[17] E. A. Gomaa, E. M. Abouelleef, E. T. Helmy, "Solvent effects on the thermodynamics of solvation of barium diphenylamine sulfonate in ethanol-water mixed solvents," *South. Braz. J. Chem*., vol. 21, no. 21, 2013.

[18] P. W. Atkins, *Physical Chemistry*, Oxford University Press, 1978.

[19] D. J. G. Ives, *Chemical Thermodynamics*, University Chemistry, Macdonald Technical and Scientific, London, 1971.

[20] E. A. Gomaa and E. T. Helmy, "Parameters influencing complexation of doxycycline acidic drug with nano sulfate salts in absolute methanol," *AASCIT Journal of Nanoscience*, vol. 1, no. 2, pp. 19-25, 2015.

[21] P. Debye and H. Huckel, "The theory of electrolyte II – The border law for electrical conductivity," *Physik. Z.*, vol. 24, p. 305, 1923.

[22] E. A. Gomaa, E. M. A. Elleef, and E. T. Helmy, "Conductance studies on complex formation between $CaCl_2$ and ampicillin in water and in methanol solvents at different temperatures," *Research & Reviews: Journal of Pharmacy and Pharmaceutical Sciences*, pp. 55-64, 2014.

[23] E. A. Gomaa, H. M. A. El-Nader, and S. E. Rashed, "Gbbs free energies for interaction of $CuCl_2$ with (E)-1-phenyl-2-(2-(4-((E)-pheyldiazenyl) phenyl hydrazono)-2-(phenylsulfonyl) ethanone in ethanol at different temperatures," *The International Journal of Engineering and Science*, vol. 3, pp. 64-73, 2014.

[24] E. A. Gomaa, K. M. Ibrahim, and N. M. Hassan, "Evaluation of thermodynamic parameters (conductometrically) for the interaction of Cu (II) ion with 4-phenyl-1-diacetyl monoxime-3-thiosemicarbazone (BMPTS) in (60% V) ethanol (EtOH-H2O) at different temperatures," *The International Journal of Engineering and Science*, vol. 3, pp. 44-51, 2014.

[25] E. A. Gomaa, K. M. Ibrahim, N. M. Hassan, "Thermodynamics of complex formation (conductometrically) between Cu (II) ion and 4-phenyl-1-diacetyl monoxime–3-thiosemicarbazone (BMPTS) in ethanol at different temperatures," *Research & Reviews: Journal of Chemistry*, vol. 3, no. 1, pp. 47-55, 2014.

[26] E. A. Gomaa, E. M. A. Elleef, M. G. Abdel-Razek, "Thermodynamics of the solvation of $CaSO_4$ in mixed DMF-H_2O at 301.15 K," *International Research Journal of Pure & Applied Chemistry*, vol. 3, no. 4, pp. 320-329, 2013.

[27] M. N. A. El-Hady, R. R. Zaky, K. M. Ibrahim, and E. A. Gomaa, "(E)-3-(2-(furan-ylmethylene) hydrazinyl)-3-oxo-N-(thiazol-2yl) propanamide complexes: Synthesis, characterization and antimicrobial studies," *Journal of Molecular Structure*, vol. 1016, pp. 169-180, 2012.

[28] E. A. Gomaa, K. M. Ibrahim, and N. M. Hassan, "Conductometric study of complex formation between Cu (II) ion and 2-hydroxyimino-3-(2'-hydazonopyridyl)-butane (HL)," *Frontiers in Science*, vol. 2, no. 4, pp. 76-85, 2012.

[29] E. A. Gomaa and B. A. M. Al-Jahdali, "Conductometric studies of calcium ions with kryptofix 221 in mixed MeOH-DMF solvents at different temperatures," *Education*, vol. 2, no. 3, pp. 37-40, 2012.

[30] E. A. Gomaa and B. A. M. Al-Jahdalli, "Electrical conductance of $Cu(NO_3)_2$ with Kryptofix-222 in mixed (MeOH-DMF) solvents at different temperatures," *American Journal of Environmental Engineering*, vol. 2, no. 2, pp. 6-12, 2012.

[31] E. A. Gomaa and B. A. M. Al-Jahdali, "Electrochemical studies on the interaction of cadmium ion with Kryptofix 22 in MeOH-DMF solutions at different temperatures," *Science and Technology*, vol. 2, no. 4, pp. 66-76, 2012.

[32] K. M. Ibrahim, E. A. Gomaa, R. R. Zaky, and M. N. El-Hady, "The association and formation constants for $NiCl_2$ stoichiometric complexes with (e)-3-(2-benzylidene hydrazinyl)-3-oxo-n-(thiazol-2-yl) propanamide," *Analele Universitatii Bucuresti: Chimie*, vol. 20, no. 2, 2011.

[33] M. M. Mostafa, E. A. H. Gomaa, M. A. Mostafa, and F. I. El-Dossouki, "Complexes of some crown ethers with Hg (II) chloride, bromide, iodide and cyanide," *Sythesis and Reactivity in Inorganic and Matel-Organic Chemistry*, vol. 30, no. 1, pp. 157-174, 2000.

[34] U. von Stockar and A. M. L. van der Wielen, "Thermodynamics in biochemical engineering," *J. Biotechnol*., vol. 59, pp. 25-27, 1997.

[35] M. F. El-Sherbiny, "Potentiometric and thermodynamic studies of 2-Thioxothiazolidin-4-one and its metal complexes," *Chem. Pap*., vol. 59, p. 332, 2005

[36] Y. Sharmeli and R. Lonibala, "Thermodynamics of the complexation of *N*-(Pyridin-2-ylmethylene) isonicotinohydrazide with lighter lanthanides," *J. Chem. Eng. Data*, vol. 54, no. 1, pp 28-34, 2009.

[37] M. M. Krunz and L. B. Pfendt, "Factor analysis of the tungsten (VI)-rutin system," *Microchem. J*., vol. 28, p. 162, 1983.

[38] H. M. Cartwright, "Factor analysis of the tungsten(VI)-rutin system," *Microchem. J.*, vol. 34, pp. 313-318, 1986.

[39] F. L. Mcleans and A. B. Hastings, "The state of calcium in the fluids of the body," *J. Biol. Chem*., vol. 108, pp. 285-322, 1935.

[40] M. F. Beeler and P. G. Catrou, "Disorders of Calcium Metabolism," in *Interpretations in Clinical Chemistry: A Textbook Approach to Chemical Pathology*, Chicago: American Society of Clinical Pathologist, 1983.

[41] A. Gupta, "Stability constant and thermodynamic parameters determination of a semi-synthetic penicillin derivative with various bivalent metal ions (Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Sn^{2+}, $Hg^{2+}$and Pb^{2+}): A potentiometric study," *International Research Journal of Pure & Applied Chemistry*, vol. 3, no. 4, pp. 441-418, 2013.