

**SYNTHESIS, SPECTRAL AND
BIOLOGICAL STUDIES OF Mn(II), Ni(II),
Cu(II), AND Zn(II) COMPLEXES WITH A
TETRADENTATE SCHIFF BASE LIGAND.
COMPLEXATION STUDIES AND THE
DETERMINATION OF STABILITY
CONSTANTS (K_e)**

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ABSTRACT

The complexes Mn(II), Ni(II), Cu(II) and Zn(II) ions with a N₂O₂ Schiff base derived from 1,4-diaminobutane and salicylaldehyde, N,N'-bis(salicylidene)-1,4-diaminobutane (LH₂), have been prepared and characterized by elemental analyses, molar conductivities, spectral (IR, NMR, visible, UV) and

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magnetic moment measurements. Stability constants were measured by means of a conductometric method. Furthermore, the stability constants for complexation between ZnCl_2 , $\text{Cu}(\text{NO}_3)_2$ and AgNO_3 salts and the ligand in 80% dioxane-water and pure methanol were determined by conductance measurements. The magnitudes of these ion association constants are related to the nature of the solvation of the cation and the complexed cation. The mobilities of the complexes are also dependent, in part, upon solvation effects. The complexes $\text{Mn}(\text{II})$, $\text{Ni}(\text{II})$, $\text{Cu}(\text{II})$ and $\text{Zn}(\text{II})$ ions with the Schiff base have been evaluated for their antibacterial activity against rec^- and rec^+ stains of *Bacillus subtilis*.

INTRODUCTION

Interest in the chemistry of metal chelates of tetradentate N_2O_2 Schiff bases has increased in recent decades¹⁻⁴, because of the wide applications of these complexes in various fields. Schiff base complexes are known to have antibacterial activity⁵. In the present study, the synthesized $\text{Mn}(\text{II})$, $\text{Ni}(\text{II})$, $\text{Cu}(\text{II})$ and $\text{Zn}(\text{II})$ complexes were tested against rec^- and rec^+ stains of *Bacillus subtilis*^{6,7}.

The bacterial response to DNA damage caused by UV light, ionising radiation, and chemical agents involves the repair of DNA by several processes. These are damage recognition, incision, excision, repair synthesis and ligation. Three proteins encoded by the *uvr A*, *uvr B* and *uvr C* genes carry out the first three steps of this process. They act in a series of steps to first recognise and bind to the damaged site and then hydrolyse two phosphodiester bonds. Collectively, these enzymes are called the ABC exonuclease enzyme complex that form part of the SOS system. In the rec^+ bacteria the SOS system is present, on the other hand, in the rec^- the SOS system is not present. The *rec* gene in the rec^+ *Bacillus subtilis* has a resistance against chemical substances. The rec^- bacteria do not contain this gene. Due to these properties, these bacteria usually can be used in order to compare the antibacterial activities of chemical substances⁸⁻¹⁰.

The present paper describes the synthesis and characterization of the $\text{N,N}'$ -bis(salicylidene)-1,4-diaminobutane Schiff base (Fig. 1.) and its $\text{Mn}(\text{II})$, $\text{Ni}(\text{II})$, $\text{Cu}(\text{II})$ and $\text{Zn}(\text{II})$ complexes and their effects on cell differentiation and the SOS repair system in *Bacillus subtilis* rec^+ YB 886 and rec^- derivatives YB 886 *rec* A4. We have used conductivity measurements to determine the stability (formation) constants for the $\text{Zn}(\text{II})$, $\text{Cu}(\text{II})$ and

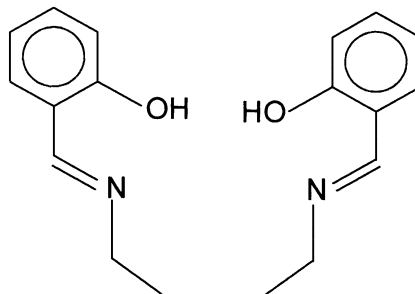


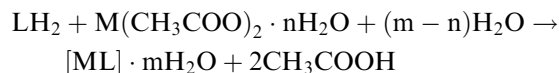
Figure 1. N,N'-bis(salicylidene)-1,4-diaminobutane (LH₂).

Ag(I) ion-LH₂ ligand interaction¹¹. This method also yields accurate values for the ion association constants for the cation-ligand complexes with various anions. Our results suggest that a number of cation-LH₂ ligand complexes undergo ion association and that this phenomenon is highly dependent on the nature of ion-solvent and ion-ligand interactions.

RESULTS AND DISCUSSION

The Schiff base ligand is readily formed by the condensation of salicylaldehyde and 1,4-diaminobutane (Fig. 1). The ligand LH₂, on interaction with Mn(II), Ni(II), Cu(II) and Zn(II) salts, yields complexes corresponding to the general formula [ML]·mH₂O. Some physical properties such as colours, melting points and yields *etc.* of the synthesized complexes are given in Table I.

The metal complexes Mn(II), Ni(II), Cu(II) and Zn(II) were prepared according to the following equation:

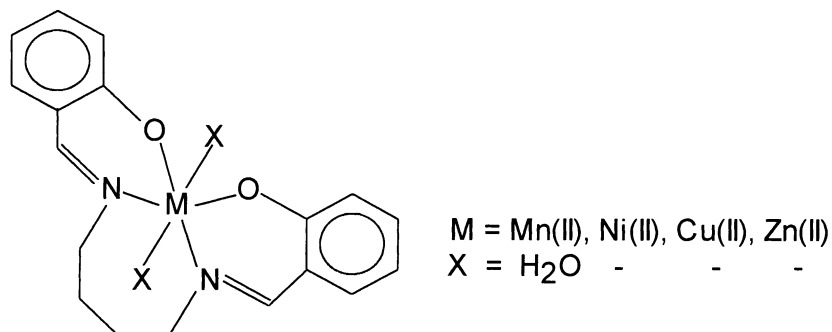


M =	Zn(II),	Mn(II),	Ni(II),	Cu(II)	LH ₂ =	C ₁₈ H ₂₀ N ₂ O ₂
n =	2	4	4	1		
m =	1	2	1	1		

The metal to ligand ratio of the Mn(II), Ni(II), Cu(II) and Zn(II) complexes was found to be 1:1; in addition, the Zn(II), Ni(II) and Cu(II)

Table I. The Colours, Formulas, Formula Weight, Yields, Melting Points, and Elemental Analyses of the Ligand and the Complexes

Compounds	F.W g/mol	M.p. (°C)	Yield (%)	Elemental Analyses Calculated (found), %			μ_{eff} (B.M)
				C	H	N	
Ligand (LH ₂) (Yellow) C ₁₈ H ₁₈ N ₂ O ₂	294.00	88.0	81.0	73.47 (73.40)	6.12 (6.30)	9.52 (9.40)	–
CuL (Brown) C ₁₈ H ₁₈ N ₂ O ₃ Cu	373.55	240.0	65.0	57.52 (57.40)	4.81 (4.70)	7.80 (7.65)	2.01
MnL (Purple) C ₁₈ H ₂₀ N ₂ O ₄ Mn	384.94	285.0	59.0	56.11 (56.30)	5.20 (5.04)	7.27 (7.40)	5.52
NiL (Red) C ₁₈ H ₁₈ N ₂ O ₃ Ni	370.71	285.0	61.0	58.27 (58.20)	4.86 (4.80)	7.55 (7.64)	Dia
ZnL (Light Yellow) C ₁₈ H ₁₈ N ₂ O ₃ Zn	377.38	290.0	60.0	57.24 (57.20)	4.77 (4.67)	7.41 (7.30)	Dia

**Figure 2.** Suggested structure of tetrahedral Zn(II), square-planar Ni(II), and Cu(II), and octahedral Mn(II) complexes of the ligand LH₂.

complexes contain one molecule of water of crystallization but the Mn(II) complex contains two molecules of water of crystallization (Fig. 2). In addition, Zn(II) complex was found in the earlier study and suggested structure of tetrahedral Zn(II) was determined to be consistent with literature¹².

IR Spectra

Important IR spectral bands of the ligand and metal complexes are given in Table II. The broad band in the IR spectrum of the Schiff base at 2868 cm^{-1} is assigned to the stretch vibration of the intramolecularly hydrogen bonded OH group in the molecule. Similar bands were observed at the same frequency in the IR spectra of salicylideneanilines¹³. This band is not present in the IR spectra of the complexes.

A high-intensity band around 1284 cm^{-1} in the Schiff base is due to the phenolic C–O stretching frequency. In the complexes the C–O stretching vibration appears at a slightly lower frequency, at $1280\text{--}1283\text{ cm}^{-1}$ confirming the coordination through the phenolic oxygen atom^{14–15}.

The C=N azomethine band occurs at 1638 cm^{-1} in the Schiff base ligand. This band shifts to lower frequency by $2\text{--}17\text{ cm}^{-1}$ on complexation, indicating the coordination of the azomethine nitrogen to the metal ion^{16,17}.

In the complexes, the coordination of the water molecule is indicated by the appearance of a broad band in the region $3550\text{--}3400\text{ cm}^{-1}$. A band at $1605\text{--}1660\text{ cm}^{-1}$ in the complexes is assigned to $\delta(\text{H}_2\text{O})$ of coordinated water¹⁸.

The coordination of the phenolic oxygen and azomethine nitrogen is further supported by the appearance of two non-ligand bands at $500\text{--}560$ and $400\text{--}500\text{ cm}^{-1}$ due to $\nu(\text{M}\text{--}\text{O})$ and $\nu(\text{M}\text{--}\text{N})$, respectively, in all of the complexes^{1,5,18,19}.

¹H NMR Spectra of the Schiff Base (LH₂), Zn(II), and Ni(II) Complexes

¹H NMR spectrum of LH₂ (DMSO-d₆): $\delta = 2.55$ (m, 4H, $-\text{CH}_2-$); 3.47 (t, 4H, $J = 10.00$ Hz, $-\text{CH}_2-$); 3.76 (s, 2H, $-\text{OH}$); 6.89 (d, $J = 8.00$ Hz, 2H, aromatic H₆); 7.00 (dd, $J_1 = 12.00$ Hz, $J_2 = 1.75$ Hz, 2H, aromatic H₄); 7.33 (dd, 2H, $J_1 = 7.00$ Hz, $J_2 = 2.00$ Hz, aromatic H₅); 7.55 (d, $J = 9$ Hz, 2H, aromatic H₃); 13.35 (s, 2H, $-\text{HC}=\text{N}-$).

¹H NMR spectrum of the Zn(II) complex (DMSO-d₆): $\delta = 0.90$ (s, 6H, $\text{CH}_3\text{COO}-$); 1.75 (m, 4H, $-\text{CH}_2-$); 2.50 (bs, 4H, H_2O); 3.20 (t, $J = 24.00$ Hz, 4H, $-\text{CH}_2-$); 7.12 (d, $J = 8.00$ Hz, 2H, aromatic H₆); 7.35 (d, $J = 8.00$ Hz, 2H, aromatic H₄); 7.44 (dd, $J_1 = 8.00$ Hz, $J_2 = 1.75$ Hz, 2H, aromatic H₅); 7.87 (d, $J = 8.00$ Hz, 2H, aromatic H₃); 10.01 (s, 2H, $-(\text{H})\text{C}=\text{N}-$).

¹H NMR spectrum of the Ni(II) complex (DMSO-d₆): $\delta = 0.93$ (s, 6H, $\text{CH}_3\text{COO}-$); 2.16 (m, 4H, $-\text{CH}_2-$); 2.23 (bs, 4H, H_2O); 3.01 (t, $J = 27.00$ Hz, 4H, $-\text{CH}_2-$); 7.00 (d, $J = 7.75$ Hz, 2H, aromatic H₆); 7.31

Table II. Characteristic IR Bands (cm^{-1}) of the Ligand and Complexes in KBr Pellets

Ligand (LH_2)	CuL	NiL	MnL	ZnL	Assignment
2895 m	—	—	—	—	Intramolecular H-bounded—OH
1631 s	1624 s	1616 s	1627 s	1629 s	v(C=N) Azomethine,
1285 m	1280 m	1283 w	1283 w	1281 w	v(C—O) Phenolic
3049 w	3049 w	3049 w	3049 w	3049 w	v(C—H) Aromatic
2950–2958 m	2950–2958 m	2950–2958 m	2950–2958 m	2950–2958 m	v(C—H) Aliphatic
—	3400–3150 m	3400–3150 m	3400–3150 m	3400–3150 m	H_2O
—	540	541	542	540	v(M—O)
—	420	480	470	490	v(M—N)

s: strong, m: medium, w: weak

(dd, $J_1 = 8.00$ Hz, $J_2 = 2.00$ Hz, 2H, aromatic H₄); 7.37 (dd, $J_1 = 8.00$ Hz, $J_2 = 0.75$ Hz, 2H, aromatic H₅); 7.50 (d, $J = 8.25$ Hz, 2H, aromatic H₃); the resonance of $-(\text{H})\text{C}=\text{N}-$ has not been observed.

Electronic Spectra

The electronic spectra of all of the complexes were recorded in 10^{-3} M DMF solution at room temperature (Table III). The spectra of the free Schiff base exhibit two absorption bands in the regions 252–275 and 305–324 nm. These bands are attributed to $\pi \rightarrow \pi^*$ transitions, the first band is due to transitions of the benzene ring and the second to the imino group. In the complexes, the imino $\pi \rightarrow \pi^*$ transition is shifted to longer wave length as a consequence of coordination to the metal, confirming the formation of Schiff base metal complexes²⁰.

The electronic spectrum of the Cu(II) complex shows an absorption band at 640 nm attributed to the ${}^2\text{T}_{2g} \rightarrow {}^2\text{E}_g(\text{G})$ transition, which is compatible with this complex having a square-planar structure^{3,13}.

The electronic spectrum of the Zn(II) complex shows an absorption band at 368 nm attributed to the L \rightarrow M (charge transfer) transition, which is compatible with this complex having a tetrahedral structure^{3,14}.

The electronic spectrum of the Ni(II) complex shows an absorption band at 450 nm attributed to the ${}^1\text{A}_{1g} \rightarrow {}^1\text{B}_{1g}$ transition, which is compatible with this complex having a square-planar structure³.

The complex $\text{MnL} \cdot 2\text{H}_2\text{O}$ exhibits electronic spectral bands at 529 nm and 383 nm corresponding to the ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{1g}(\text{P})$ (ν_3) transition, typical of an octahedral manganese(II) complex. The room temperature magnetic moment (5.52 B.M.) of $\text{MnL} \cdot 2\text{H}_2\text{O}$ corresponds to five unpaired electrons, also indicating a high-spin manganese (II) octahedral complex^{20,21}.

Table III. Electronic Spectra Data of the Complexes and Ligand

Compounds	$\lambda_{\text{max}}/\text{nm}$ ($\epsilon/\text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$)
LH ₂	305 (3653), 319 (2679)
CuL	252 (670), 275 (841), 311 (3040), 364 (3208), 398 (2273), 523 (500), 640 (115)
MnL	254 (487), 324 (3094), 361 (3095), 366 (3107), 383 (1723), 520 (460)
NiL	270 (300), 378 (360), 450 (50)
ZnL	256 (451), 262 (486), 368 (1888)

Magnetic Properties

The magnetic susceptibilities of the Mn(II) and Cu(II) complexes are 5.52 and 2.01 B.M., respectively. Since the Mn(II) and Cu(II) complexes are paramagnetic^{22–23}, their ¹H NMR spectra could not be obtained. The Zn(II) and Ni(II) complexes are diamagnetic, their ¹H NMR spectra could be obtained

Conductivity

The Mn(II), Ni(II), Cu(II) and Zn(II) complexes are non-electrolytes as shown by their molar conductivity (Λ_M) as measured in DMF, they are in the range^{6,19,24} 8.2–13.2 $\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$.

The Conductometric Study of the Ligand LH₂ with Ag(I), Zn(II), and Cu(II) Salts

Molar conductivities, Λ_M ($\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$), were calculated from the infinite frequency electrolytic conductances, κ , after correcting for the pure solvent conductance, *i.e.* $\Lambda_M = 1000 \kappa / C_{MX}$ where C_{MX} is the total concentration of the metal salt. The experimental molar conductance equations and all calculations for stability constants and Gibbs free enthalpy values have been published in our previous work¹¹. In 80% dioxane-water (see Table IV) the stability constants (log Ke) increase as the crystal radii increased of the complexed cation in the order Cu(II) < Zn(II) < Ag(I) but the opposite behaviour has been observed for these metal complexes with LH₂ in methanol [see Table V; Ag(I) < Zn(II) < Cu(II)]. This order of stability constants is attributed to the degree of solvation where smaller cations with a high charge density are more solvated than the successively larger cations.

Table IV. Log Ke and $-\Delta G^0$ (kcal/mol) Values for the Interaction of LH₂ with ZnCl₂, Cu(NO₃)₂ and AgNO₃ in 80% Dioxane-Water at 25 °C as Determined by a Conductometric Study

Ligand	Value	Ag ⁺	Zn ²⁺	Cu ⁺²
LH ₂	Log Ke	4.70 ± 0.10	2.67 ± 0.25	1.41 ± 0.21
	$-\Delta G^0$	6409.86 ± 0.08	3638.50 ± 0.06	5573.61 ± 0.16

Table V. Log Ke and $-\Delta G^0$ (kcal/mol) Values for the Interaction of LH₂ with ZnCl₂, Cu(NO₃)₂ and AgNO₃ in Methanol at 25 °C by a Conductometric Study

Ligand	Value	Ag ⁺	Zn ²⁺	Cu ⁺²
LH ₂	Log Ke	2.46 ± 0.02	3.35 ± 0.26	3.58 ± 0.15
	$-\Delta G^0$	3353.62 ± 0.25	4561.38 ± 0.09	4888.02 ± 0.13

The ligand LH₂ showed different complexation ability in 80% dioxane-water compared to methanol.

The Effect of Schiff Base Complexes on Bacteria

Bacillus subtilis YB 886 (*rec*⁺) and YB 886 A4 (*rec*⁻) were incubated in the medium that contains different concentrations the Schiff base complexes for 12 hours. It has been found that the amount of DNA in *rec*⁻ bacteria were decreased more than the *rec*⁺ bacteria in the presence of Cu(II) and Mn(II) complexes. On the other hand, the total protein content in *rec*⁻ bacteria was decreased more than the total protein of *rec*⁺ bacteria. It can be concluded that both bacteria were affected by the Schiff base.

The DNA amount decreased more for the Cu(II) and Mn(II) complexes in comparison to the Ni(II) and Zn(II) complexes. The total protein concentration is decreased in the presence of the Schiff base in both bacteria when compared with the control. This decrease is more in the *rec*⁻ bacteria than the *rec*⁺ bacteria²⁵⁻²⁹.

EXPERIMENTAL

Reagents and Measurements

The electronic spectra of the complexes in the UV-VIS region were recorded in DMF solutions using a Shimadzu Model 160 UV-Visible spectrophotometer. The IR spectra of the complexes in KBr pellets were recorded with Midac 1700 instrument. ¹H NMR spectra in DMSO-d₆ were recorded on a Bruker GmbH DPX-400 MHz Digital FT-NMR spectrometer; magnetic susceptibilities were determined on a Sherwood Scientific magnetic susceptibility balance (Model No: MK1) at room temperature (23 °C) using Hg[Co(SCN)₂] as calibrant; diamagnetic corrections were calculated from Pascal's constants³⁰. The elemental analyses were obtained

on a Carlo Erba instrument. All experiments were carried out in an air-conditioned room maintained at 25 ± 1 °C.

Synthesis of Schiff Base (LH₂)

The Schiff base ligand was prepared as previously reported³¹ (Fig. 1).

Synthesis of the Complexes

The following general procedure was used to prepare all of the complexes. A solution containing 20 mmol of metal acetate in 25 mL of absolute ethanol was added slowly and dropwise into 25 mL of the ethanol solution of the Schiff base (1.76 g, 20 mmol) and stirred at 50 °C on a water bath for 2–3 h. The resulting precipitate was filtered, washed with ether and water, and was recrystallized from absolute ethanol and dried in *vacuo* at room temperature.

Complexation Studies and the Determination of the Stability Constants (K_e)

Anhydrous AgNO₃, ZnCl₂ and Cu(NO₃)₂ of highest purity were used. Stability constants were measured by means of a conductometric method¹¹. The water used in the conductometric studies was redistilled from alkaline potassium permanganate. Dioxane was dried over sodium metal, and anhydrous methanol was used without further purification (Merck; H₂O content less than 0.01%). The solutions were prepared at constant 1:1 ratio of metal salt to ligand (LH₂) in an 80% dioxane-water mixture and in methanol. All solutions were prepared in a dry box and transferred to the dry conductivity cell. The conductances were measured at 25 ± 0.05 °C. The measuring equipment consisted of a glass vessel (type Ingold) with an external jacket. At the same time, the system was connected to a thermostatted water-bath (25 ± 0.05 °C) and a conductivity cell (Cole Parmer 19050–66) with a conductometer (Suntex SC-170 Model). The cell constant was determined as 0.769 cm^{-1} at 25 °C, measuring the conductivity of aqueous potassium chloride solutions of various concentrations¹¹. Log K_e and $-\Delta G^0$ values for the reaction of the ligands with the cations were determined by a conductometric procedure outlined previously¹¹. Results are reported as the average and standard deviation from the average of four-six independent experimental determinations.

Table VI. The Effect of Schiff Base Complexes on *Rec*⁻ and *Rec*⁺ *Bacillus subtilis*

Concentration	1 µg/mL	2 µg/mL	3 µg/mL	4 µg/mL	5 µg/mL	Control
Cu Complex						
OD 550 Growth ^a (<i>rec</i> ⁻)	1.025	0.986	0.980	0.970	0.966	1.251
OD 550 Growth ^a (<i>rec</i> ⁺)	1.281	1.277	1.256	1.251	1.244	1.301
DNA Contents						
µg/mL (<i>rec</i> ⁻)	122	116	105	100	98	128
µg/mL (<i>rec</i> ⁺)	130	129	125	123	122	132
Protein Contents						
µg/mL (<i>rec</i> ⁻)	311	275	251	228	223	326
µg/mL (<i>rec</i> ⁺)	345	339	336	332	331	351
Ni Complex						
OD 550 Growth ^a (<i>rec</i> ⁻)	1.105	0.970	0.921	0.910	0.900	1.251
OD 550 Growth ^a (<i>rec</i> ⁺)	1.296	1.291	1.280	1.276	1.271	1.301
DNA Contents						
µg/mL (<i>rec</i> ⁻)	125	123	120	119	118	128
µg/mL (<i>rec</i> ⁺)	131	130	130	129	129	132
Protein Contents						
µg/mL (<i>rec</i> ⁻)	301	260	251	245	241	326
µg/mL (<i>rec</i> ⁺)	345	341	339	337	335	351
Mn Complex						
OD 550 Growth ^a (<i>rec</i> ⁻)	1.201	1.196	1.190	1.186	1.180	1.251
OD 550 Growth ^a (<i>rec</i> ⁺)	1.300	1.298	1.296	1.291	1.290	1.301
DNA Contents						
µg/mL (<i>rec</i> ⁻)	121	120	116	110	108	128
µg/mL (<i>rec</i> ⁺)	132	131	131	130	126	132
Protein Contents						
µg/mL (<i>rec</i> ⁻)	331	321	320	318	316	345
µg/mL (<i>rec</i> ⁺)	350	350	348	347	346	351
Zn Complex						
OD 550 Growth ^a (<i>rec</i> ⁻)	1.001	0.821	0.796	0.770	0.750	1.251
OD 550 Growth ^a (<i>rec</i> ⁺)	1.291	1.280	1.261	1.253	1.248	1.301
DNA Contents						
µg/mL (<i>rec</i> ⁻)	105	96	91	76	73	128
µg/mL (<i>rec</i> ⁺)	130	130	121	116	112	132
Protein Contents						
µg/mL (<i>rec</i> ⁻)	297	246	222	210	202	326
µg/mL (<i>rec</i> ⁺)	333	314	312	308	301	351

^aOptical density at 550 nm.

Antibacterial Studies

Bacterial Cultures and Growth Conditions

Wild-type strains *Bacillus subtilis* YB 886 (met B5, trpC2xin, 1SP β -) and *rec* derivatives YB 1015 (YB 886 *rec* A4) were obtained from Dr. Charles M. Lowett, Williams College, O'Gara Lab. Williamstown, and Massachusetts. Bacteria were grown on Luria Bertoni (LB) medium (10 g tryptone, 5 g yeast extract and 10 g NaCl, 1 L) at 37 °C until the optical density reaches 0.8 at 550 nm. The cultures were centrifuged at 10,000 \times g for 15 minutes. Pelleted cells were resuspended in 5 mL of lysis buffer (2 mM tris of pH 7.5, 10% sucrose, 1 mM dithioereitol, 0.1 mM EDTA). 0.6 mg/mL PMSF (phenylmethylsulfonyl fluoride) were added to the pellets in order to stop the activity of protease. Later on, 2 mg/mL of lysozyme were added to the pellets and incubated on ice for 30 minutes, and sonicated for 1 minute and again incubated at 37 °C for 15 minutes. Debris was removed by centrifugation at 100,000 \times g for 45 minutes at 4 °C.

The purpose of the biological studies was to see whether these types of complexes have antibacterial activity. If this is the case, then more studies can be conducted on the mechanism of these complexes on the bacteria.

Bacterial DNA and Protein Determination

The protein content was determined by the Lowry method³² with bovine serum albumin (BSA) as standard. The DNA content was determined by optical density analytical measurement with an UV spectrophotometer³³.

Purification of Bacterial DNA

Bacterial chromosomal DNA was isolated by modification of the phenol extraction procedure³⁴.

Treatment of Cu, Ni, Mn, and Zn Complexes

The Schiff Base complexes were added to the growth medium in various concentrations (1,2,3,4 and 5 μ g/mL) (see Table VI).

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