Moroccan Journal of Chemistry
ISSN: 2351-812X
http://revues.imist.ma/?journal=morjchem&page=login
Nartop & al. / Mor. J. Chem. 5 N°4 (2017) 560-572

Synthesis, characterization and investigation of tautomeric, potentiometric and antimicrobial properties of a novel unsymmetric Schiff base and its Fe(III) and Ni(II) complexes

D. Nartop[(a)*], Ö. Özdemir[(b)], P. Gürkan[(b)]

[(a)]Department of Polymer Engineering, Faculty of Techonology, Düzce University, 81620 Düzce, Turkey
[(b)]Department of Chemistry, Faculty of Science, Gazi University, 06500 Ankara, Turkey

** Corresponding author:*
dileknartop@duzce.edu.tr
Received 14 Feb 2017, Revised 10 Sept 2017, Accepted 14 Sept 2017

Abstract

A new unsymmetric Schiff base [(2OH)R-CH=N-(C_6H_4)-CH=N-R'(2OH) R=phenyl, R' = naphthyl] with its Fe(III) and Ni(II) complexes were synthesized by a two step method. Diimine Schiff base and its complexes were characterized by elemental analysis, mass spectra, IR, $^{1}H/^{13}C$-NMR spectra, TGA analysis, electronic and magnetic measurements. The phenol-imine and keto-amine tautomerism of the unsymmetric Schiff base was investigated with NMR techniques and UV-visible spectra in different solvents. Also, the protonation constants of the ligand and the stability constants of its Ni(II) and Fe(III) complexes were determined potentiometrically in 1:1(v/v) ethanol-water mixture at an ionic strength of 0.5 mol·L^{-1} KCl and at 25.0 ± 0.1 ^{o}C. The antifungal, antimicrobial activities and the minimum inhibitory concentration (MIC) values of the compounds were evaluated against *Escherichia coli* (0157:H7), *Micrococcus luteus* (NRRLB 4975), *Bacillus cereus* (RSKK 863) and *Candida albicans* (ATCC 16231).

Keywords: *Unsymmetric Schiff base; Reduction; Tautomerism; Potentiometry; Antimicrobial activity.*

1. Introduction

Schiff bases are one of the well known classes of ligands in coordination chemistry due to their ease of preparation, structural discrepancies and capability of complexes with metal ions. They have wide application areas e.g. antibacterial, antitumor activities, photochromic, thermochromic properties and catalytic features [1-12]. Symmetrical Schiff bases can be synthesized simply by condensation reaction between aromatic diamines with aldehydes or aromatic dialdehydes with amines as the type of (~CH=N-Ar-N=CH~) or (~N=HC-Ar-CH=N~). However, (~CH=N-Ar-CH=N~) type unsymmetrical Schiff bases cannot be synthesized directly. Recently, we reported these type unsymmetrical diimines and some of their metal complexes [13-17], and herein we report a new unsymmetrical Schiff base (L) and its Ni(II) and Fe(III) complexes (NiL and FeL). Phenol-imine and keto-amine tautomers occur when forming (O-H…N) and (O…H-N) type hydrogen bonding in the molecule. The presence of ortho-hydroxy group in Schiff bases has been considered as one of the important factors for the existence of intramolecular hydrogen bonds and tautomerism [18-20]. In this article, phenol-imine and keto-amine tautomerism of the unsymmetric Schiff base is investigated both in different solutions with UV-visible spectra and in solid state with $^{1}H/^{13}C$-NMR and IR spectra. Potentiometric studies provide valuable information about the basicity, acidity or complex formation features of the compounds in the solution [21]. Herein, potentiometric protonation constants of the ligand and stability constants of its ML-type Ni(II) and Fe(III) complexes are also determined in 1:1(v/v) ethanol-water mixture. Furthermore, the unsymmetrical Schiff base and its complexes are evaluated for antibacterial activity by the well-diffusion method against *Escherichia coli* (0157:H7), *Micrococcus luteus* (NRRLB 4975), *Bacillus cereus* (RSKK 863) and for antifungal activity against *Candida albicans* (ATCC 16231). Complexes, especially FeL, showed good antibacterial effect and low MIC values against gram positive bacteria and the fungus. Its efficiency against fungus is especially important as there are only a small number of antifungal agents in clinical use because of their ineffectiveness or toxicity [22,23].

2. Experimantal

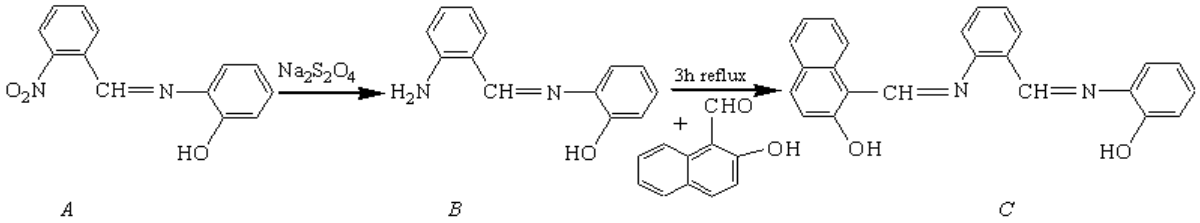
2.1. Materials and instrumentation

All reagents were commercially available and used without further purification. Elemental analysis was carried out with a LECO-CHNS-9320 analyzer. ^{1}H and ^{13}C-NMR spectra were recorded with a Bruker Avance DPX-400 using TMS as internal standard and DMSO-d_6 as solvent. IR spectra were recorded on a Mattson-1000 FTIR instrument in KBr pellets. UV-vis spectra were recorded on a Shimadzu UV-160 A spectrophotometer. Mass spectra were recorded at 70 eV on a Agılent 1100 MSD mass spectrometer. The TGA curves were obtained using a Du Pont Instrument 951 between 35-800 ^{o}C at a heating rate of 10 ^{o}C min^{-1} in nitrogen. Metal contents were determined with a Perkin Elmer Analyst 800 model AAS. Magnetic measurements were determined using a Sherwood Scientific MKI model Evans magnetic balance. Molar conductivities in DMF (10^{-3} mol/L) at 20 ^{o}C were measured using a Siemens WPA CM 35 apparatus. Melting points were determined with a Gallenkamp melting point apparatus. Antimicrobial activities and MIC values of the compounds were determined in Gazi University MOBAM (Molecular Biology Research and Training Center).

2.2. Synthesis of the ligand

The starting Schiff base 2-hyhroxy-N-(2-nitrobenzylidene)aniline (**A**) was synthesized by reacting equimolar amounts of 2-nitro-benzaldehyde with 2-hydroxy aniline in EtOH, as reported in the literature [24,25] (Scheme 1). The unsymmetric diimine (L) was prepared by using our two-stage method, as described previously [14]. Firstly, 2 mmol of (**A**) was dissolved in 80 mL ethanol-water solution (1:1) at 70 ^{o}C. 5 mmol of sodium dithionite was slowly added to

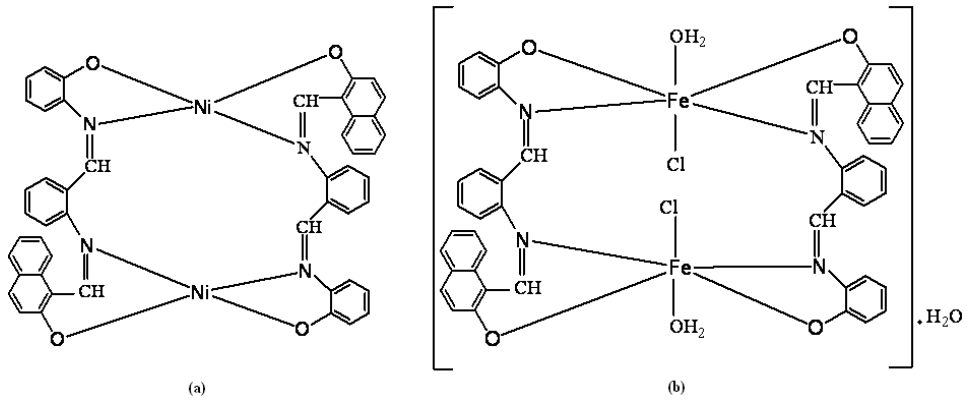
this solution in small portions over the course of 1 hour and the solution was stirred for 1 hour at 50 °C for finishing the reducing process. Thus, the amino derivative of the Schiff base (B) was obtained in solution. Secondly, 2 mmol of 2-hydroxy-1-naphthaldehyde in 50 mL ethanol was added to the solution B and was heated to reflux for 3 hours. The resulting solution (C) was evaporated at room temperature for 1 day. The orange crude product was treated with warm water and was recrystallized from ethanol.



Scheme 1. Reactions scheme of unsymmetric Schiff base (L).

2.3. Synthesis of the complexes

The Ni(II) and Fe(III) complexes were prepared by the same general procedure. The solution of metal chloride in ethanol (10 mL) was added to unsymmetric diimine ligand solution (30 mL). The mixture was stirred and heated for about 4 hours at 70 °C. The resulting solution was evaporated at room temperature for approximately 4-5 days, until a precipitate was formed. The solid was removed by filtration, washed with hot water, ethanol and ether, respectively. The product was dried in a vacuum desiccator over anhydrous $CaCl_2$. The suggested structures of NiL and FeL complexes are given in Scheme 2.



Scheme 2. Suggested structure of metal complexes: NiL (a), FeL (b).

2.4. Absorption measurements of L for tautomeric equilibria

For the determination of tautomeric equilibria, the solutions of **L** in cyclohexane, toluene, chloroform, ethanol and DMSO were prepared in pure solvents, acidic and basic media. 0.2 mL trifluoroacetic acid and triethylamine were added into the pure solutions for the acidic and basic media. Spectra were taken between 250-550 nm.

2.5. Potentiometric reagents and solutions

The potentiometric measurements were carried out in (1:1) aqueous-ethanol media (v/v). Ethanol was purchased from Merck.Doubly distilled and CO_2 free water was used exclusively. Stock solutions of strong acid and base were prepared using analytical reagent grade 0.1 M hydrochloric acid (Merck) and 0.1 M potassium hydroxide (Merck) solutions. The KOH solution (0.0054 M) was standardized by titration against the primary standard oxalic acid dihydrate (Aldrich) and then the aqueous HCl solution (0.0527 M) was standardized by titration with the KOH (0.0054 M). During each titration the ionic strength was maintained at 0.5 M KCl (Merck, extra pure). All metal ion

solutions were prepared from their analytical grade chlorides. They were standardized by atomic absorption spectroscopy method.

2.6. Potentiometric measurements

All potentiometric measurements were carried out with a double walled glass cell that was thermostated at 25.0 ± 0.1 ^{o}C. A Jenway pH-meter equipped with an ISE combined glass electrode. Electrode system was kept in 1:1 (v:v) aqueous-ethanol media. According to Gran's method, the system was calibrated with titration of HCl solution with KOH solution in 0.50M KCl ionic strength for read the hydrogen ion concentration [26,27]. Small amounts of titrant (5 µL) KOH solution were added with a microburette. The following solutions were titrated potentiometrically against 0.0054 M KOH:

- HCl + KCl (for cell calibration)
- HCl + KCl + ligand (for the determination of protonation constants)
- HCl + KCl + ligand + metal ion (for the determination of stability constants).

The metal-to-ligand ratio were 1:4 for Ni(II) and 1:8 for Fe(III). The Ni(II) concentration was held at 0.0050 M and Fe(III) concentration was held 0.0020 M. The computations of the protonation constants of the unsymmetric diimine ligand from potentiometric data was performed with the PKAS computer program [28]. The experimental method of Bjerrum and Calvin as modified by Irving and Rossoti was used to determine ň, $\check{n}_A$ and p*L* values [29]. The stability constants of the Ni(II) and Fe(III) complexes were calculated using the equation:

logK = p*L* / log(ň / 1-ň)

2.7. Antibacterial and antifungal activities

The unsymmetric ligand and its complexes were tested for their in vitro antibacterial and antifungal activities against three bacteria and a yeast for three different concentrations (1000, 500 and 250 µg/mL) by the agar-well diffusion method. Suspensions of activated microorganisms were adjusted to 0.5 McFarland and poured into Nutrient broth agar at the concentration of 1%. 20 mL of these samples were transferred the sterilized petri dishes and were allowed to solidify. Then holes of 12 mm diameter were punched using a sterile cork borer and they were filled with 100 µl of the stock solutions. The plates were incubated for 24 h at 37 °C and inhibition zones generated around the holes were measured with a compass. Gentamicin (30 µg) and amikacin (10 µg) disks were used as a reference. The mean values of two tests were taken for each sample. Determination of MIC (µg/mL) values were done using serial dilutions (1, 2, 4, 8, 16, 32, 63, 125, 250, 500, 1000 µg/mL).

3. Results and Discussions

3.1. Structure of the unsymmetric diimine Schiff base

The newly synthesized ONNO type tetradentate ligand is unsymmetrical with respect to the 'direction' of the imine bond (i.e. –CH=N-aryl-CH=N–). Structure of the ligand was characterized by elemental analysis, IR, ^{1}H/^{13}C-NMR spectroscopy and mass spectra. Phenol-imine and keto-amine tautomerism is one of the most characteristic properties of the 2-hydroxy Schiff bases, which can be investigated using several techniques e.g. NMR, FT-IR, mass, X-ray, UV-vis spectroscopies [30-35]. The IR spectral data of the ligand is given in Table 1. The strong broad band within the range 2000–3500cm^{-1}, centered at 3428 cm^{-1}, show the presence of (O-H…N) intramolecular hydrogen bonding due to phenolic OH groups of the phenol-imine forms [36,15]. It is possible to assign the C=O and the C=N vibrations which are either specific to the keto or the phenol forms, from IR spectra of the compounds. Herein, C=O vibration are not observed but two strong bands are observed at 1587 cm^{-1} and 1625 cm^{-1} because of the unsymmetrical imine groups.

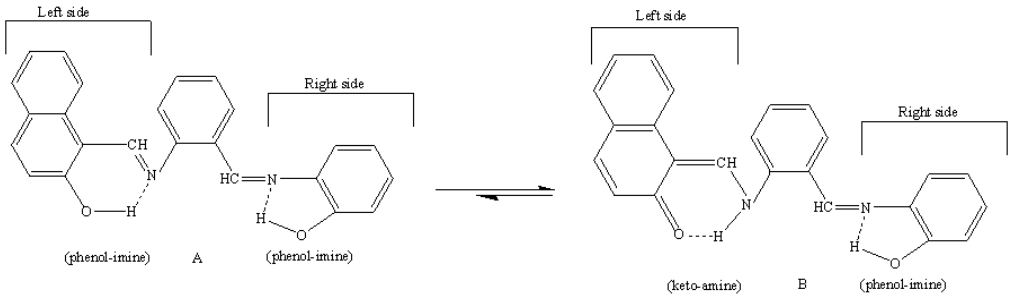
These findings are proved that diimine exists in the phenol form [37]. Furtermore, the existence of the strong phenolic C–O band at 1237 cm^{-1} and nonexistence of the NH band is the other evidence for the phenol-imine form of the diimine in the solid state [15]. The νCH(aromatic) and νC=C(ring) are observed at 3025 and 1638 cm^{-1}, respectively.

Table 1. Analytical and physical properties, selected IR bands and electronic spectral data of the ligand and its Ni(II) and Fe(III) complexes.

Comp.	Empirical Formula (Mol. weight)	Colour $\mu_{(BM)}$	Found (Calcd) % C H N M	λ_{max}(nm)[a] (ε/(mol.cm^{-1}))x10^4	ν(OH) ν(H_2O)	ν$(CH)_{arom.}$	ν(C=N)	ν$(C=C)_{ring}$	ν$(CO)_{arom.}$	νM-N νM-O
L	$C_{24}H_{18}N_2O_2$ (366 g/mol)	orange -	78.33 5.19 7.74 - (78.69) (4.92) (7.65) -		3428[c], 2195[b] -	3025	1625 1587	1638	1237	- -
NiL	$[Ni(C_{24}H_{16}N_2O_2)]_2$ (854 g/mol)	green 0	68.33 3.85 6.24 13.99 (68.25) (3.79) (6.63) (13.74)	233 (1.3), 260 (9.0) 348 (0.7), 477(0.2)	- -	3028	1598 1562	1622	1307	454 479
FeL	$[Fe(C_{24}H_{16}N_2O_2)ClH_2O]_2.H_2O$ (964 g/mol)	brown 5.80	59.60 4.28 5.37 11.87 (59.75) (3.94) (5.81) (11.62)	234 (1.9), 250 (1.1) 311 (0.7), 503(0.3)	- 3403	3044	1600 1574	1631	1316	455 481

a: in ethanol, c: center, b: broad

^{1}H and ^{13}C-NMR spectral data of the ligand are given in Table 2. NMR spectral data of the compounds give information concerning the proton transfer equilibrium and intramolecular hydrogen bonds. Tautomerism studies of α-hydroxynaphtaldimine Schiff bases show that downfield shift of the OH proton is clearly due to strong intramolecular (O-H…N) hydrogen bonding [33]. L ligand can be supposed to be composed of two parts bound to central 1,2 phenylene ring:naphthaldimine parts in the left side and phenylazomethine parts in the right side. A six membered ring is formed by the intramolecular hydrogen bonding in the left side, while a five membered ring occurs in the right side. O…H-N type hydrogen bonding is formed in the keto-amine tautomer, but O-H…N type hydrogen bonding is formed in the phenol-imine tautomer. Tautomeric forms of the L are given in Scheme 3.



Scheme 3. Tautomeric forms of the L.

^{1}H-NMR and ^{13}C-NMR spectral data of the ligand in DMSO-d_6 show that keto-amine tautomer is dominant in the six membered chelate ring (left side) and tautomer exists mostly in the five membered chelate ring (right side) (Figure 1). As seen in Table 2, amino (NH) and enamine (=CH-) protons related with the keto-amine tautomer in the left side are observed at δ 15.68 ppm and at δ 8.38 ppm as a doublet (1H, $^3J_{NHCH}$ = 8.391 Hz). In the right side of molecule**,** phenolic (OH) and imine (CH=N) protons of phenol-imine form occur at δ 10.28 ppm (1H) and at δ 9.49 ppm (1H) as dublet peaks. NH and OH signals are also defined with D_2O exchange. Aromatic protons resonate at the range 6.76-7.94 ppm (multiplet). In the proton de-coupled ^{13}C-NMR data of L, the peaks at δ 177.9 ppm and δ 138.3 ppm belong to (**C**=O) and (=**C**-N) carbon atoms of the keto-amine tautomer of the left side, respectively. The phenolic (**C**-O) and imine (**C**=N) carbon atoms of the right side (five membered ring) are observed at δ 150.0 ppm and δ 149.0 ppm, respectively. Aromatic carbon atoms resonate at the range 116.5-134.4 ppm respectively.

Table 2. ^{1}H and ^{13}C-NMR data of the L and ^{1}H-NMR of the NiL.

Comp.	Left side (six membered ring)					Right side (five membered ring)		
	Phenol-imine form		Keto-amine form			Phenol-imine form		
	C**H**=N	O-**H**	=C**H**-	^{3}J [b]	N-**H**	C**H**=N	O-**H**	Ar-H
	C=N	**C**-O	=**C**-N		**C**=O	**C**=N	**C**-O	Ar-C
L	-	-	8.38 (d,1H)	8.391	15.68 (d, 1H)	9.49 (d,1H)	10.28(d,1H)	6.76-7.94 (m)
	-	-	138.3	-	177.9	149.0	150.0	116.5-134.4
NiL	8.30 (d,1H)	-				9.39 (d, 1H)	-	6.69-7.85 (m)

d:dublet, m:multiplet

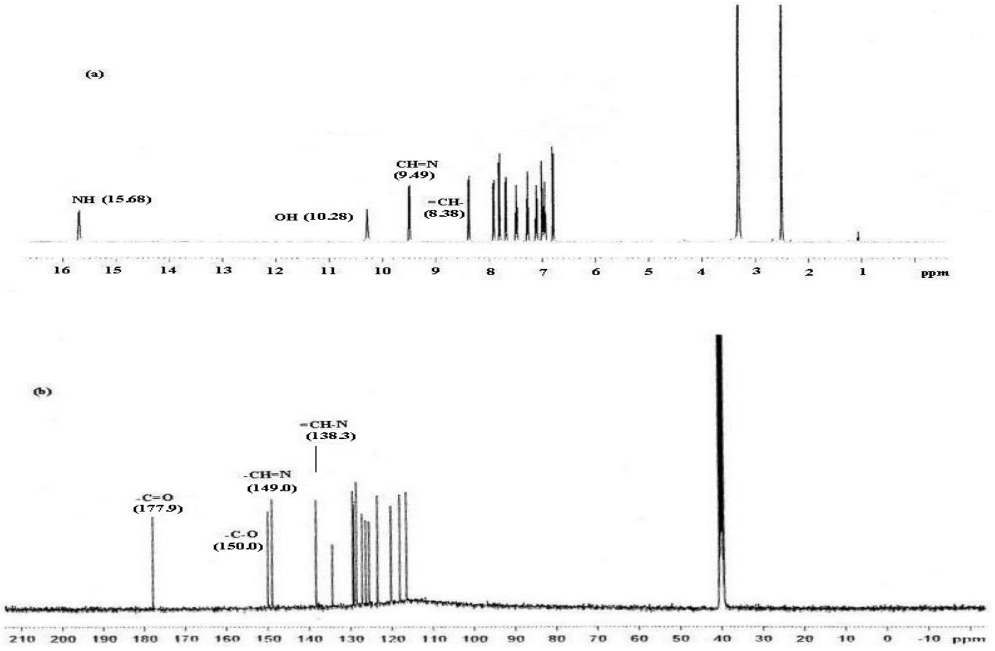
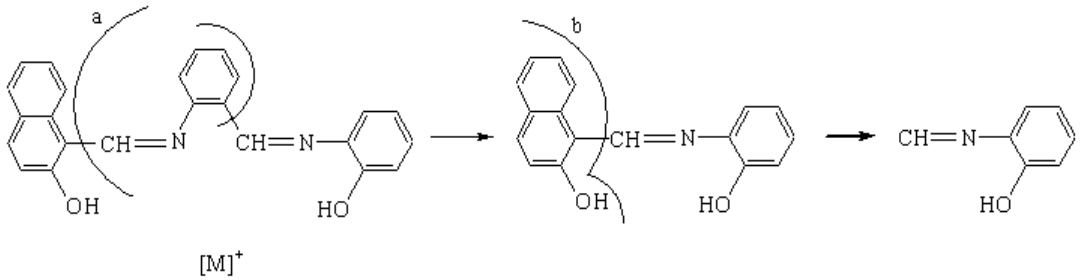


Figure 1. ^{1}H-NMR (a) and ^{13}C-NMR (b) spectra of the L.

In the LC-Mass spectrum of L, the principal peak presented at m/z: 264.0 may be ascribed to the elimination of the fragment ion $[C_7H_5N]^+$ from the molecular ion $[M+H]^+$ (Scheme 4-a). The other appreciably peak at m/z: 120.1 (2.5%) may be attributed the expulsion of $[C_{10}H_7O]^+$ (Scheme 4-b). Molecular ion peak of the compound could not be determined.



Scheme 4. Fragmentation pathways of the L.

3.2. Structures of the complexes

Structure of the complexes were characterized by elemental analysis, IR, ^{1}H/^{13}C-NMR spectroscopy, TGA analysis,

UV-visible and mass spectra. Some analytical and physical results of the complexes are given in Table 1. Reaction of ONNO type tetradentate unsymmetric diimine with nickel(II) chloride and iron(III) chloride, gave dimeric and binuclear complexes (Scheme 2). The diamagnetic behavior of the $[NiL]_2$ type suggests square planar geometry. $[FeL(H_2O)Cl]_2.H_2O$ type is paramagnetic and octahedral complex. Magnetic moment value of the Fe(III) complex is 5.80 BM. This value is indicative of interaction between two Fe(III) centers in the dimeric molecules [39]. All of the complexes are amorphous solids and their melting points are above 360°C. They are stable at room temperature and nonelectrolyte nature. They are slightly soluble in acetone and methanol, soluble in DMF, DMSO and insoluble in water. The IR spectral data of the complexes are given in Table 1. In the spectra of the complexes, two strong bands are observed in 1598 and 1562 cm^{-1} for NiL, 1600 and 1574 cm^{-1} for FeL, due to the unsymmetric imine groups. The νC=N vibrations are shifted to lower frequencies ca 13-27 cm-1 as compared to the ligand. These shifts confirm that imine nitrogen atoms are coordinated to the metal ions. The new bands appeared at 454 cm^{-1} and 479 cm^{-1} for NiL and 455 cm^{-1} and 481 cm^{-1} for FeL can be assigned to the νM-N and νM-O respectively. ^{1}H-NMR spectral data of the NiL complex are given in Table 2. The ^{1}H-NMR spectra of the complex exhibits two signals at 8.30 and 9.39 ppm, because of the different chemical environments of the unsymmetrical imine protons. These signals are observed in the lower field than ligand and phenolic protons in the ^{1}H-NMR spectrum disappears on complexations, suggesting the coordination through phenolic oxygen atoms and azomethine groups. The aromatic protons are also observed in the range 6.69-7.85 ppm. In the LC-mass spectra of NiL and FeL complexes, the molecular ion peaks are observed at the predicted values of m/z: 844.4 [M]+ (4.5%) and m/z: 964.5 [M]+ (0.8%), respectively. The values of the molecular ion peaks are consistent with the proposed dimeric structures of unsymmetric Schiff bases. The TGA curve of the FeL consists of two decomposition steps at ca 75-173 °C and 290-800 °C (Figure 2). The first step seems to be consistent with loss of hydration and coordination water. NiL complex decomposes in one step. The residues at the end of the decomposition for FeL and NiL complexes are found to be Fe_2O_3 and NiO, respectively. The general UV-vis bands of the complexes, which are recorded in ethanol, are given in Table 1. The higher energy bands at 233 nm and 234 nm are assigned to Π-Π* transition of aromatic rings. The medium energy bands at 250 nm and 260 nm are attributed to n-Π* transition of the imine groups. The lower energy bands at 311 nm and 348 nm are assigned to charge transfer transition. In the electronic spectra of square-planar NiL complex, a weak absorption band at 477 nm assigned to $^1A_1g \rightarrow {}^1A_2g$ transition [40]. The electronic spectra of FeL shows the new band at 503 nm. It may be due to the L→M charge transfer, obscuring the low intensity, spin forbidden d–d bands [41].

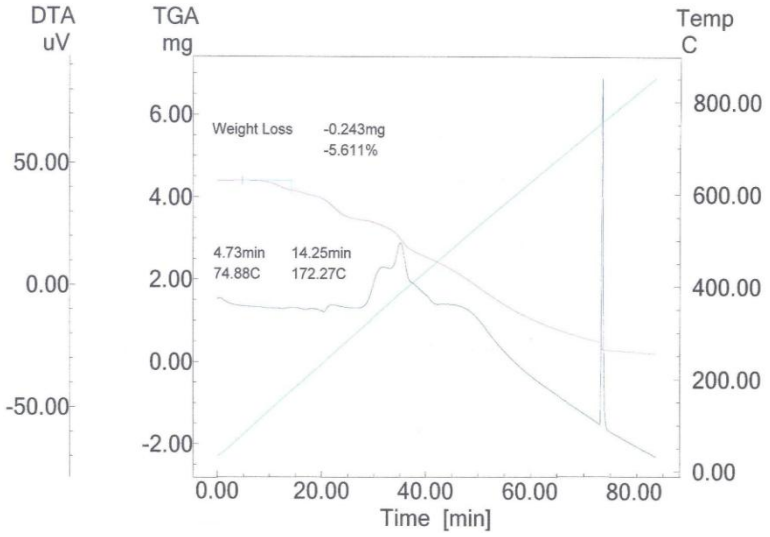


Figure 2. The TGA curves of FeL.

3.3. Tautomeric equilibria with UV-visible spectroscopy

UV-visible spectroscopy is known to be a very sensitive method for studying o-hydroxy salicylaldimine or naphthaldimine derivatives. Presence of a new band above 400 nm indicates the existence of keto-amine tautomer of the Schiff bases. The band below at 400 nm shows the phenol-imine tautomer [42]. For tautomeric studies, the UV-vis spectra of the unsymmetric ligand is measured in polar (DMSO, ethanol and chloroform) and non-polar (toluene and cyclohexane) solvents and also in acidic and basic media at a concentration of approximately 10^{-5} M. Trifluoroacetic acid and triethylamine are added to achieve acidic and basic media, respectively. The absorption maxima (λ_{max}), molar extinction coefficients (ε) and calculated percentages of the keto-amine tautomers are given in Table 3. The molar extinctio-n coefficients for toluene and cyclohexane were not calculated, because of their low solubility. Figure 3 shows UV spectra of ligand in different solvents (a), in acidic (b) and basic media (c), respectively.

Table 3. Absorption maxima (λ_{max}) and percentage of keto-amine tautomer for the L in various solvents, acidic and basic solutions.

Comp.	Tautomer	$\lambda_{max.}$, nm (Є x 10^4, M^{-1} cm^{-1})														
		DMSO			Ethanol			Chloroform			Toluene			Cyclohexane		
		Pure solvent	Acidic media	Basic media	Pure solvent	Acidic media	Basic media	Pure solvent	Acidic media	Basic media	Pure solvent	Acidic media	Basic media	Pure solvent	Acidic media	Basic media
L	Phenol-imine	319(2.4) 352(1.8)	317(2.0) 357(1.6	289(2.5)	322(1.1) 340(1.2)	366(0.9)	326(1.1)	327(1.4) 397(1.5)	305(0.8) 378(1.4)	326(1.3)	322 372	327 372	322 362	323 350 385	304 377	318
	Keto-amine	450(2.7) 471(2.6)	442(0.9) 471(0.7)	429(2.5) 473(2.4)	452(3.1) 461(3.0)	441(2.1)	451(2.6) 472(2.4)	449(1.6) 469(1.4)	447(3.6) 469(2.7)	450(1.8) 469(1.6)		445 468	451 473		471	449 468
	%[a]	53	31	51	74	66	72	52	71	57	-	54	35	-	60	41

a: as keto-amine percent

The absorption spectra of L shows four absorption maxima between 319-327 nm, 340-397 nm, 449-452 nm and 461-471 nm in pure polar solvents (DMSO, ethanol, chloroform) as shown in Figure 3-a and the Table 4. The bands in the range of 319-327 nm and 340-397 nm are attributed to the phenol-imine tautomer, while the bands in the range of 449-452 nm and 461-471 nm are assigned to the keto-amine tautomer. These results suggest that unsymmetric ligand may exist as a mixture of phenol-imine and keto-amine tautomeric forms in pure polar solvents. The keto-amine tautomer is dominant in pure solvents of ethanol, DMSO and chloroform with the ratio of 74%, 53% and 52%, respectively. The absorption spectra of the L shows two absorption maxima in the range of 322-323 nm and 350-372 nm in non-polar solvents (toluene and cyclohexane), respectively. According to these findings, it can be said that the phenol-imine tautomeric forms is predominant in pure non-polar solvents. The unsymmetric ligand exists as a mixture of phenol-imine and keto-amine tautomeric forms in all acidic and basic solutions (Figure 3-b,c). That's because that the new bands above 400 nm. The keto-amine tautomer is dominant in basic solvents of ethanol, chloroform and DMSO with the ratio of 72%, 57% and 51%, respectively, except in toluene and cyclohexane. The keto-amine tautomer is also dominant (between 54 and 71%) in all of the asidic solutions, except in DMSO.

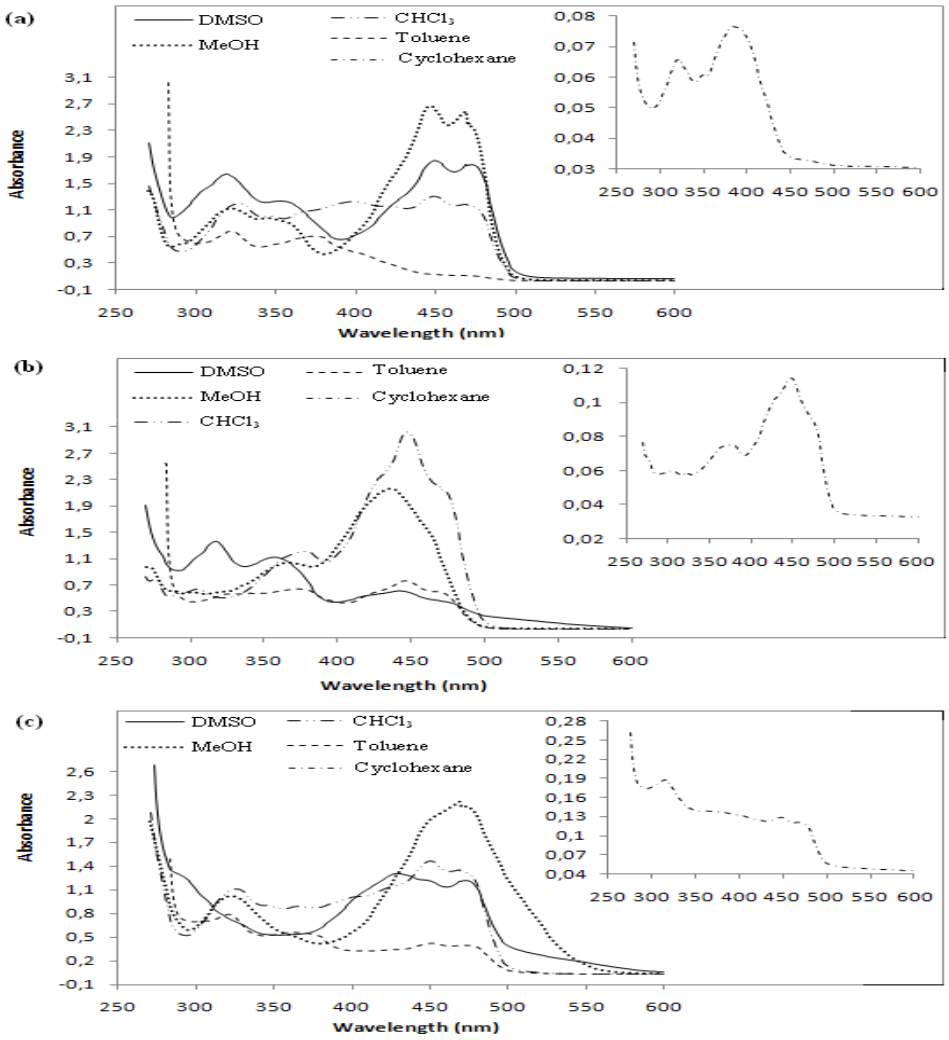


Figure 3. Absorption spectra of the L in various solvents (a), in acidic (b),in basic (c) solutions.

3.4. Potentiometric Studies

Protonation constants of the unsymmetric ligand and stability constants of its Ni(II) and Fe(III) complexes are presented in Table 4. The ligand has four protonation constants: first and second constants ($\log K_1^H$ and $\log K_2^H$) correspond to the protonation of phenolate and naphtholate anions, third and fourth constants ($\log K_3^H$ and $\log K_4^H$) refer to protonation of imine nitrogen atoms. According to the results of NMR and UV-visible spectroscopy, for the ligand, keto-amine tautomer was dominant on the left side, while phenol-imine tautomer was formed on the right side of the molecule, in ethanol solution. Hence, a phenolic proton is titrated on the right side (related to $\log K_1^H$), but there is a less acidic proton, bounded to keto oxygen atom, on the left side (related to $\log K_2^H$). It is expected that, the lower constant belongs to the protonation of the nitrogen atom on the left side, the higher to the possibility of (O-H…N) intramolecular hydrogen bonding in six membered chelate ring. The $\log K_3^H$ value is also in agreement with the value of the starting Schiff base, in the literature [43]. Figure 4 shows the titration curves of the ligand and the complexes obtained in 1:1 ethanol- water medium. The order of the stability constants are found to be: Ni (II) < Fe (III).

Table 4. Protonation and stability constants of the all compounds at 25.0 ± 0.1 °C, for 50% ethanol-50% water mixture (μ = 0.5 M KCl).

Compound	L				NiL	FeL
LogK	$LogK_1^H$	$LogK_2^H$	$LogK_3^H$	$LogK_4^H$	$LogK_{ML}$	$LogK_{ML}$
	8.36±0.003	7.53±0.003	4.34±0.003	2.99±0.004	8.29±0.14	13.88±0.18

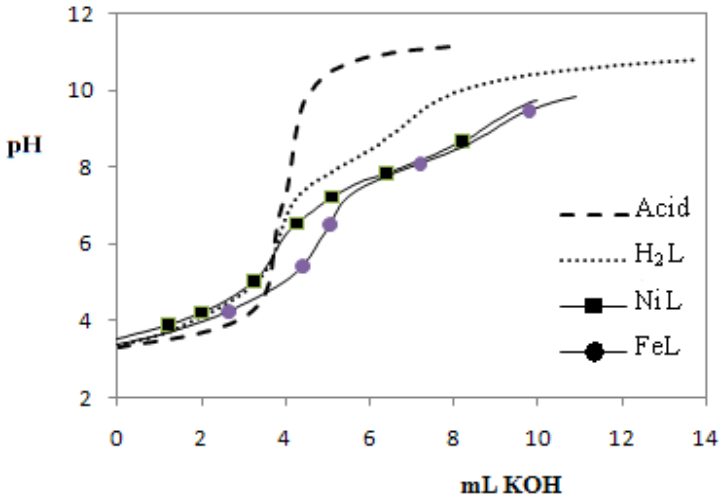


Figure 4. Potentiometric titration curves of the L and its complexes.

3.5. Antibacterial and antifungal activities

The antifungal activity, antibacterial activities and MIC values of the compounds are screened against gram negative *Escherichia coli (*0157:H7), gram positive *Micrococcus luteus* (NRRLB 4975), *Bacillus cereus* (RSKK 863) and the fungus *Candida albicans* (ATCC 16231). The results are given in Table 5. The unsymmetric ligand and its complexes show moderate antimicrobial activity against the studied microorganisms. Generally, NiL and FeL complexes are more active than the L ligand. This enhancement in activity depends on some factor such as nature of the metal ion, lipophilic character, concentration, chelation [44-46]. FeL complex is more active than NiL, for all of the bacteria and the fungus. Its MIC values are also dramatically low against G^+ *M. luteus* and *B. cereus*. This case may be resulting from the bigger stability or the higher oxidation state of Fe(III) ion than Ni(II). For FeL, the diameter of the inhibition zones and MIC value against *B. Cereus* (from left to right; 1000, 500, 250, 125, 63, 32, 16, 8, 4, 2, 1 μg/mL) are given in Figure 5 and Figure 6, respectively. UV-visible spectroscopy is known to be a very sensitive method for studying o-hydroxy salicylaldimine or naphthaldimine derivatives. Presence of a new band above 400 nm indicates the existence of keto-amine tautomer of the Schiff bases. The band below at 400 nm shows the phenol-imine tautomer [42].

Table 5. Antibacterial activities (inhibition zone diameter as mm) and MIC values (µg/mL) of the compounds.

Compound	Concentration	*E. coli* G^-	*M. luteus* G^+	*B. cereus* G^+	*C. albicans (Fungus)*
L	250 µg/ml	4,0 ± 0,0	7,0 ± 0,0	7,0 ± 0,4	16,0 ± 0,7
	500 µg/ml	6,0 ± 0,0	8,0 ± 0,0	10,0 ± 0,0	20,0 ± 0,3
	1000 µg/ml	8,0 ± 0,2	12,0 ± 0,2	10,0 ± 0,0	21,0 ± 0,4
	MIC	≥ 1000	≥ 1000	≥ 125	≥ 500
NiL	250 µg/ml	5,0 ± 0,4	9,0 ± 0,0	8,0 ± 0,0	18,0 ± 0,3
	500 µg/ml	8,0 ± 0,1	12,0 ± 0,0	9,0 ± 0,7	20,0 ± 1,2
	1000 µg/ml	8,0 ± 0,4	13,0 ± 0,0	12,0 ± 0,4	22,0 ± 0,4
	MIC	≥ 250	≥ 1000	≥ 63	≥ 250
FeL	250 µg/ml	7,0 ± 0,0	12,0 ± 0,4	10,0 ± 0,4	19,0 ± 0,4
	500 µg/ml	8,0 ± 0,4	14,0 ± 0,4	14,0 ± 0,4	22,0 ± 0,8
	1000 µg/ml	9,0 ± 0,2	15,0 ± 0,4	14,0 ± 0,2	26,0 ± 0,4
	MIC	≥ 250	≥ 63	≥ 32	≥ 63
Gentamisin [b]	30 µg	23,5 ± 0,7	33,0 ± 1,4	30,0 ± 1,7	[a]
Amikacin [b]	10 µg	17,0 ± 0,7	29,0 ± 1,2	24,5 ± 0,8	[a]

a:Fungus *C. albicans* not studied with antibiotics **b:** Antibiotics disks

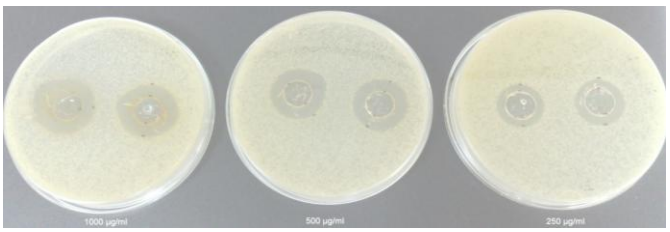


Figure 5. Diameter of the inhibition zones of FeL against B. cereus.

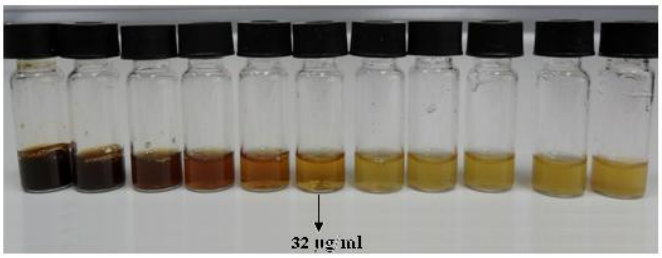


Figure 6. The minimum inhibitory concentration (MIC) value of FeL against *B. Cereus* (from left to right; 1000, 500, 250, 125, 63, 32, 16, 8, 4, 2, 1 µg/mL).

4. Conclusion

A novel unsymmetrical Schiff base with its Fe(III) and Ni(II) complexes were synthesized by using a two-stage method. Herein, the phenol-imine and keto-amine tautomerism study reported. Furthermore, the protonation constants of the diimine and the stability constants of its Ni(II) and Fe(III) complexes were determined potentiometrically.

Additionally, the antimicrobial activities of the unsymmetric ligand and its complexes were screened against *Escherichia coli, Micrococcus luteus, Bacillus cereus* and *Candida albicans.*

Acknowledgements- The authors are grateful to Research Foundation of Gazi University.

References

[1] X. D. Jin, Y. H. Jin, Z. Y. Zou, Z. G. Cui, H. B. Wang, P. L. Kang, C. H. Ge, K. Li, *J. Coord. Chem.* 64 (2011) 1533-1543.
[2] M. S. Alam, L. Liu, Y. E. Lee, D. U. Lee, *Chem. Pharm. Bull.* 59 (2011) 568-573.
[3] R. Rao, K. R. Reddy, K. N. Mahendra, *Bulg. Chem. Commun.* 46, (2014) 11-17.
[4] L. A. Saghatforoush, A. Aminkhani, F. Chalabian, *Trans. Met. Chem.* 34 (2009) 899-904.
[5] S. M. Jadhav, V. A. Shelke, A. S. Munde, S. G. Shankarwar, V. R. Patharkar, T. K. Chondhekar, *J. Coord. Chem.* 63 (2010) 4153-4164.
[6] X. Zhong, J. Yi, J. Sun, H. L. Wei, W. S. Liu, K. B. Yu, *Eur. J. Med. Chem.* 41 (2006) 1090-1092.
[7] M. M. Kamel, H. Ali, M. M. Anwar, N. A. Mohamed, A. M. Soliman, *Eur. J. Med. Chem.* 45 (2010) 572-580.
[8] E. Hadjoudis, I. M. Mavridis, *Chem. Soc. Rev.* 33 (2004) 579-588.
[9] V. R. Souzaa, H. R. Rechenbergb, J. A. Bonacinc, H. E. Tomac, *Spectrochim. Acta A.* 71 (2008) 1296-1301.
[10] M. E. Hanhan, *Appl. Organometal. Chem.* 22 (2008) 270-275.
[11] R. Csuk, A. Barthel, T. Brezesinski, C. Raschke, *Bioorg. Med. Chem. Lett.* 14 (2004) 4983-4985.
[12] N. S. Youssef, E. A. El-Zahany, B. N. Barsoum, A. M. A. El-Seidy, *Trans. Met. Chem.* 34 (2009) 905-914.
[13] D. Nartop, P. Gürkan, N. Sarı, S. Çete, *J. Coord. Chem.* 61 (2008) 3516-3524.
[14] D. Nartop, P. Gürkan, *Chinese J. Inorg. Chem.* 6 (2013) 1227-1234.
[15] Ö. Güngör, P. Gürkan, *Spectrochim. Acta A.* 77 (2010) 304-311.
[16] D. Nartop, W. Clegg, R. W. Harrington, R. A. Henderson, C. Y. Wills, *Dalton Trans.* 43 (2014) 3372-3382.
[17] Ö. Güngör, P. Gürkan, *Arab. J. Chem.* doi: 10.1016/j.arabjc.2015.02.009.
[18] S. Bilge, Z. Kılıç, Z. Hayvalı, T. Hökelek, S. Safran, *J. Chem. Sci.* 121 (2009) 989-1001.
[19] M. Flores-Leonar, N. Esturau-Escofet, J. M. Mendez-Stivalet, A. Marı´n-Becerra, C. Amador-Bedolla, *J. Mol. Struc.* 1006 (2011) 600-605.
[20] Ö. Güngör, P. Gürkan, *J. Mol. Struc.* 1074 (2014) 62-70.
[21] M. R. Shehata, A. A. El-Sherif, M. M. Shoukry, M. H. Barakat, *Spectrochim. Acta A.* 96 (2012) 889-897.
[22] T. C. White, K. A. Marr, R. A. Bowden, *Clin. Microbiol. Rev.* 11 (1998) 382-390.
[23] R. A. Fromtling, *Drug New Perspect* 12 (1999) 557- 569.
[24] R. Poholoudek-Fabini, D. Fröhling, *Arch. Pharm.* **298,** 423, (1965).
[25] M. Nakamura, K. Komatsu, Y. Gondo, K. Ohta, Y. Ueda, *Chem. Pharm. Bull.* 15 (1967) 585-592.
[26] G. Gran, *Analyst* 77 (1952) 661-671.
[27] N. Sarı, P. Gürkan, S. Arslan, *Trans. Met. Chem.* 28 (2003) 468-474.
[28] A. E. Martell, R. Motekaitis, Determination and Use of the Stability Constants, VCH Publisher, New York, 1988.
[29] H. M. Irving, H. S. Rossotti, *J. Chem. Soc.* (1954) 2910-2918.
[30] N. S. Golubev, S. N. Smirnov, P. M. Tolstoy, S. Sharif, M. D. Toney, G. S. Denisov, H. H. Limbach, *J. Mol. Struc.* (2007) 844-845 319-327.
[31] D. Nedeltcheva, B. Damyanova, S. Popov, *J. Mol. Struc.* 749 (2005) 36-44.
[32] D. Nedeltcheva, F. S. Kamounah, L. Mirolo, K. M. Fromm, L. Antonov, *Dyes and Pigments*, 83 (2009) 121-126.

[33] S. Saha, D. Mallick, R. Majumdar, M. Roy, R. R. Dighe, E. D. Jemmis, A. R. Chakravarty, *Inorg. Chem.* 50 (2011) 2975-2987.
[34] G. Türkoğlu, H. Berber, H. Dal, C. Öğretir, *Spectrochim. Acta A.* 79 (2011) 1573-1583.
[35] G. Kaştaş, *J. Mol. Struc.* 1017 (2012) 38-44.
[36] H. Nazır, M. Yıldız, H. Yılmaz, M. N. Tahir, D. Ülkü, *J. Mol. Struct.* 524 (2000) 241-250.
[37] Ö. Özdemir, *J. Mol. Struc.* 1125 (2016) 260-271.
[38] Z. Popovic, V. Roje, G. Pavlovic, D. M. Calogovic, G. Giester, *J. Mol. Struct.* 597 (2001) 39- 47.
[39] R. K. Dubey, U. K. Dubey, C. M. Mishra, *Trans. Met. Chem.* 31 (2006) 849-855.
[40] A. B. P. Lever, Inorganic Electronic Spectroscopy, 2nd ed., Elsevier, Amsterdam, 1984.
[41] H. Katırcıoğlu, Y. Beyatlı, B. Aslım, Z. Yüksekdağ, T. Atıcı, *Internet J. Microbiol.* 2, (2006).
[42] H. Ünver, M. Yıldız, D. M. Zengin, S. Özbey, E. Kendi, *J. Chem. Crystallogr.* 31 (2001) 211-216.
[43] F. Köseoğlu, E. Kılıç, D. Uysal, *Talanta* 42 (1995) 1875-1882.
[44] S. J. Lippard, J. M. Berg, Principles of Bioinorganic Chemistry; University Science Books, Mill Valley, California, 1994.
[45] A. Chaudhari, S. C. Joshi, R. V. Singh, *Main Group Met. Chem.* 27 (2004) 59-70.
[46] N. Gupta, R. Swaroop, R. V. Singh, *Main Group Met. Chem.* 20 (1997) 387-392.