Acid Dissociation Constants of 5,5-Diphenylpyrrolidine *N*-Aroylthioureas and Stability Constants of their Pt(II) and Ni(II) Complexes in Acetonitrile-Water Hydroorganic Solvent

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Abstract

In this study, acid dissociation constants of 5,5-diphenylpyrrolidine *N*-aroylthiourea derivatives, exhibiting anti(myco)bacterial activity, were determined by potentiometric titration in 30% (v/v) acetonitrile-water hydroorganic solventat 25 ± 0.1 °C, at an ionic background of 0.1 mol / L of NaCl using the HYPERQUAD computer program. Three acid dissociation constants were determined for each compound **1a-e** and we suggest that these acid dissociation constants are related to the carboxyl, enol and enthiol groups. Stability constants of their Pt(II) and Ni(II) complexes were also determined by potentiometric titration under the same conditions stated above using the HYPERQUAD computer program. The ligands behave as bidentate and bind to the metal atom *via* the S and O atoms. In various pH conditions, the different complex forms were formulated as ML_2, MHL_2, MH_2L_2, MH_3L_2, MH_4L_2, MH_5L_2 and $MH_{-4}L_2$ between Pt^{2+}/Ni^{2+} ions and 5,5-diphenylpyrrolidine *N*-aroylthioureas. Stability constants of the complexes show that the ligands **1a-e** form complexes with Pt(II) and Ni(II) metals as 2:1. The stability of the complexes formed by the ligands and platinum were found to follow the order: **3d** > **3a** > **3e** > **3c** > **3b**. The stability of the complexes formed by the ligands and nickel were found to follow the order: **4d** > **4c** > **4a** > **4b** > **4e**. As a result, generally the platinum complexes formed with these ligands are more stable than their nickel complexes.

Keywords—Acid dissociation constant, Aroylthiourea, 5,5-Diphenylpyrrolidine, Nickel, Platinum, Stability constant

1 Introduction

Physicochemical parameters contain critical information to understand the nature of molecules and one of them is the acid dissociation constant (pK_a) [1]. pK_a is an indispensable parameter for the pharmaceutical scientist because it provides information about many features of molecules such as acidity, solubility and hydrogen bonding capacity [2-4]. The majority of drugs are organic-based mol-ecules and numerous new organic molecules containing acidic or basic groups have been synthesized for the determination of their pharmacological activities. Most of the pharmacologically active compounds contain one or more ionizable groups and are capable of gaining and/or losing a proton in aqueous media [2, 5-7]. pK_a values help to predict the degree of ionization of pharmacologically active molecules in different pH regions of the living systems [2, 6]. Furthermore, pK_a values are cru-

cial for drug design and molecular modeling studies [8, 9], selection of the optimum conditions for analysis methods [6] and understanding of the stereochemical structures of active centers of enzymes [10]. For these reasons, many studies have been reported about the determination of pK_a values of pharmacological active compounds by using various analytical methods such as potentiometry [1, 11], spectroscopy [6, 12], chromatography [13, 14] and electrophoresis [15, 16] etc.

The stability constant is another physicochemical parameter which contains very critical information for drug development. The stability constant provides information to understand the formation of bonds in a complex, the bonding mechanisms and the stability of the complex in different pH conditions, to determine the concentration of the components present in a mixture in equilibrium and to reveal the presence of complexes in media [17, 18]. Furthermore, the mechanism of anticancer activity described by dissociation of the ligand and subsequent binding to DNA, resulting in antitumor activity, is also associated with the stability of the organometallic pharmaceuticals [19]

Transition metals play a vital role in living systems and many pharmaceuticals containing transition metals such as platinum, nickel, ruthenium, gold, copper, cobalt, zinc and iron are widely used in the clinical field. Moreover, transition metals have been intensively studied in bioorganometallic chemistry to develop new metal based drugs [20, 21]. Many studies have been reported in the literature about the determination of stability constants of transition metal complexes with various organic ligands with potential pharmacological activity [22-26]. The ability of complex formation between organic ligands and transition metals depends on a number of factors, such as the type of solvent, pH value of the solution and temperature [19, 27].

N-Substituted-*N'*-aroyl(acyl)thiourea derivatives with general formula $R_1C(O)NHC(S)NR_2R_3$ have been extensively studied as ligands in bioorganometallic and coordination chemistry [28], due to their wide range of pharmacological activities such as antibacterial, antifungal [29, 30], antitubercular [31] anticancer [32] and carbonic anhydrase inhibi-

tor [33] activities, and due to the carbonyl and thiocarbonyl groups in their structures, which have a huge bonding potential [28]. Many metal complexes such as Ni(II) [34-36], Pd(II) [36-38], Pt(II) [31, 38], Cu(II) [35, 36] and Co(III) [39, 40] of *N*-aroylthiourea derivatives have been synthesized within the scope of their wide range of pharmacological properties. Furthermore, these types of ligands have been studied in pharmaceutical chemistry as bioactive molecules [41] and intermediate molecules for the synthesis of potential bioactive heterocyclic compounds [42-44]. The pyrrolidine ring is an indispensable scaffold for drug research. The pyrrolidine ring is present in many drug molecules such as procyclidine [45] and bepridil [46]. Pyrrolidines bearing modifiable substituents can be easily synthesized regio- and stereospecifically *via* a 1,3-dipolar cycloaddition reaction [47-53]. Grigg et al. [47-50] and Najera et al [51-53] have published many studies about the synthesis of pyrrolidine derivatives with potential pharmacological properties.

In the previous work [54], we have reported the synthesis and antimicrobial activity of a series of 5,5-diphenylpyrrolidine *N*-aroylthioureas. In this study, the determination of acid dissociation constants of 5,5-diphenylpyrrolidine *N*-aroylthiourea derivatives and the stability constants of their Pt(II) and Ni(II) complexes in acetonitrile-water hydroorganic solvent are reported.

2 Materials and Methods

2.1 Apparatus and Materials. The ligands were prepared according to a literature method [54]. All reagents had analytical grade and were used without further purification. Potassium hydrogen phthalate (Fluka) and sodium tetraborate (Fluka), required for calibration of the electrode systems, were dried and used for the preparation of 0.05 mol / kg potassium hydrogen phthalate and 0.01 mol / kg sodium tetraborate solutions. The ligand solutions were prepared as 1.10^{-3} mol / L in acetonitrile and a 0.025 mol / L NaOH solution served as titrant. Solutions of Pt(II) and Ni(II) ions (1.10^{-3} mol / L) in deionized water were prepared from K_2PtCl_4 and $NiCl_2 \cdot 6H_2O$, respectively, and then standard-

ized with ethylenediaminetetraacetic acid (EDTA) [55]. 0.1 mol / L HCl (Titrisol[R] Merck) and 1.0 mol / L NaCl (Merck) stock solutions were also prepared. An aquaMAX™-Ultra water purification system (Young Lin Inst.) was used for obtaining deionized water with a resistance of 18.2 MΩ.cm.

Titration was performed by using set of the Molspin pH-mV-meter, with an Orion 8102BNUWP ROSS ultra combination pH electrode having an automatic micro burette, and the system was controlled by a PC computer. During the titrations, the temperature in the double-wall glass titration vessel was controlled using a thermostat (DIGITERM 100, SELECTA) and kept at 25.0 ± 0.1 ºC, and the vessel solution was stirred. Calibration of the combination pH electrode was performed by using the buffer solutions of pH 4.005 (potassium hydrogen phthalate) and 9.018 (sodium tetraborate) at 25.0 ± 0.1 ºC in water as required in the instructions of the Molspin Manual [56]. After then, the combination pH electrode was calibrated by using the buffer solution of pH 5.015 (potassium hydrogen phthalate) at 25.0 ± 0.1 ºC in 30 % (w/w) acetonitrile-water [57]. During all of the titrations, the titration vessel was purged through nitrogen (99.9 %). In this study, HYPERQUAD, one of the most useful computer programs which are used to determine acid dissociation constants with high precision and accuracy from potentiometric data [58], was used for calculate the acid dissociation constants and stability constants from potentiometric data.

2.2 Procedure. As described in our previous study [59], the potentiometric titrations were performed in the double-wall glass titration vessel, kept at 25.0 ± 0.1 °C, the titration solution was magnetically stirred. Before and after each titration process, the double-wall glass titration vessel was rinsed and dried. The titration vessel was capped by a lid, containing three holes. Air bubbles were removed during dropwise addition of the alkali solution. The syringe was rinsed with deionized water and several times with the alkali solution before adding the alkali solution. In the first part of this study, to determine of the pK_a values of **1a-e** in 30% (v/v) acetonitrile-water mixture, added 10 mL of the ligand solution, 0.5 mL of the HCl solution and 5 mL of the NaCl solution from their stock solutions

to the titration cell. After that, 5 mL acetonitrile added to titration cell and the titration cell was filled to 50.00 mL with deionized water. In the second part of this study, to determine of the stability constants of the Pt(II) and Ni(II) complexes of **1a-e** ligands in 30% (v/v) acetonitrile-water mixture, added 10 mL of the ligand solution, 5 mL of the K$_2$PtCl$_4$ or NiCl$_2$·6H$_2$O solution, 0.5 mL of the HCl solution and 5 mL of the NaCl solution from their stock solutions to the titration cell. After that, 5 mL acetonitrile added to titration cell and the titration cell was filled to 50.00 mL with deionized water. The data were obtained by titrating 50.00 mL of the prepared titration cell with standardized NaOH solution and three titrations were performed for each compound. Standard deviations quoted only refer to random errors. Volume increment of the NaOH solution was 0.03 mL and for each titration process, 200 data points were obtained. The pK_w value, defined as $-\log[H^+][OH^-]$ for the aqueous system was obtained as 13.98 at the ionic strength employed.

3 Results and Discussion

3.1 Protonation Constants of the Ligands

Acid dissociation constants of 5,5-diphenylpyrrolidine *N*-aroylthioureas **1a-e** were determined potentiometrically at 25.0 ± 0.1 ºC in a 30 % (v/v) acetonitrile-water hydroorganic solvent system. Solutions of the ligands were prepared in acidic media and NaCl solution was used to keep the ionic strength constant at 0.1. As a result of the calculations, three different pK_a values were found for each ligand. pK_{a1}, pK_{a2} and pK_{a3} values were obtained in a range of 3.49 ± 0.07 - 5.19 ± 0.04, 6.96 ± 0.03 - 7.84 ± 0.04 and 8.29 ± 0.02 - 9.94 ± 0.08 respectively. We proposed that pK_{a1} values arise due to carboxylic acid formation by hydrolysis of the methyl ester of **1a-e** in acidic aqueous media (**Fig. 1**) and the result for acidic aqueous media is consistent with the literature [60]. The formation of carboxylic acid derivatives **2a-e** from hydrolysis of methyl esters of **1a-e** in acidic media is shown in **Fig. 1**. According to the protonation of **2a-e** in acidic aqueous media, given in **Fig. 2**, the 5,5-diphenylpyrrolidine *N*-aroylthioureas containing

carboxylic acid groups (**2a-e**) may have five ionizable centers as two carboxyl groups, enthiol, enol and amide NH groups.

According to the obtained pK_a values, it can be suggested that pK_{a1} value is related to the carboxyl group, pK_{a2} and pK_{a3} values are related to the enol and enthiol group respectively. The expected pK_a value range of the amide NH group of **2a-e** is around 10-12. Difference in the structure of **2a-e** originates from the substituent at the 4-position of the aromatic ring of the aroyl group and **2a-e**, given alphabetically, have at that position H, C(CH₃)₃, OCH₃, Cl and Ph respectively. If we examine the changing pK_a values of **1a-e** and make comments about the pK_a values among themselves; in the presence of OCH₃ group as substituent, the highest pK_{a1} value was obtained and decreased significantly the acidity of 1c with 5.19 ± 0.04. The lowest pK_{a1} value, 3.49 ± 0.07, was obtained for **2b** containing a C(CH₃)₃ group as substituent. We can anticipate that the C(CH₃)₃ group should increase the pK_a value. This unexpected result can be explained by the fact that the substituents are at a distance from the carboxyl groups, so the changing pK_{a1} values stem from intra(inter)-molecular interactions. It can be also said that the carboxyl groups interact with the solvent system as another explanation. When comparing the pK_{a2} values of **2a-e**, the lowest pK_{a2} value, 6.96 ± 0.03, was obtained for **2d** containing a Cl group as substituent as expected. The highest pK_{a2} value, 7.84 ± 0.04, was obtained in the presence of a OCH₃ group as substituent. When comparing the pK_{a3} values of **2a-e**, the lowest pK_{a3} value was obtained for **2d** with 8.29 ± 0.02 in the presence of a Cl group as substituent, and the highest pK_{a3} value was obtained for **2e** with 9.94 ± 0.08 in the presence of a phenyl group as substituent. pK_a values for each C(O)NHC(S)N type of ligands in a dioxane-water (50% (v/v)) mixture have been reported [61]. In the previous study [59], we reported that three acid dissociation constants exist for each fused ring thiohydantoin-pyrrolidine derivative having a C(O)NHC(S)N cyclic moiety and suggested that the pKa values were related to their carboxyl, enol andamide NH groups. Herein, we have reported that three pK_a values for each 5,5-

diphenylpyrrolidine *N*-aroylthioureas were determined in acetonitrile-water hydroorganic solvent by using the HYPERQUAD computer programme.
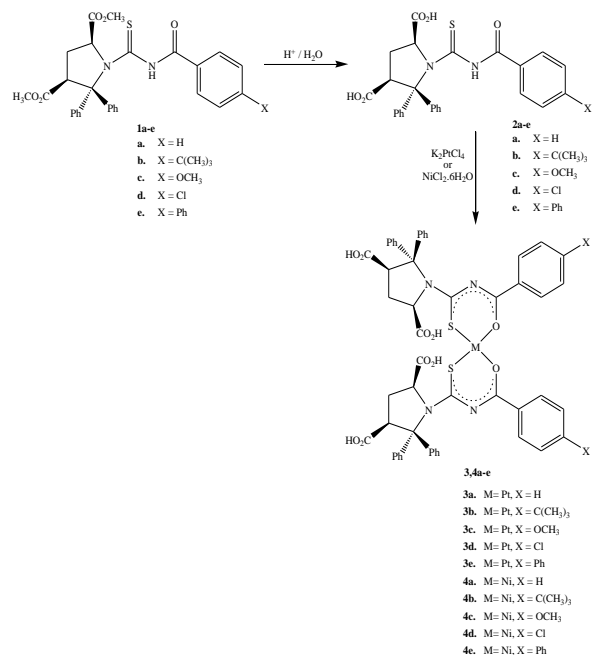

Fig. 1 Hydrolysis of the methyl ester of 5,5-diphenylpyrrolidine *N*-aroylthioureas **1a-e** and their complexation with Pt(II) and Ni(II) ions **3,4-a-e** in acidic media

The data, used for calculation of acid dissociation constants, were potentiometrically obtained by three individual measurements. As a result of calculations, performed with the HYPERQUAD program, three deprotonated species formulated as LH₃, LH₂ and LH were determined. The deprotonation equilibrium for the ligands is given in the following equation 1 (charges are omitted for simplicity) [62].

$$LH_n \rightleftharpoons LH_{n-1} + H \qquad (1)$$

and the deprotonation constants (K_n) are given by equation 2;

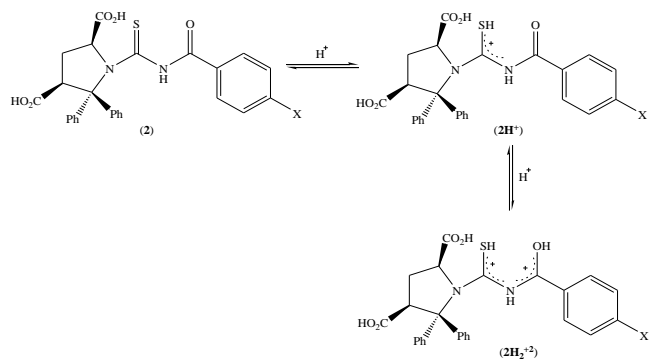$$K_n = [LH_{n-1}][H]/[LH_n] \qquad (2)$$

Fig. 2 A scheme showing the proposed mechanism for protonation of 5,5-diphenylpyrrolidine *N*-aroylthioureas **2a-e**
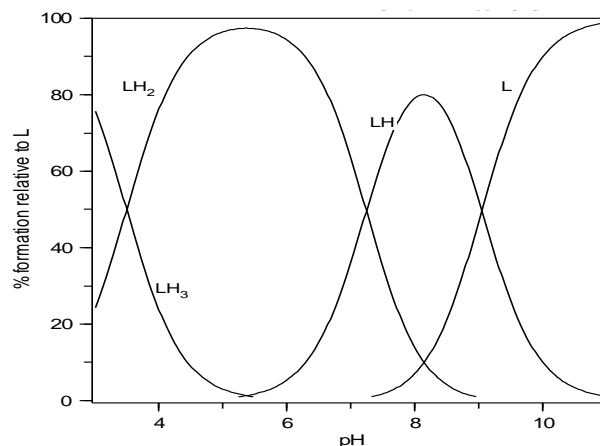
The protonated ligand loses its protons with increasing pH and converts into another species of the ligand (see **Fig. 3**). The titration curves of **2a-e** and the distribution curves of species H in a 30% (v/v) acetonitrile-water mixture are given in **Fig. 3**.
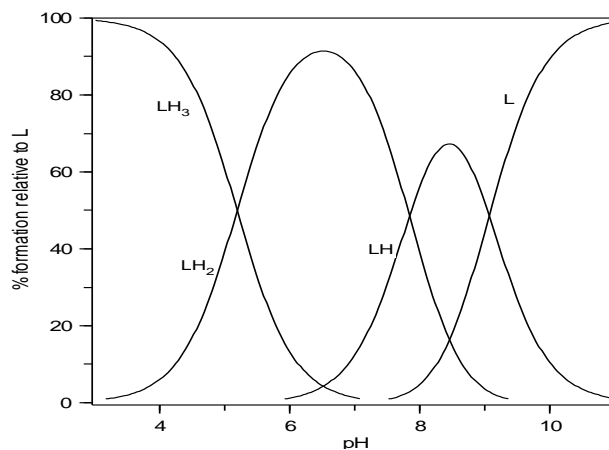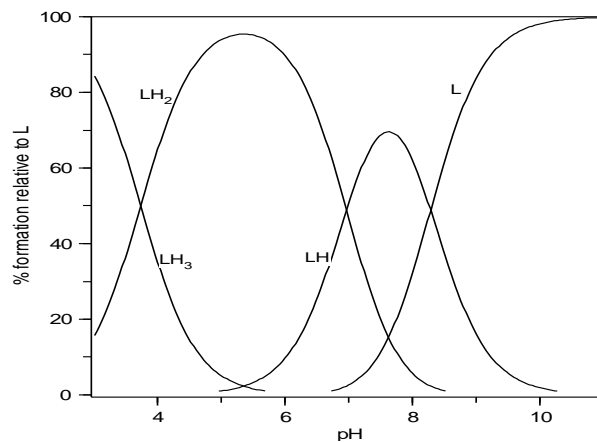

(**a**)

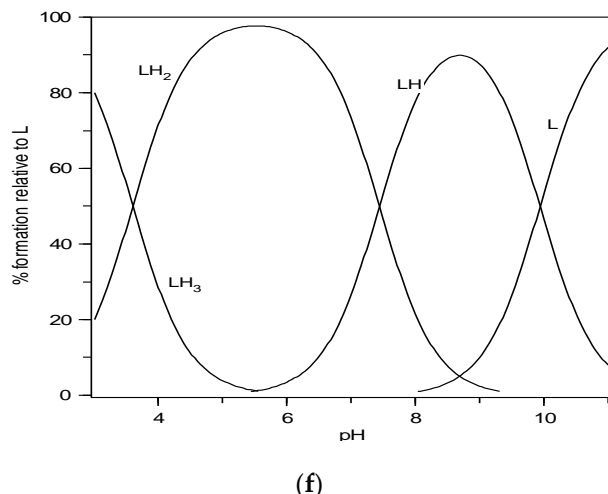(**b**)

(**c**)

(**d**)

(**e**)

(f)
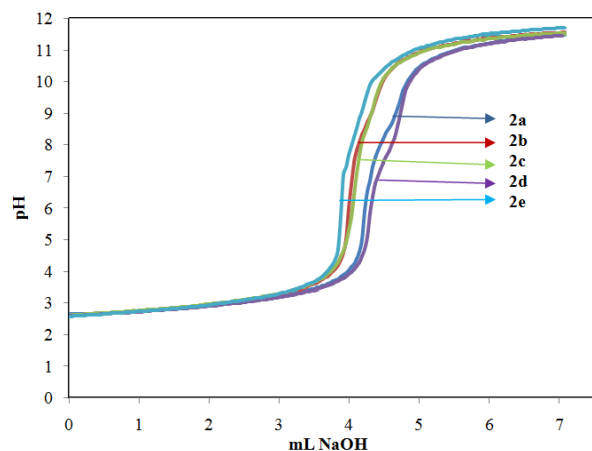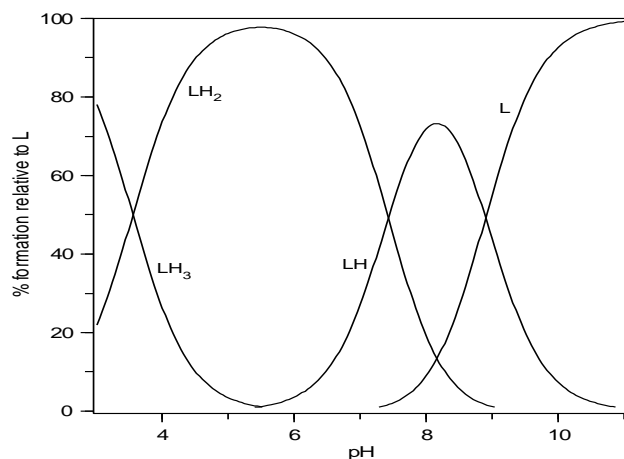
Fig. 3 Potentiometric titration curves (a) and distribution curves of the ligands [(b) **2a** (c) **2b** (d) **2c** (e) **2d** (f) **2e** in a 30% (v/v) acetonitrile-water mixture]

Acid dissociation constants of **2a-e** were computed from data, obtained by potentiometric titrations in acidic media, by using the HYPERQUAD computer program. The determined acid dissociation constants of **2a-e** are given in **Table 1**.

Table 1 pK_a values of the ligands (30% (v/v) acetonitrile-water mixture, 25.0 ±0.1 ºC, I=0.1 mol / L by NaCl) (log$_{10}\beta$ is cumulative acid dissociation constants)

Ligand	Species	log$_{10}\beta$	pK_a Values
	LH$_3$	19.88±0.03	3.55±0.03
2a	LH$_2$	16.33±0.03	7.43±0.03
	LH	8.90±0.03	8.90±0.03
	LH$_3$	19.79±0.03	3.49±0.07
2b	LH$_2$	16.30±0.07	7.25±0.07
	LH	9.05±0.05	9.05±0.05
	LH$_3$	22.10±0.03	5.19±0.04
2c	LH$_2$	16.91±0.03	7.84±0.04
	LH	9.07±0.04	9.07±0.04
	LH$_3$	18.98±0.05	3.73±0.05
2d	LH$_2$	15.25±0.03	6.96±0.03
	LH	8.29±0.02	8.29±0.02
	LH$_3$	20.99±0.03	3.61±0.10

The top-right table (continuation):

2e	LH$_2$	17.38±0.10	7.44±0.10
	LH	9.94±0.08	9.94±0.08

3.2 Stability Constants

The stability constants of 5,5-diphenylpyrrolidine *N*-aroylthioureas **1a-e** with the Pt(II) and Ni(II) ions in 30% (v/v) acetonitrile-water hydroorganic solution were interpreted with the HYPERQUAD computer program.
The cumulative stability constants *(β$_{mlh}$)* are defined in the following equations 3 and 4 [62]:
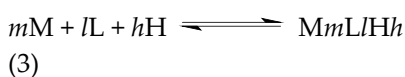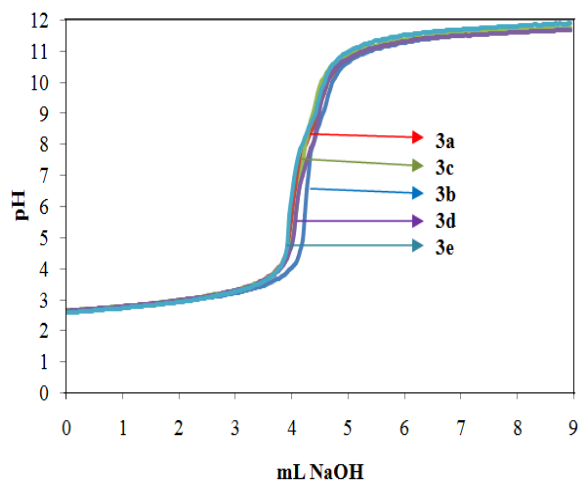
$$m\text{M} + l\text{L} + h\text{H} \rightleftharpoons \text{M}_m\text{L}_l\text{H}_h$$
(3)

$$\beta mlh = [\text{M}_m\text{L}_l\text{H}_h] / [\text{M}]^m[\text{L}]^l[\text{H}]^h$$
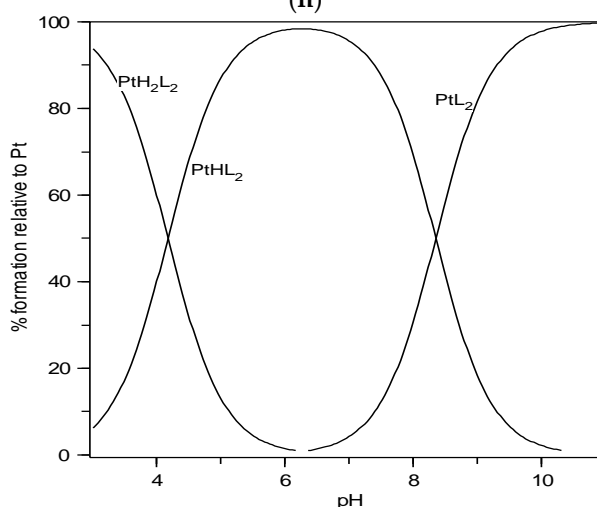(4)

M, L and H denotes the metal ion, the ligand and H, respectively. Respective stoichiometric coefficients are specified as m, l and h. Pt(II)-ligand and Ni(II)-ligand titration curves are given in **Fig. 4** and **Fig. 5**, respectively.
A number of species formulated as PtH$_5$L$_2$, PtH$_4$L$_2$, PtH$_3$L$_2$, PtH$_2$L$_2$, PtHL$_2$ and PtL$_2$of Pt(II)-**2a-e** are formed in acetonitrile-water solution at the different pH ranges. Three different species of **3a** exist during the complexation of the Pt(II) ions with **2a**. The first occurring type is PtH$_2$L$_2$ in acidic media and disappears around pH 6. During addition of base to the titration cell, PtHL$_2$ species also begin to exist in addition to PtH$_2$L$_2$ species in the solution and up to pH 6, where it reaches a maximal occurrence of about 95% and the species disappears around pH 10. PtL$_2$ species start to exist around pH 7 and reaches a maximal occurrence of about 100% over pH 10 and it is the only species in the solution at a pH above 10. During the complexation of the Pt(II) ions with **2b**, six different species of **3b** were observed. The first occurring type is PtH$_5$L$_2$ in acidic media and during addition of base to the titration cell, PtH$_4$L$_2$, PtH$_3$L$_2$, PtH$_2$L$_2$, PtHL$_2$ and PtL$_2$ species, respectively, exist and these species reach a maximal occurrence of about 40%, 95%, 80%, 35% and 100%, respectively, at the appropriate pH points. PtH$_5$L$_2$ and PtH$_4$L$_2$ species
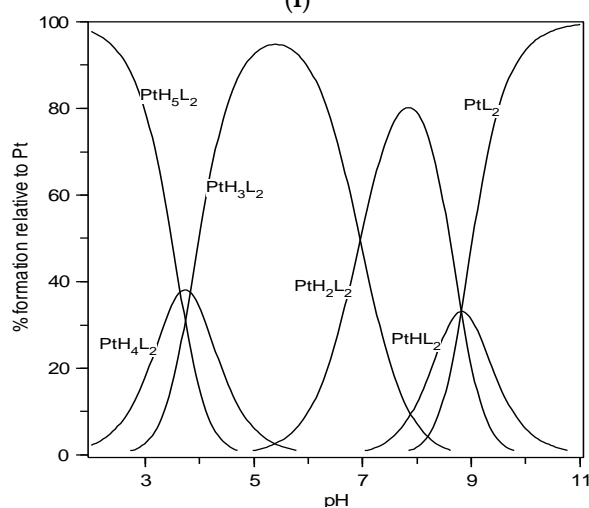
occur in acidic media and disappear before pH 6. PtH_3L_2 and PtH_2L_2 occur in both acidic and basic media, but $PtHL_2$ and PtL_2 species only occur in basic media. PtL_2 species is the only species in the solution at a pH above 11. During the complexation of the Pt(II) ions and **2c**, six different complex species of **3c** exist, by analogy with **3b**. The first occurring type is also the PtH_5L_2 species in acidic media and during addition of base to the titration cell, PtH_4L_2, PtH_3L_2, PtH_2L_2, $PtHL_2$ and PtL_2 species begin to exist, respectively, and the species reach a maximal occurrence of about 75%, 80%, 60%, 58% and 95%, respectively at the appropriate pH points. The PtL_2 species is also the only species in the solution at a pH above 11 in the complexation of the Pt(II) ions and **2c**. The marked differences between complexation of Pt(II) ions with **2b** or **2c** are the maximal occurrence of the species, and the PtH_4L_2 species of **3c** disappears around pH 7 instead of pH 6. Five species formulated as PtH_4L_2, PtH_3L_2, PtH_2L_2, $PtHL_2$ and PtL_2 of **3d** occur during the complexation of the Pt(II) ions and **2d**. PtH_4L_2 and PtH_3L_2 species occur and disappear in acidic media. PtH_2L_2 and $PtHL_2$ occur in both acidic and basic media, but PtL_2 species only occur in basic media. Four different species of **3e** occur during the complexation of the Pt(II) ions and **2e**. The first occurring type is PtH_3L_2 in acidic media and disappears around pH 9. During addition of base to the titration cell, other species formulated as PtH_2L_2, $PtHL_2$ and PtL_2, having a maximal occurrence of about 75%, 15% and 95%, respectively, at the appropriate pH points, of **3e** begin to exist in the media. All the species exist in basic media, but $PtHL_2$ and PtL_2 are never absent in acidic media. The PtL_2 is the only species in the solution at a pH above 10. According to the results, to obtain only PtL_2 species of Pt(II) compexes of the 5,5-diphenylpyrrolidine *N*-aroylthioureas in 30% (v/v) acetonitrile-water hydroorganic solution, the pH value of the media must be higher than approximately pH 11 (**Fig. 4**).
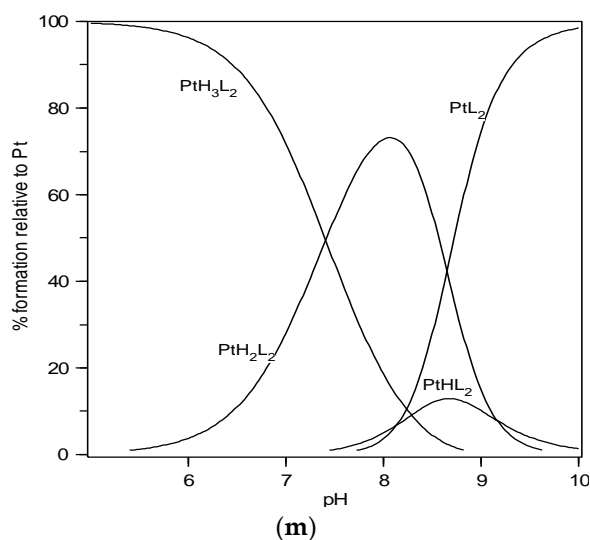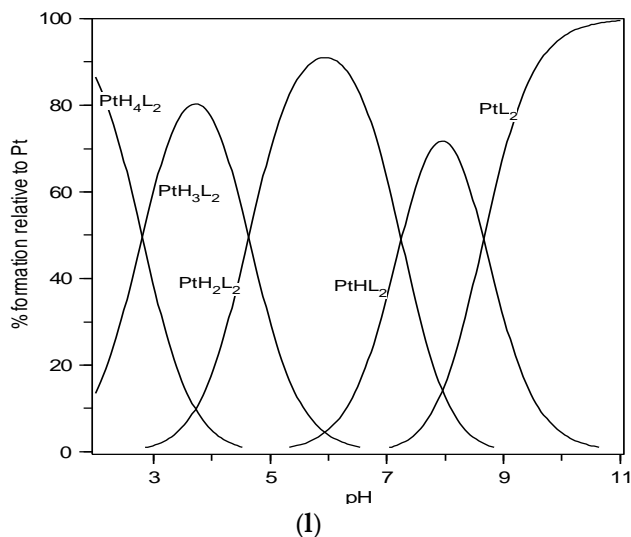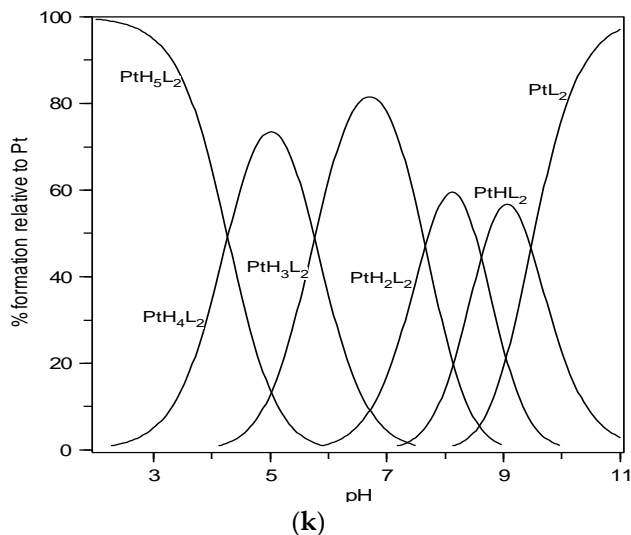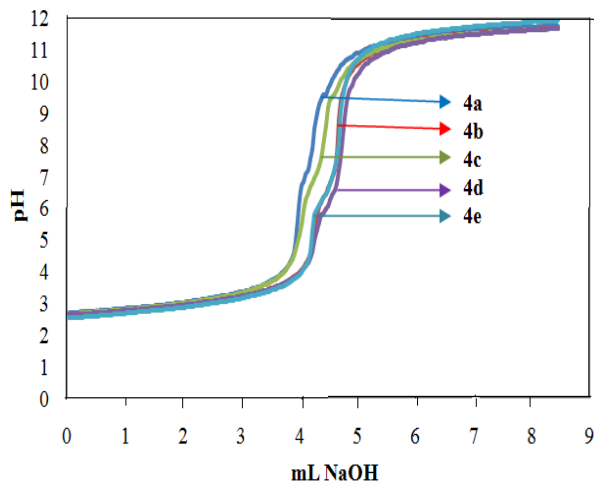

(h)

(i)

(j)

(k)

(l)

(m)

Fig. 4 Potentiometric titration curves (h) of Pt(II) complexes with the ligands and distribution curves

of Pt(II) complexes with the ligands [(i) **3a**, (j) **3b**, (k) **3c**, (l) **3d**, (m) **3e** in a 30% (v/v) acetonitrile-water mixture]

A number of species, formulated as NiH_5L_2, NiH_4L_2, NiH_3L_2, NiH_2L_2, $NiHL_2$, NiL_2 and $NiH_{-4}L_2$, of Ni(II)-**2a-e** are observed in the media at the different pH ranges. Six species, formulated as NiH_5L_2, NiH_4L_2, NiH_3L_2, NiH_2L_2, $NiHL_2$ and NiL_2, of **4a** are observed during the complexation of the Ni(II) ions with **2a**. The NiH_5L_2 species is observed only in acidic media and disappears around pH 6. During addition of base to the titration cell, NiH_4L_2, NiH_3L_2 and NiH_2L_2 species, respectively, are observed in both acidic and basic media and their maximal occurrence is around 95%, 35% and 90%, respectively, at the appropriate pH points. Additionally, $NiHL_2$ and NiL_2 species were observed only in basic media and these species reached a maximal occurrence of about 40% and 95%, respectively, at the pH value of approximately 9.5 and 11, respectively. During the complexation of the Ni(II) ions with **2b**, **2c**, **2d**, seven species were observed, formulated as NiH_5L_2, NiH_4L_2, NiH_3L_2, NiH_2L_2, $NiHL_2$, NiL_2 and $NiH_{-4}L_2$, related to **4b, 4c, 4d** complexes, respectively. NiH_5L_2 species of **4b, 4c, 4d** complexes run out around pH 7. NiH_3L_2 and NiH_2L_2 species of **4b, 4c, 4d** complexes were observed both in the acidic and basic media. $NiHL_2$ and NiL_2 species of **4b, 4c, 4d** complexes were observed only in the basic media and $NiH_{-4}L_2$ species were observed for the three compounds above pH 11. Five different species of **4e** occur during the complexation of the Ni(II) ions and **2e**. The first occurring type is NiH_5L_2 in acidic media and disappears around pH 7. During addition of base to the titration cell, other species formulated as NiH_4L_2, NiH_3L_2, NiH_2L_2 and NiL_2, having a maximal occurrence of about 30%, 90%, 80% and 95%, respectively, at the appropriate pH points, of **4e** begin to exist in the media. All the species exist in the basic media, but NiH_2L_2 and NiL_2 were never absent in acidic media (**Fig. 5**). Furthermore, the $NiHL_2$ species of **4e** could not be determined in these conditions.
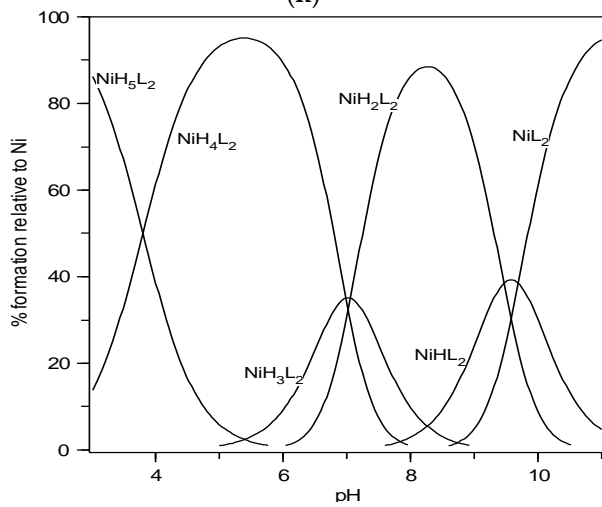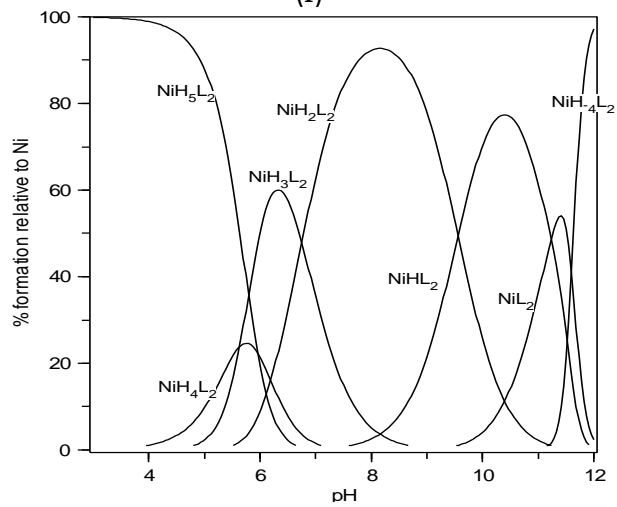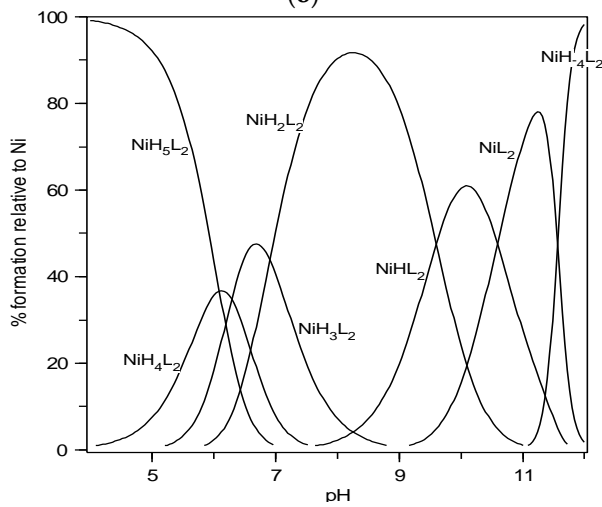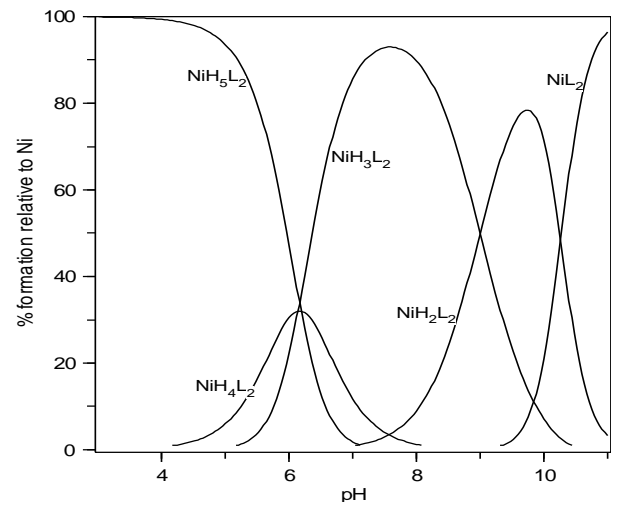
(n)
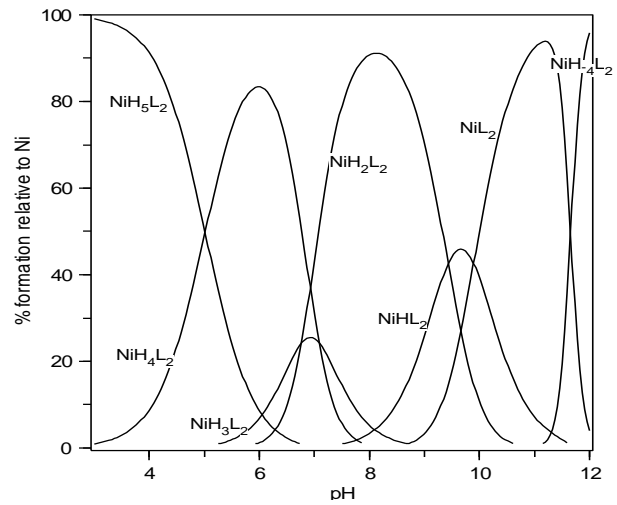
(r)

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(t)

Fig. 5 Potentiometric titration curves (n) of Ni(II) complexes with the ligands and distribution curves of Ni(II) complexes with the ligands [(o) **4a**, (p) **4b**,

(r) **4c**, (s) **4d**, (t) **4e**, in a 30% (v/v) acetonitrile-water mixture]

When we evaluated the stability constants of the Pt(II) and Ni(II) complexes (**Table 2**); the stability constants of the PtL$_2$ and NiL$_2$ species of **3a-e** and **4a-e** complexes, they can be sorted as **3d** > **3a** > **3e** > **3c** > **3b** and **4d** > **4c** > **4a** > **4b** > **4e**. These results show that bonding a Cl as substituent at the 4-position of the aromatic ring increased the stability

of the complex more than other substituents. In other words, to bind electron-withdrawing groups to the aromatic ring of the *N*-aroylthiourea moiety increases the stability of Pt(II) and Ni(II) complexes with 5,5-diphenylpyrrolidine *N*-aroylthioureas.

Table 2 Stability constants of the species of Pt(II) and Ni(II) complexes (30% (v/v) acetonitrile-water mixture, 25.0 ± 0.1 ℃, I=0.1 mol / L by NaCl)

Complex	ML$_2$	MHL$_2$	MH$_2$L$_2$	MH$_3$L$_2$	MH$_4$L$_2$	MH$_5$L$_2$	MH$_{-4}$L$_2$
3a	25.96±0.05	34.31±0.10	38.49±0.07	-	-	-	-
3b	21.30±0.06	30.12±0.06	38.94±0.04	45.89±0.06	49.71±0.14	53.36±0.15	-
3c	21.88±0.08	31.35±0.03	39.97±0.03	47.63±0.04	53.39±0.04	57.66±0.04	-
3d	26.90±0.06	35.56±0.03	42.81±0.04	47.44±0.05	50.25±0.05	-	-
3e	24.36±0.04	32.50±0.10	41.66±0.03	49.07±0.04	-	-	-
4a	21.77±0.07	31.46±0.10	40.93±0.09	47.99±0.09	54.97±0.09	58.77±0.10	-
4b	21.75±0.05	32.34±0.04	41.94±0.05	48.84±0.06	55.13±0.06	61.21±0.06	-24.49±0.11
4c	22.13±0.03	32.03±0.03	41.46±0.04	48.22±0.05	55.31±0.04	60.32±0.05	-24.50±0.11
4d	22.84±0.25	34.73±0.27	44.33±0.27	50.91±0.27	56.66±0.27	62.45±0.27	-23.56±0.25
4e	21.32±0.11	-	41.85±0.12	50.86±0.18	56.99±0.21	63.18±0.13	-

4 Conclusion

In conclusion, the acid dissociation constants of a series of 5,5-diphenylpyrrolidine *N*-aroylthioureas having anti(myco)bacterial activity and the stability constants of their Pt(II) and Ni(II) complexes were potentiometrically determined. Three acid dissociation constants, containing useful information for many different scientific areas, were interpreted with the HYPERQUAD computer program. The determination of the pK_a values was carried out in 30% (v/v) acetonitrile-water hydroorganic solvent at 25 ± 0.1℃, at ionic strength of 0.1. Under these conditions, pK_a values for **1a-e** were calculated and determined three pK_a values for the ionizable species of *N*-Substituted-*N'*-aroyl(acyl)thiourea type compounds. Furthermore, the

stability constants show that 5,5-diphenylpyrrolidine *N*-aroylthiourea ligands form stable complexes with Pt(II) and Ni(II) as 2:1 and other protonated forms of Pt(II) and Ni(II) complexes in different range of the pH value.

5 Acknowledgements

6 References

[1] Mazák, K.; Noszál, B. Advances in microspeciation

of drugs and biomolecules: Species-specific concentrations, acid-base properties and related parameters. J. Pharm. Biomed. Anal. 2016; 130, 390-403.

[2] Babic′, S.; Horvat, A.J.M.; Pavlovic′, D.M.; Kasˇtelan-Macan, M. Determination of pKa values of active pharmaceutical ingredients. Trends in Analytical Chemistry. 2007; 26, 1043–1061.

[3] Meloun, M.; Borovská, S.; Vrána, A. The thermo-dynamic dissociation constants of the anticancer drugs camptothecine, 7-ethyl-10-hydroxycamptothecine, 10-hydroxycamptothecine and 7-ethylcamptothecine by the least-squares nonlinear regression of multiwave-length spectrophotometric pH-titration data. Anal. Chim. Acta. 2007; 584, 419-432.

[4] Roda, G.; Dallanoce, C.; Grazioso, G.; Liberti, V.; De Amici, M. Determination of acid dissociation con-stants of compounds active at neuronal nicotinic ace-tylcholine receptors by means of electrophoretic and potentiometric techniques. Anal. Sci. 2010; 26, 51-54.

[5] Aslan, N.; Erden, P.E.; Doğan, A.; Canel, E.; Kılıç, E. Protonation constants of some alanyl dipeptides in mixed aqueous organic solvents. J. Solution Chem. 2016; 45, 299-312.

[6] Dohoda, D.; Tsinman, K.; Tsinman, O.; Wang, H.; Tam, K.Y. Spectrophotometric pKa determination of ionizable pharmaceuticals: Resolution of molecules with weak pH-dependent spectral shift. . Pharmaceut. Biomed. Anal. 2015; 114, 88–96.

[7] Shelley, J.C.; Cholleti, A.; Frye, L.L.; Greenwood, J.R.; Timlin, M.R.; Uchimaya, M. Epik: a software pro-gram for pKa prediction and protonation state genera-tion for drug-like molecules. J. Comput. Aided. Mol. Des. 2007; 21, 681–691.

[8] Grover, M.; Singh, B.; Bakshi, M.; Singh, S. Quanti-tative structure–property relationships in pharmaceu-tical research–Part 1. Pharma. Sci. Technol. To. 2000; 3, 28-35.

[9] Şanli, S.; Altun, Y.; Şanli, N.; Alsancak, G.; Beltran, J.L. Solvent effects on pKa values of some substituted sulfonamides in acetonitrile-water binary mixtures by the UV-spectroscopy method. J. Chem. Eng. Data. 2009; 54, 3014-3021.

[10] Öğretir, C.; Yarlıgan, S.; Demirayak, Ş.; Arslan, T. A theoretical approach to acidity-basicity behaviour of some biologically active 6-phenyl-4,5-dihydro-3(2H)-pyridazinone derivatives. J. Mol. Struc-Theochem. 2003; 666-667, 609-615.

[11] Narin, I.; Sarioglan, S.; Anilanmert, B.; Sari, H. pKa Determinations for montelukast sodium and levodro-propizine. J. Solution Chem. 2010; 39, 1582-1588.

[12] Sanli, S.; Altun, Y.; Guven, G. Solvent effects on pKa values of some anticancer agents in acetonitrile-water binary mixtures. J. Chem. Eng. Data. 2014; 59, 4015-4020.

[13] Wiczling, P.; Struck-Lewicka, W.; Siluk, D.; Markuszewski, M.J.; Kaliszan, R. The simultaneous determination of hydrophobicity and dissociation constant by liquid chromatography–mass spectrometry. J. Pharmaceut. Biomed. Anal. 2014; 94, 180-187.

[14] Demiralay, E.Ç.; Üstün, Z.; Daldal, Y.D. Estimation of thermodynamic acidity constants of some penicillinase-resistant penicillins. J. Pharmaceut. Biomed. Anal. 2014; 91, 7-11.

[15] Cabot, J.M.; Fuguet, E.; Rosés, M. Internal standard capillary electrophoresis as a high-throughput method for pKa determination in drug discovery and development. ACS Comb. Sci. 2014; 16, 518-525.

[16] Rodríguez-Barrientos, D.; Rojas-Hernández, A.; Gutiérrez, A.; Moya-Hernández, R.; Gómez-Balderas, R.; Ramírez-Silva, M.T. Determination of pKa values of tenoxicam from ^{1}H NMR chemical shifts and of oxicams from electrophoretic mobilities (CZE) with the aid of programs SQUAD and HYPNMR. Talanta. 2009; 80, 754-762.

[17] Kadhum, A.A.A. Determination and studying the factors affecting the stability constant values of some azo dyes. Journal of Al-Qadisiyah for Pure Science, 2008; 13, 1-14.

[18] Rosotti, F.J.C.; Rosotti, H. The determination of stability constants and other equilibrium constants in solution. McGraw-Hill, New York, 1961.

[19] El-Sherif, A.A.; Shehata, M.R.; Shoukry, M.M.; Mahmoud, N. Potentiometric study of speciation and thermodynamics of complex formation equilibria of diorganotin (IV) dichloride with 1-(2-aminoethyl)piperazine. J. Solution Chem. 2016; 45, 410-430.

[20] Baile, M.B.; Kolhe, N.S.; Deotarse, P.P.; Jain, A.S.; Kulkarni, A.A. Metal ion complex-potential anticancer drug. International Journal of Pharma Research Review. 2015; 4, 59-66.

[21] Allardyce, C.S.; Dorcier, A.; Scolaro, C.; Dyson, P.J. Development of organometallic (organo-transition metal) pharmaceuticals. Appl. Organometal. Chem. 2005; 19, 1-10.

[22] Shobana, S.; Subramaniam, P.; Dharmaraja, J.; Arvindnarayan, S. Stability and structural studies on Ni (II)–5-fluorouracil mixed ligand complex systems. J. Solution Chem. 2016; 45, 334-358.

[23] Fazary, A.E.; Al-Shihri, A.S.; Saleh, K.A.; Alfaifi, M.Y.; Alshehri, M.A.; Elbehairi, S.E.I. Di-and tri-valent metal ions interactions with four biodegradable hydroxamate and cataecholate siderophores: New insights into their complexation equilibria. J. Solution Chem. 2016; 45, 732-749.

[24] Fazary, A.E.; Alshihri, A.S.; Alfaifi, M.Y.; Saleh, K.A.; Elbehairi, S.E.I.; Fawy, K.F.; Abd-Rabboh, H.S.M. Gibbs energies of protonation and complexation of platinum and vanadate metal ions with naringenin and phenolic acids: Theoretical calculations associated with experimental values. J. Chem. Thermodynamics. 2016; 100, 7-21.

[25] Polat, F.; Atabey, H.; Sari, H.; Cukurovali, A. Potentiometric study of equilibrium constants of a novel triazine–thione derivative and its stability constants with Hg^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+} metal ions in ethanol and water mixed. Turk. J. Chem. 2013; 37, 439-448.

[26] Jelić, R.; Marković, S.; Petrović, B. Equilibrium studies on complex formation reactions of dichlorido [(R,R)-trans-1, 2-diaminocyclohexane] platinum (II) complex with ligands of biological significance. Monatsh. Chem. 2011; 142, 985-992.

[27] Ferraro, A.; Fabbricino, M.; van Hullebusch, E.D.; Esposito, G.; Pirozzi, F. Effect of soil/contamination characteristics and process operational conditions on aminopolycarboxylates enhanced soil washing for heavy metals removal: a review. Rev. Environ. Sci. Biotechnol. 2016; 15, 111-145.

[28]Saeed, A.; Flörke, U.; Erben, M.F. A review on the chemistry, coordination, structure and biological properties of 1-(acyl/aroyl)-3-(substituted) thioureas. J. Sulfur. Chem. 2014; 35, 318-355.

[29] Saeed, A.; Zaib, S.; Pervez, A.; Mumtaz, A.; Shahid, M.; Iqbal, J. Synthesis, molecular docking studies, and in vitro screening of sulfanilamide-thiourea hybrids as antimicrobial and urease inhibitors. Med. Chem. Res. 2013; 22, 3653-3662.

[30] Döndaş, H.A.; Nural, Y.; Duran, N.; Kilner, C. Synthesis, crystal structure and antifungal / antibacterial activity of some novel highly functionalized benzoylaminocarbothioyl pyrrolidines. Turk. J. Chem. 2006; 30, 573-583.

[31] Plutín, A.M.; Alvarez, A.; Mocelo, R.; Ramos, R.; Castellano, E.E.; da Silva, M.M.; Colina-Vegas, L.; Pavan, F.R.; Batista, A.A. Anti-Mycobacterium tuberculosis activity of platinum (II)/N,N-disubstituted-N′-acyl thiourea complexes. Inorg. Chem. Commun. 2016; 63, 74-80.

[32] Saeed, S.; Rashid, N.; Jones, P.G.; Ali, M.; Hussain, R. Synthesis, characterization and biological evaluation of some thiourea derivatives bearing benzothiazole moiety as potential antimicrobial and anticancer agents. Eur. J. Med. Chem. 2010; 45, 1323-1331.

[33] Saeed, A.; Al-Rashida, M.; Hamayoun, M.; Mumtaz, A.; Iqbal, J. Carbonic anhydrase inhibition by 1-aroyl-3-(4-aminosulfonylphenyl)thioureas. J. Enzyme Inhib. Med. Chem. 2014; 29, 901-905.

[34] Rauf, M.K.; Yaseen, S.; Badshah, A.; Zaib, S.; Arshad, R.; Ud-Din, I.; Tahir, M.N.; Iqbal, J. Synthesis, characterization and urease inhibition, in vitro anticancer and antileishmanial studies of Ni (II) complexes with N,N,N′-trisubstituted thioureas. J. Biol. Inorg. Chem. 2015; 20, 541-554.

[35] Xie, J.; Cheng, Z.; Yang, W.; Liu, H.; Zhou, W.; Li, M.; Xu, Y. Crystal structures and antimicrobial and cytotoxic activities of zinc (II), nickel (II) and copper (II) complexes of N-(piperidylthiocarbonyl)benzamide. Appl. Organomet. Chem. 2015; 29, 157-164.

[36] Nural, Y.; Kilincarslan, R.; Dondas, H.A.; Cetinkaya, B.; Serin, M.S.; Grigg, R.; Ince, T.; Kilner, C. Synthesis of Ni (II), Pd (II) and Cu (II) metal complexes of novel highly functionalized aroylaminocarbo-N-thioyl pyrrolidines and their activity against fungi and yeast. Polyhedron. 2009; 28, 2847-2854.

[37] Maurya, M.R.; Uprety, B.; Avecilla, F.; Tariq, S.; Azam, A. Palladium (II) complexes of OS donor N-(di

(butyl/phenyl) carbamothioyl) benzamide and their antiamoebic activity. Eur. J. Med. Chem. 2015; 98, 54-60.

[38] Nkabyo, H.A.; Hannekom, D.; McKenzie, J.; Koch, K.R. Light-induced cis/trans isomerization of cis-[Pd (L-S, O)₂] and cis-[Pt (L-S,O)₂] complexes of chelating *N,N*-dialkyl-*N′*-acylthioureas: Key to the formation and isolation of trans isomers. J. Coord. Chem. 2014; 67, 4039-4060.

[39] San Tan, S.; Al-Abbasi, A.A.; Tahir, M.I.; Kassim, M.B. Synthesis, structure and spectroscopic properties of cobalt (III) complexes with 1-benzoyl-(3,3-disubstituted) thiourea. Polyhedron. 2014; 68, 287-294.

[40] Yang, W.; Liu, H.; Li, M.; Wang, F.; Zhou, W.; Fan, J. Synthesis, structures and antibacterial activities of benzoylthiourea derivatives and their complexes with cobalt. J. Inorg. Biochem. 2012; 116, 97-105.

[41] Lain, S.; Hollick, J.J.; Campbell, J.; Staples, O.D.; Higgins, M.; Aoubala, M.; McCarthy, A.; Appleyard, V.; Murray, K.E.; Baker, L.; Thompson, A.; Mathers, J.; Holland, S.J.; Stark, M.J.; Pass, G.; Woods, J.; Lane, D.P.; Westwood, N.J. Discovery, in vivo activity, and mechanism of action of a small-molecule p53 activator. Cancer Cell. 2008; 13, 454-463.

[42] Abdelaziz, M.A.; El-Sehrawi, H.M.; Mohareb, R.M. Synthesis, cytotoxicity and toxicity of thieno [2,3-*d*] pyrimidine derivatives derived from 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo [*b*] thiophene. Med. Chem. Res. 2015; 24, 3932-3948.

[43] Stasevych, M.; Zvarych, V.; Lunin, V.; Halenova, T.; Savchuk, O.; Dudchak, O.; Vovk, M.; Novikov, V. Novel anthraquinone-based derivatives as potent inhibitors for receptor tyrosine kinases. Indian J. Pharm. Sci. 2015; 77, 634-637.

[44] Nural, Y.; Döndaş, H.A.; Grigg, R.; Şahin, E. Polysubstituted fused ring bicyclic thiohydantoins from aminocarbo-*N*-thioylpyrrolidines derived from azomethine ylide 1,3-dipolar cycloadditions. Heterocycles, 2011; 83, 2091-2114.

[45] Ettinger, U.; Kumari, V.; Zachariah, E.; Galea, A.; Crawford, T.J.; Corr, P.J.; Taylor, D.; Das, M.; Sharma, T. Effects of procyclidine on eye movements in schizophrenia. Neuropsychopharmacol. 2003; 28, 2199-2208.

[46] Fujiki, A.; Tsuneda, T.; Sugao, M.; Mizumaki, K.; Inoue, H. Usefulness and safety of bepridil in converting persistent atrial fibrillation to sinus rhythm. Am. J. Cardiol. 2003; 92, 472-475.

[47] Elboray, E.E.; Grigg, R.; Fishwick, C.W.G.; Kilner, C.; Sarker, M.A.B.; Aly, M.F.; H. Abbas-Temirek, H. X Y–ZH compounds as potential 1, 3-dipoles. Part 65: Atom economic cascade synthesis of highly functionalized pyrimidinylpyrrolidines. Tetrahedron. 2011; 67, 5700-5710.

[48] Dondas, H.A.; Fishwick, C.W.G.; Gai, X.; Grigg, R.; Kilner, C.; Dumrongchai, N.; Kongkathip, B.; Kongkathip, N.; Polysuk, C.; Sridharan, V. Stereoselective palladium-catalyzed four-component cascade synthesis of pyrrolidinyl-, pyrazolidinyl-, and isoxazolidinyl isoquinolines. Angew. Chem. Int. Edit. 2005; 44, 7570-7574.

[49] Dondas, H.A.; Fishwick, C.W.; Grigg, R.; Kilner, C. 1,3-Dipolar cycloaddition of stabilised and non-stabilised azomethine ylides derived from uracil polyoxin C (UPoC): Access to nikkomycin analogues. Tetrahedron. 2004; 60, 3473-3485.

[50] Dondas, H.A.; Grigg, R.; Thornton-Pett, M. Spiro (pyrrolidinyl-2,3′-benzodiazepines) related to MK-329. Tetrahedron. 1996; 52, 13455-13466.

[51] Cayuelas, A.; Ortiz, R.; Nájera, C.; Sansano, J.M.; Larrañaga, O.; de Cózar, A.; Cossío, F.P. Enantioselective synthesis of polysubstituted spiro-nitroprolinates mediated by a (*R,R*)-me-DuPhos AgF-catalyzed 1,3-dipolar cycloaddition. Org. Lett. 2016; 18, 2926-2929.

[52] Costa, P.R.R.; Sansano, J.M.; Cossío, U.; Barcellos, J.C.F.; Dias, A.G.; Nájera, C.; Arrieta, A.; de Cozar, A.; Cossio, F.P. Synthesis of chromen [4,3-*b*] pyrrolidines by intramolecular 1, 3-dipolar cycloadditions of azomethine ylides: An experimental and computational assessment of the origin of stereocontrol. Eur. J. Org. Chem.2015; 2015, 4689-4698.

[53] Nejera, C.; Sansano, J.M. Asymmetric 1,3-dipolar cycloadditons of stabilized azomethine ylides with nitroalkenes. Curr. Top. Med. Chem. 2014; 14, 1271-1282.

[54] D. Ersen, MSc Thesis, Mersin University, Mersin, 2016.

[55] Jeffery, G.H.; Bassett, J.; Mendham, J.; Denney, R.C. Vogel's Textbook of Quantitative Chemical

Analysis, 5th Edn. Longman, London, 1989.

[56] Pettit, L.D. Academic Software, Sourby Farm, Timble, Otley, LS21 2PW, UK, 1992.

[57] Barbosa, J.; Sanz-Nebot, V. Assignment of reference pH-values to primary standard buffer solutions for standardization of potentiometric sensors in acetonitrile-water mixtures. Fresen. J. Anal. Chem. 1995; 353, 148-155.

[58] Gans, P.; Sabatini, A.; Vacca, A. Investigation of equilibria in solution. Determination of equilibrium constants with the HYPERQUAD suite of programs. Talanta. 1996; 43, 1739-1753.

[59] Nural, Y.; Döndaş, H.A.; Sarı, H.; Atabey, H.; Belveren, S.; Gemili, M. Determination of acid dissociation constants (pK_a) of bicyclic thiohydantoin-pyrrolidine compounds in 20% ethanol-water hydroorganic solvent. Int. J. Anal. Chem. 2014; 2014, Article ID 634194, 6 pages.

[60] Clayden, J.; Greeves, N.; Warren, S.; Wothers, P. Organic chemistry, Oxford University Press, UK, 2007.

[61] Binzet, G.; Zeybek, B.; Kılıç, E.; Külcü, N.; Arslan, H. Determination of the ionization constants of some benzoyl thiourea derivatives in dioxane-water mixture. Journal of Chemistry. 2013; 2013, Article ID 201238, 7 pages.

[62] Atabey, H.; Sari, H.; Al-Obaidi, F.N. Protonation equilibria of Carminic acid and stability constants of its complexes with some divalent metal ions in aqueous solution. J. Solution Chem. 2012; 41, 793-803.