

Synthesis, Characterization and Spectrophotometric Studies of Seven Novel Antibacterial Hydrophilic Iron(II) Schiff Base Amino Acid Complexes

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ABSTRACT. A series of new Iron(II) Schiff base amino acid complexes derived from the condensation of amino acid and sodium 2-hydroxybenzaldehyde-5-sulfonate have been synthesized. The complexes were characterized by elemental, electronic, IR spectral analyses and conductance measurements. The stability and solubility of the prepared complexes were determined. Two spectral methods used to determine the stoichiometry of the prepared complexes which exhibited divalent tridentate coordination and formed chelates of octahedral structures. The antibacterial activity of the prepared complexes has been tested against *Bacillus cereus*, *Pseudomonas aeruginosa* and *Micrococcus* bacteria. The effect of HCl on investigated complexes studied spectrophotometrically.

Key words: Schiff base complex, Amino acid, Bio-inorganic chemistry, Spectrophotometric study

INTRODUCTION

Salicylaldehyde-amino acid Schiff base complexes are used as non-enzymatic models for the metal - pyridoxal (vitamin B6) amino acid Schiff base systems which are the key intermediates in many metabolic reactions of amino acids catalyzed by enzymes which require pyridoxal as a cofactor (transamination, decarboxylation, elimination, racemization, etc.).¹ Over the past few years, there have been many reports on Schiff base applications in homogeneous and heterogeneous catalysis,² e.g., epoxidation of alkenes,³ cyclopropanation reaction,⁴ asymmetric hydrosilylation of ketones,⁵ selective oxidation of alcohols to corresponding carbonyl compounds,⁶ controlled ring-opening polymerization,⁷ hydrogenation of benzene under mild conditions,⁸ hydrogenation of ketones,⁹ Suzuki cross-coupling reaction under ambient condition¹⁰ and enantioselective oxidation of methyl aryl sulfides.¹¹ In the area of bio-inorganic chemistry, transition metal complexes of Schiff bases have attracted a lot of interest due to their potent biological activities such as antifungal, antibacterial, anticancer and herbicidal applications.¹²⁻¹⁴ These studies have shown that complexation of metals to Schiff base ligands improves the antimicrobial and anticancer activities of the ligands.¹³ Some research groups found that the Schiff base metal complexes derived from salicylaldehyde can specially cleave the DNA.¹⁵⁻¹⁷ Water soluble complexes of sulfonato-substituted Schiff base ligands used as catalytic antioxidants.¹⁸ Comparatively very little effort has been expended

to prepare Iron(II) Schiff base amino acid complexes¹⁹⁻²¹ despite their importance as complexes containing a metal in unstable, low oxidation state, as well as involving unstable ligands, the Schiff base amino acid, that forms through coordination to Fe(II) metal ion. Therefore, in this work we prepared seven novel Fe(II) Schiff base amino acid complexes and characterized their structures by various possible physical methods to obtain more information about their structures.

EXPERIMENTAL

Reagents and Instrumentation

All the used amino acids and $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ in the present study are of analytical grade purchased from Sigma Aldrich, sodium 2-hydroxybenzaldehyde-5-sulfonate was prepared according to literature.²² Conductivity measurements were made at 25 °C on Jenway Conductivity meter 4320 using bidistilled water as solvent. The electronic spectra of the complexes were monitored using matched 1 cm silica cells on Perkin Elmer Lambda 35 spectrophotometer and IR spectra of the metal chelates recorded over the 400–4000 cm^{-1} range on a Shimadzu FTIR-8101 Fourier transform infrared spectrophotometer using KBr discs. Elemental analyses were carried out at the Micro analytical Center, Assiut University, Egypt.

Synthesis of Schiff Base Metal Complexes

2 mmol (0.448 g) of sodium 2-hydroxybenzaldehyde-5-sulfonate in de-ionized water (20 ml) was added dropwise

to an equimolar aqueous solution of amino acid; **1**, 0.15 g Glycine in de-ionized water (10 ml); **2**, 0.178 g L-alanine in de-ionized water (10 ml); **3**, 0.262 g L-leucine in de-ionized water (15 ml); **4**, 0.262 g L-Isoleucine in de-ionized water (15 ml); **5**, 0.298 g DL-methionine in de-ionized water (15 ml); **6**, 0.21 g DL-serine in de-ionized water (10 ml); **7**, 0.33 g L-phenylalanine in de-ionized water (10 ml) then the mixture was stirred at 100 °C for 3 h to give yellow color. Then the obtained ligand solution was mixed with an equimolar aqueous solution of ferrous ammonium sulphate. In order to avoid the oxidation of ferrous to ferric, a few drops of Glacial acetic acid were added.^{19–21} The resulting solution was stirred at 40 °C for 10 h (Its color becomes deep violet), then the obtained solution was evaporated under room temperature. The solid obtained was filtered-off, washed several times with ether, and then recrystallized from ethanol - water mixture (2:1). All the complexes were prepared by similar procedure.

Antibacterial Activity

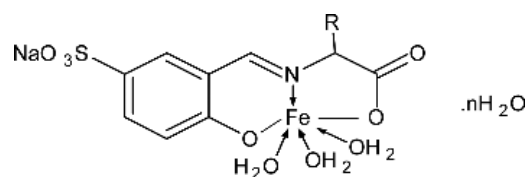
The Inhibitory effect of the prepared Schiff base complexes on the in vitro growth of bacteria representing one gram negative bacterium, *Pseudomonas aeruginosa* and two gram positive bacteria, *Bacillus Cereus* and *Micrococcus* was evaluated using agar diffusion method^{23,24} by measuring the zone of inhibition on agar plates at two different concentrations 3 mg/ml and 6 mg/ml. De-ionized water was used as solvent control. All plates were incubated at 37 ± 0.5 °C for 24 h.

RESULTS AND DISCUSSION

All the complexes are soluble in bidistilled water and in

Table 1. General properties of the prepared complexes

No.	Empirical formula	Molar conductance (ohm ⁻¹ cm ² mol ⁻¹)	M.P. (°C)	Electronic spectra	
				λ_{\max} , nm	ϵ_{\max} , M ⁻¹ cm ⁻¹
1	FeC ₉ H ₁₄ NO ₁₀ SNa	106	>350 °C	505	644.71
				320	3596.34
2	FeC ₁₀ H ₁₆ NO ₁₀ SNa	108	>350 °C	504	668.73
				321	3809.51
3	FeC ₁₃ H ₂₂ NO ₁₀ SNa	112	>350 °C	502	694.46
				324	3969.14
4	FeC ₁₃ H ₂₂ NO ₁₀ SNa	114	>350 °C	500	711.57
				321	4010.23
5	FeC ₁₂ H ₂₄ N O ₁₂ S ₂ Na	120	>350 °C	499	727.03
				320	4286.97
6	FeC ₁₀ H ₂₀ NO ₁₃ SNa	125	>350 °C	508	637.87
				322	3709.21
7	FeC ₁₆ H ₂₄ NO ₁₂ SNa	98	>350 °C	509	768.19
				322	4685



Scheme 1.

two organic solvents (ethanol and methanol), but partially soluble in acetone. The suggested structures of the prepared complexes are listed in *Scheme 1* and general properties listed in *Table 1*.

Infrared Spectra

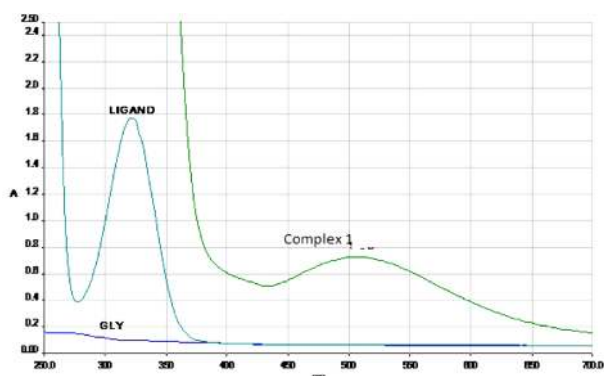
The IR spectral data containing the relevant vibrational bands of Fe(II) complexes are listed in *Table 2*. The absorption bands around 1625 cm⁻¹ is corresponding to azomethine group (N=C)^{25,26} and around 1100 cm⁻¹ is related to S=O bond,²⁷ the disappearance of the peak around 1650 cm⁻¹ shows that the product does not contain 5-sulfosalicylaldehyde.²⁸ The IR results showed that the metal coordinated to nitrogen (azomethine) and oxygen (carboxylato) atoms of the amino acid groups besides water molecules.

Electronic Spectra

The electronic spectra of the aqueous solutions of the prepared Fe(II) complexes display maximum absorption bands around 325 nm ($\epsilon = 3596\text{--}4685$ dm³ mol⁻¹ cm⁻¹).

Table 2. The infrared absorption frequencies (cm^{-1}) of the investigated Fe (II) Schiff base amino acid complexes

Complex	H ₂ O	C=N	C=C	S=O	M-O	M-N
1	3114.5	1625.2	1435.2	1135.2	741.7	620.2
2	3205.1	1626.2	1416.9	1118.8	819.8	614.4
3	3217.7	1635.8	1409.2	1112.1	731.1	612.5
4	3209.9	1636.8	1439.1	1097.6	817.9	616.3
5	3192.6	1632.0	1413.0	1116.0	823.7	615.4
6	3442.4	1637.8	1399.5	1128.5	815.4	687.7
7	3199.3	1616.5	1437.1	1138.1	746.5	612.5

**Figure 1.** The electronic spectra of complex **1** and its components.

This band can be ascribed to an intramolecular charge transfer transition taking place in the complexed ligand. In addition, a band shown around 500 nm ($\epsilon = 637\text{--}769 \text{ M}^{-1}\text{cm}^{-1}$) may be attributable to a d-d transition in an octahedral

structure for such complexes. *Fig. 1* shows the comparison between complex **1**, its ligand and the corresponding amino acid (Glycine).

Elemental Analysis and Electrical Conductivity

The obtained elemental analyses of the metal complexes are listed in *Table 3*. The obtained data are in agreement with the calculated values showing that the complexes have (1:1) metal/ligand ratio as in *Scheme 1*.

The observed molar conductance values of the 1:1 complexes of $1 \times 10^{-3} \text{ M}$ aqueous solutions of the prepared complexes at 25°C are consistent with the electrolytic nature of the (1:1) complexes due to the presence of $-\text{SO}_3\text{Na}$ group in the proposed structures of the Schiff base metal complexes.

The electrical conductivity of the prepared complexes is determined at different temperatures (*Table 4*), the acti-

Table 3. The elemental analyses and magnetic moment of the investigated complexes

Complex	Found (calculated) (%)				μ_{eff} (BM)
	C	H	N	S	
1	27.11 (26.55)	2.97 (3.47)	3.39 (3.44)	7.16 (7.88)	3.96
2	27.91 (28.52)	3.23 (3.83)	2.97 (3.33)	7.11 (7.61)	3.99
3	33.98 (33.71)	4.32 (4.79)	2.59 (3.02)	6.40 (6.92)	4.01
4	33.91 (33.71)	4.42 (4.79)	2.64 (3.02)	6.39 (6.92)	4.03
5	27.37 (27.86)	4.38 (4.68)	2.51 (2.71)	11.95 (12.40)	4.06
6	24.91 (25.38)	3.91 (4.26)	2.99 (2.96)	6.38 (6.78)	4.1
7	35.54 (36.04)	4.94 (4.54)	2.34 (2.63)	5.95 (6.01)	4.18

Table 4. The observed conductivity (σ , μS) and activation energy of the prepared complexes

Complex T, K	σ , μS						
	1	2	3	4	5	6	7
283	99	100	101	102	103	104	91
288	102	102	104	106	108	110	93
293	104	106	107	110	114	117	95
298	106	109	113	114	120	124	98
303	109	112	116	118	126	130	101
308	112	114	119	123	131	134	103
313	116	117	121	127	135	138	106
E, kJ/mol	7.45	7.88	9.40	10.78	13.68	14.24	7.60

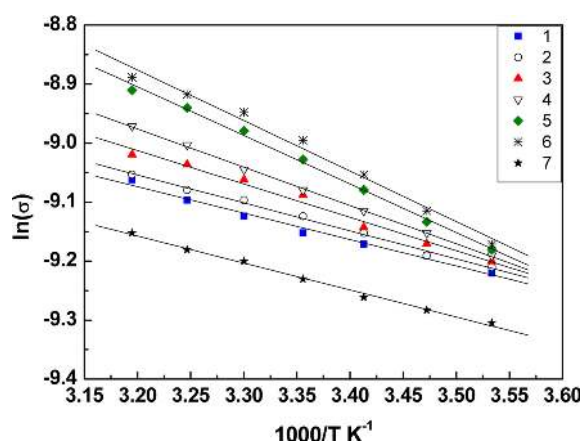


Figure 2. The relation between $\ln \sigma$ against $1/T$ for all the investigated complexes.

vation energies of charged ions in the complexes solutions were calculated from the following equation:

$$\sigma = \sigma_0 e^{(-E/2KT)}$$

where σ , σ_0 , E , and k are the observed conductance, conductivity constant, activation energy, and Boltzman constant, respectively^{29,30} Fig. 2 shows the relation between $\ln \sigma$ against $1/T$ for all the investigated complexes, From the slopes of the linear plots, the values of E were calculated. The conductivity of the investigated complexes increases with increasing temperature, hence all complexes exhibit semiconducting properties.

Determination of the Stoichiometry of the Complexes

The stoichiometry of the tested complexes were determined by applying the spectrophotometric molar ratio^{19,21}

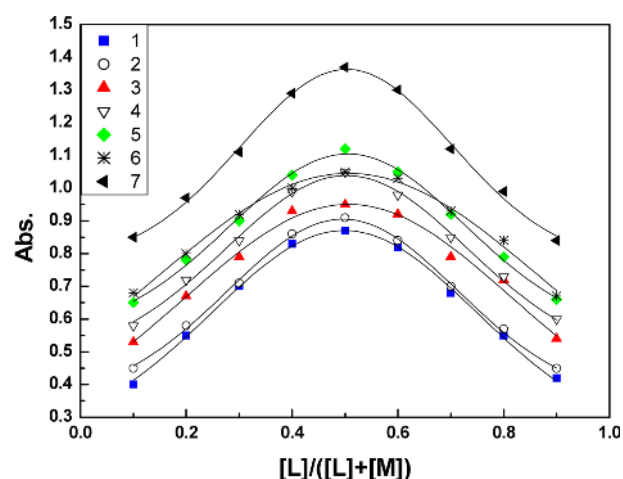


Figure 3. Continuous variation plot of Fe(II) Schiff base amino acid complexes in aqueous medium at $[\text{Fe}^{2+}] + [\text{L}] = 5 \times 10^{-3} \text{ mol dm}^{-3}$ and 298 K against $[\text{Fe}^{2+}\text{-ald.}]$, as a blank, with the same concentration as in the test solution.

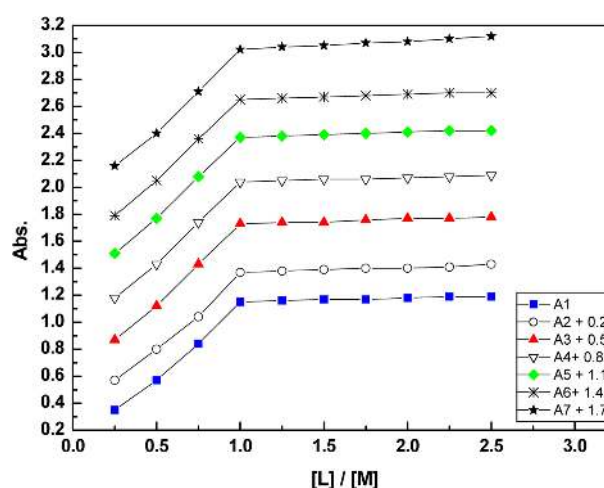


Figure 4. Molar ratio plots of Fe(II) Schiff base amino acid complexes in aqueous medium at $[\text{Fe}^{2+}] = 7.5 \times 10^{-3} \text{ mol dm}^{-3}$ and 298 K against $[\text{Fe}^{2+}\text{-ald.}]$, as a blank, of the same concentration as in the test solution.

and continuous variation methods^{19,21} (cf. Figs. 3 and 4). The results suggested the possible formation of 1:1 complexes (Iron(II): Schiff base amino acid ligand).

Magnetic Moment Measurements

Magnetic susceptibility measurements showed that the prepared complexes have paramagnetic character and suggested high spin values i.e., the studied Schiff base amino acid ligands are so weak that they exhibited low t_{2g} and e_g d-splitting of the octahedral structures of the complexes.^{31,32}

Apparent Formation Constant

The apparent formation constants (K_f) of the synthesized complexes formed in solution were determined from spectrophotometric measurements using the continuous variation method²¹ (cf. Table 5). The obtained K_f values indicate the stability of these complexes.

Stability pH Range of the Investigated Complexes

The stability range of the studied complexes was found

Table 5. The formation constants (K_f), stability constants (pK) and Gibbs free energy (ΔG) values of the prepared complexes in aqueous medium at 298 K

Complex	$10^{-5} K_f$	pK	ΔG , kJ/mol
1	1.01	5.01	-28.55
2	1.86	5.27	-30.06
3	2.07	5.32	-30.33
4	1.43	5.16	-29.41
5	2.86	5.46	-31.13
6	5.51	5.74	-32.75
7	1.58	5.20	-29.66

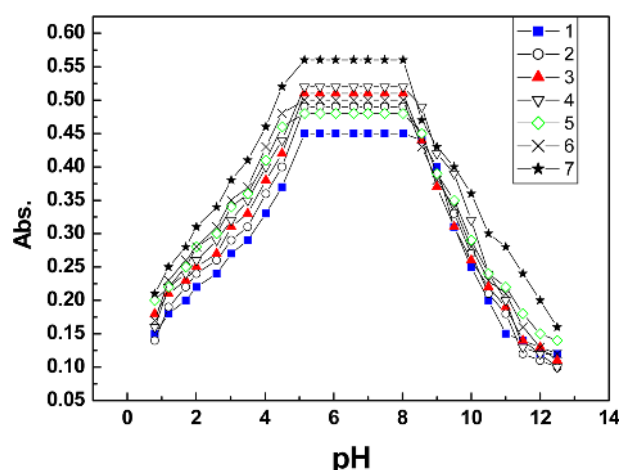


Figure 5. pH-absorbance plots of Fe (II) Schiff base amino acid complexes at 1×10^{-3} M in aqueous medium at 298 K.

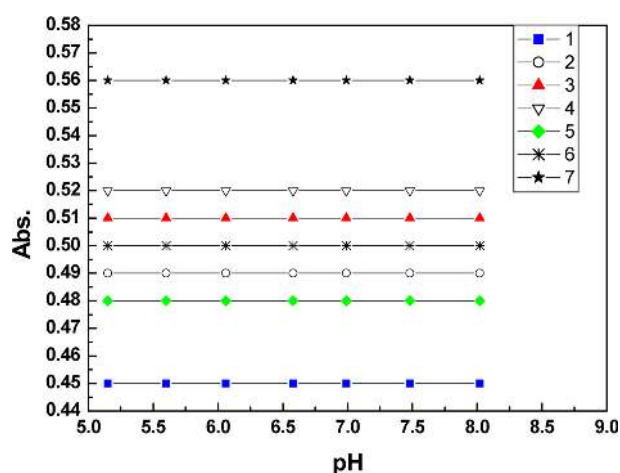


Figure 6. pH-absorbance plots of Fe (II) complexes in aqueous medium at 298 K in stability range (pH=5–8).

to be from pH = 5 to pH = 8 according to the obtained pH-absorbance curves (cf. Figs. 5 and 6). This means that Fe(II) ion greatly stabilizes the tested Schiff base amino acid ligands in this range. Accordingly, these ligands can be used as masking reagents of Fe^{2+} ions in that range of pH.

Table 6. Solubility (mol dm^{-3}) and transfer chemical potential ($\delta_m \mu^\ominus$) values of the prepared complexes in water, 10%, 30% and 50% aqueous-methanol mixtures at 25 °C

Complex	MeOH, %				$\delta_m \mu^\ominus$, kJ/mol		
	0	10	30	50	10	30	50
1	0.52	0.44	0.37	0.30	0.38	0.83	1.39
2	0.48	0.41	0.34	0.26	0.39	0.83	1.52
3	0.35	0.29	0.21	0.17	0.45	0.61	1.82
4	0.39	0.33	0.27	0.20	0.41	0.92	1.61
5	0.33	0.28	0.24	0.22	0.41	0.76	1.05
6	0.40	0.34	0.31	0.20	0.43	0.65	1.77
7	0.28	0.23	0.18	0.14	0.48	1.04	1.67

Determination of the Solubility of the Investigated Complexes

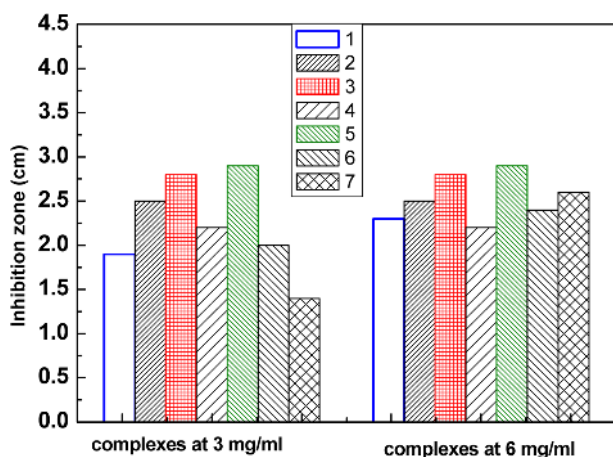
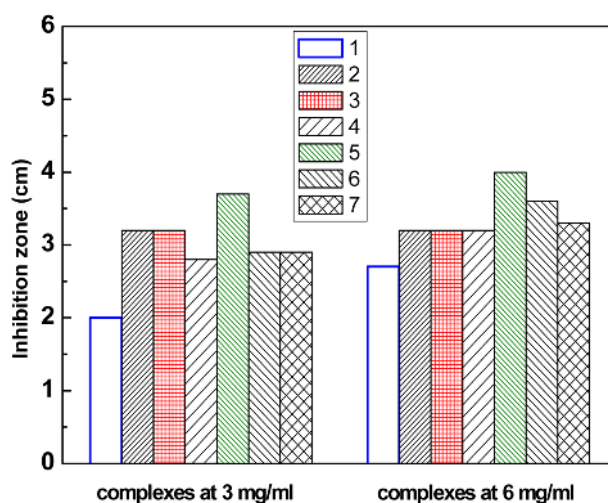
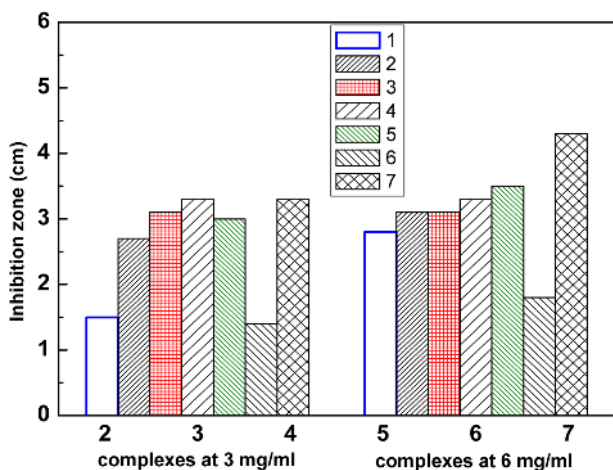
The solubility of the prepared complexes was measured in aqueous solution, 10%, 30% and 50% aqueous methanol as a solvent. A saturated solution of the solid was agitated with the appropriate solvent at 298 K. The mixture was centrifuged then an aliquot of the saturated solution removed. The obtained complexes solutions were diluted and their absorbance values measured at λ_{max} for each one.^{19,33} The solubility values and transfer chemical potentials are cited in Table 6, on the assumption that the ratio of mean activity co-efficients in the aqueous and aqueous methanol mixtures are in all cases unity. The values of the transfer chemical potentials $\delta_m \mu^\ominus$, of the investigated complexes from water to water-methanol binary mixtures, were estimated by applying the following equation: $\delta_m \mu^\ominus = -RT \ln (S_s/S_w)$ where S_s is the solubility in 10%, 30% and 50% aqueous-methanol binary mixtures and S_w the solubility in the aqueous medium. According to the solubility data, Table 3, the following ascending order of the hydrophobicity of the examined complexes was deduced: $1 < 2 < 6 < 3 < 4 < 5 < 7$.

Antibacterial Activity

The results of antibacterial activity are shown in Table 7. Investigated Schiff base complexes exhibited good activity against three types of bacteria: *Bacillus cereus*, *Pseudomonas aeruginosa*, *Micrococcus* (cf. Figs. 7–9). Complex derived from methionine showed the highest inhibitory effect against examined bacteria. In general the inhibition zones of the complexes were increased with increasing their concentrations. The highest inhibition zone was obtained by complex 7 against *Pseudomonas aeruginosa* where the minimum inhibition zone obtained by complex 7 against *Bacillus cereus*. Figs. 9–11 show the inhibition zone of complex 1 against the tested bacteria.

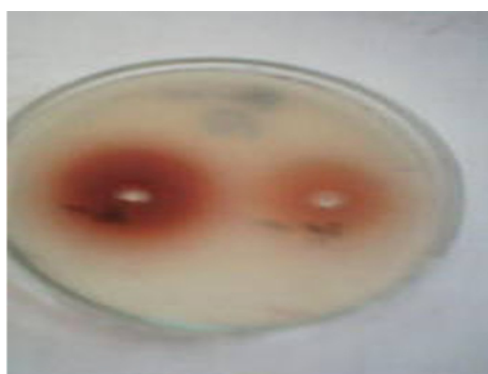
Table 7. Results of anti-bacterial evaluation of Schiff base amino acid complexes. Inhibition zone diameter in cm

Bacteria	<i>Bacillus cereus</i>		<i>Pseudomonas aeruginosa</i>		<i>Micrococcus</i>	
[complex] ^a	3	6	3	6	3	6
1	1.9	2.3	1.5	2.8	2	2.7
2	2.5	2.5	2.7	3.1	3.2	3.2
3	2.8	2.8	3.1	3.1	3.2	3.2
4	2.2	2.2	3.3	3.3	2.8	3.2
5	2.9	2.9	3	3.5	3.7	4
6	2	2.4	1.4	1.8	2.9	3.6
7	1.4	2.6	3.3	4.3	2.9	3.3

^a[complex] in mg/ml.

Figure 7. Plots of Inhibition zones of prepared complexes in the presence of *Bacillus cereus* bacteria.

Figure 9. Plots of Inhibition zones of prepared complexes in the presence of *Micrococcus* bacteria.

Figure 8. Plots of Inhibition zones of prepared complexes in the presence of *Pseudomonas aeruginosa* bacteria.

Effect of HCl on Investigated Complexes Spectrophotometric Study

Completed repeated spectral scans of prepared complexes at [complex] = 1.67×10^{-3} mol dm⁻³ were carried


Figure 10. The inhibition zone of complex 1 against *Bacillus cereus*.

out before and just after addition of hydrochloric acid (0.1 M) for a period of time with time interval 1 min at 298 K, cf. Figs. 13 and 14. These results were supported by the naked eye observation that the color of the complex rapidly changed from violet to pink then decayed slowly until the solution turned colorless and optically clear at the end of

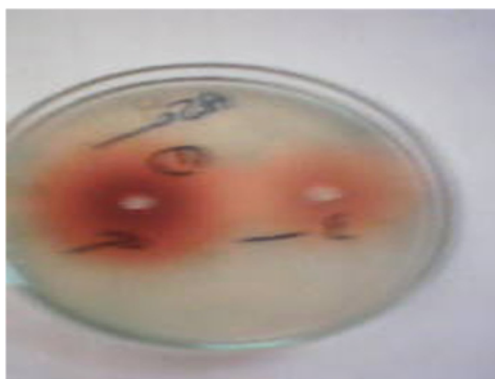


Figure 11. The inhibition zone of complex 1 against *Pseudomonas aeruginosa*.



Figure 12. The inhibition zone of complex 1 against *Micrococcus*.

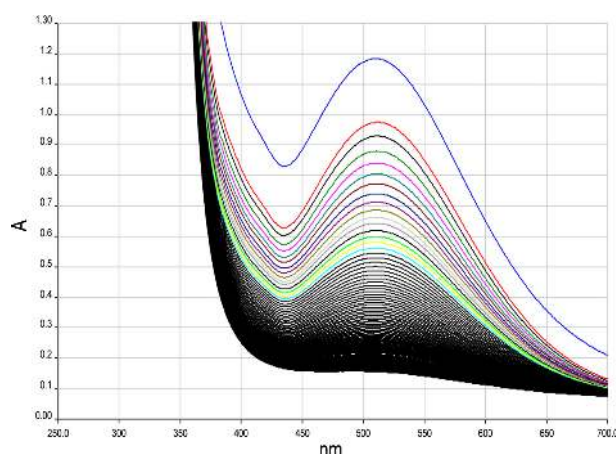


Figure 13. Repeated spectral scans of complex 4 before and after addition of HCl in aqueous media at $[\text{complex}] = 1.67 \times 10^{-3} \text{ mol dm}^{-3}$, $[\text{HCl}] = 0.1 \text{ mol dm}^{-3}$, $I = 0.5 \text{ mol dm}^{-3}$, $T = 298 \text{ K}$ and interval time 1 min.

each run. The first-order rate constants (k_{obs}) obtained by a least-squares procedure from the spectral scans, as shown

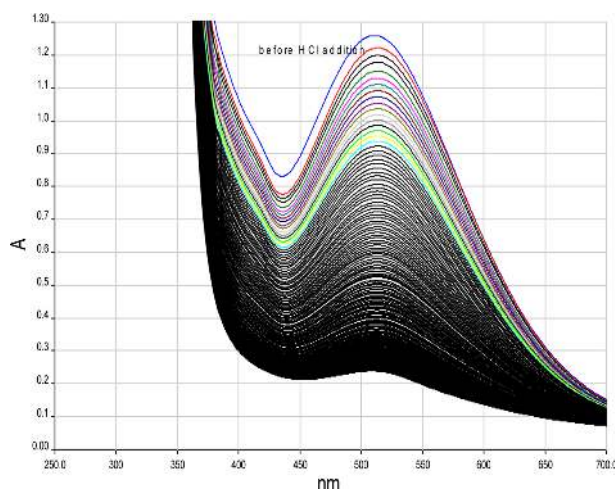


Figure 14. Repeated spectral scans of complex 7 before and after addition of HCl in aqueous media at $[\text{complex}] = 1.67 \times 10^{-3} \text{ mol dm}^{-3}$, $[\text{HCl}] = 0.1 \text{ mol dm}^{-3}$, $I = 0.5 \text{ mol dm}^{-3}$, $T = 298 \text{ K}$ and interval time 1 min.

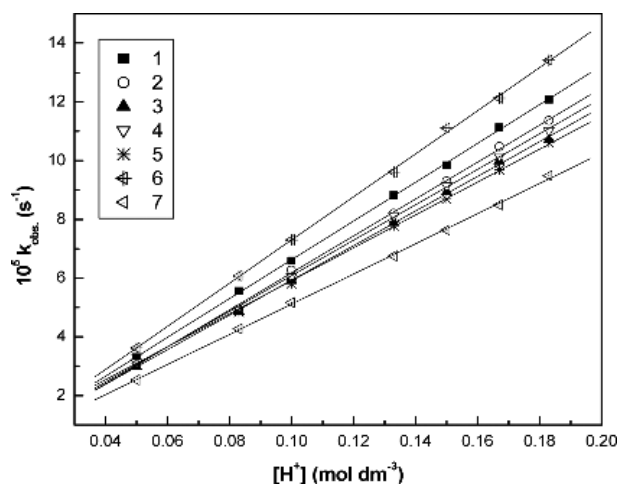


Figure 15. The relationship between $[\text{H}^+]$ and $10^5 k_{\text{obs}}$. Values for hydrolysis of investigated complexes in aqueous medium with $[\text{complex}] = 1.67 \times 10^{-3} \text{ mol dm}^{-3}$, $I = 0.5 \text{ mol dm}^{-3}$ and 298 K .

in Figs. 13 and 14. Linear plots of k_{obs} vs $[\text{H}^+]$ displayed (cf. Fig. 15) are in a good correlation with the following equation: $\text{Rate} = k_{\text{obs}} [\text{complex}]$; $k_{\text{obs}} = k_1 + k_2 [\text{H}^+]$.

CONCLUSION

Novel hydrophilic ferrous Schiff base amino acid complexes were prepared and characterized by elemental, electronic, IR spectral analyses and conductance measurements. The stability pH range of complexes was found to be from pH = 5 to pH = 8. The solubility and transfer chemical potentials of the complexes were determined to show

the hydrophilicity of these complexes. The complexes showed good antibacterial activity against *Bacillus cereus*, *Pseudomonas aeruginosa* and *Micrococcus* bacteria. The complexes studied spectrophotometrically in the presence of HCl.

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