Ball milling: a simple and efficient method for quantitative solvent-free synthesis of new potential bioactive Ni(II) and Co(II) complexes

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ABSTRACT

A simple and efficient technique for quantitative solvent-free synthesis of Ni (II) and Co (II) complexes by using ball milling as a green strategy is studied. The isolated solid compounds will be characterized using micro-analytical, thermogravimetric, magnetic and spectroscopic studies. Theoretical studies were carried out by applying DFT theory to predict the optimized configuration for all isolated solid complexes. Moreover, the in vitro antimicrobial activity assay versus fungi and bacteria via MIC technique has higher potency of Ni (II) than Co (II). The in vitro cytotoxicity of human muscle rhabdomyosarcoma (RD) via MTT technique and the antioxidant by DPPH free radical scavenging has higher inhibitory effect on complexes than free ligand. The cyclic voltammetry of Co(II) in absence/presence of H2BHNH was investigated to realize the significant effect of complex formation on the electrochemical manners of Co(II).

INTRODUCTION

Cancer is one of the most feared diseases in the world today because it is not easy to treat; this is due to cancer that comes as a result of uncontrolled multiplication of modified normal cell of human. So, the potential way of cancer treatment is drug therapy. Whereas, the drugs used to battle cancer join one of two wide types. The first is cytotoxic drugs in which the cell is killed and the second is cytostatic in which the cell stabilizes the drugs. Both types cause a decrease in the tumor size because cancer cell has a high humanity rate, which only preventing them from isolating will cause a decrease in the inhabitants [1–4].

Metals complexes are fundamental cellular components chosen to function in numerous essential biochemical processes for living organisms. Generally, metals have unique characteristics that involve reactivity toward organic substrates, variable coordination modes, and redox activity. For these reasons, the design of special coordination complexes, whichever as drug or pro-drug, is considered the main target to get a powerful tool in cancer diagnosis [5–8]. Based on the interface between molecular bioinorganic chemistry and biological system, the design of coordination complexes for cancer treatment in the current research is extensive in scope, targets multiple cellular and biological properties of many types of tumor. Recently,
the improvement of anticancer drugs stimulated from cytotoxicity toward the design of selective agents, which act on definite cellular targets [9–11].

So according to the previous facts, Ni (II) and Co(II) complexes with high biological efficiency will be prepared in an efficient and environmentally friendly way, which is to grind the reactors using ball milling technique as a green approach [12,13]. The current advance in nanotechnology led to the improvement of new metallic nanoparticles that eventually increase the environmental hazards. The main aim is to minimize the hazard of synthetic procedures, which are associated with derivative compounds and chemicals used.

**Experimental**

**Apparatus**

The apparatus used to propose the configuration for prepared compounds was displayed in Scheme 1S.

**Synthesis of H₂BHNH and its metal complexes**

The Schiff base ligand was formed as displayed in Scheme 1. Also, the waste-free and facile solid state ball milling technique was used to prepare Ni (II) and Co (II) complexes as a green strategy of chemistry in pure state with high yield (99%) as shown in Table 1.

![Scheme 1](image)

*Scheme 1*. The outline of the synthesis of H₂BHNH and its Ni(II) & Co(II) complexes.
Table 1. Analytical and physical data of H$_2$BHNH and its metal complexes.

| No. | Compound                  | (E. F.) (F. Wt.) | Yield%  | M.P. (°C) | %found (Calculated) | C   | H   | M  | $\lambda_{\text{m}*}$ |
|-----|---------------------------|------------------|---------|-----------|--------------------|-----|-----|----|------------------------|
| 1   | H$_2$BHNH                 | C$_{18}$H$_{12}$O$_2$N$_2$ (290.324) | 78      | 260       | 74.41 (74.46)      | 4.91 | -   | -  | -                      |
| 2   | [Ni(HBHNH)$_2$].2H$_2$O   | NiC$_{38}$H$_{24}$O$_2$N$_2$ (673.374) | 99      | 285       | 64.11 (64.21)      | 4.48 | 8.83 | 4  | 7                      |
| 3   | [Co(HBHNH)$_2$].2H$_2$O   | CoC$_{38}$H$_{24}$O$_2$N$_2$ (673.594) | 99      | 285       | 64.15 (64.19)      | 4.51 | 8.78 | 7  | 8.75                   |

* In DMSO ($\text{ohm}^{-1}\text{cm}^{2}\text{mol}^{-1}$)

**Conformational calculations**

The quantum chemical parameters were estimated by applying DMOL3 program [14] in Materials Studio package [15]. The configuration of the ligand and its metal complexes was proposed by applying Density Functional [16].

**Antimicrobial activity assay**

In microbiology, the MIC technique was generally the initial point for larger pre-clinical evaluations of novel antimicrobial agents [17,18]. So, for drug discovery, the first step is the screening of records drug candidate for MICs versus tested bacteria or fungi as shown in Scheme 2S.

**In vitro cytotoxicity assay**

The cytotoxic effect of tested compounds on Rhabdomyosarcoma cell lines (RD) were performed by determining the absorbance of each well at 570 nm via applying ELISA (Enzyme-linked immunosorbent assay) microplate reader (Thermo Electron Corporation Multiskan-EX reader) [19] and mean optical density for each concentration was measured (Scheme 3S). The effect is expressed as % cell viability as shown in this relation:

\[
\text{% Cell Viability} = \frac{OD \ (\text{Test})}{OD \ (\text{Control})} \times 100
\]

**Antioxidant Activity**

The free radical-scavenging activity of tested compounds was determined by applying Blois method [20] using DPPH as a stable radical scavenging (Scheme 4S). The ability to scavenge the DPPH radical was determined by applying this equation [21]:

\[
\frac{\% \text{DPPH scavenging activity}}{= \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100}
\]

$A_{\text{control}}$: The absorbance of control
$A_{\text{sample}}$: The absorbance of the sample

**Cyclic voltammetry**

The cyclic voltammetry was estimated for Co(II) in absence/presence of ligand by using three types of electrodes which are reference electrode (Ag/AgCl/KCl), the glassy carbon working electrode (GCWE) and an auxiliary electrode (platinum wire) that immersed in 30 ml 0.1 M KCl as supporting electrolyte which connect with potentiostat of the type DY 2100.

**Results and discussion**

**General characteristics**

The isolated solid complexes of Ni (II) and Co (II) were physically and analytically characterized to set up their chemical formula and also have non-hygroscopic nature. The non-conducting aspect was proposed for Ni (II) and Co (II) complexes, owing to molar conductivity values for $10^{-3}$ M are 4, 7 $\Omega^{-1}\text{cm}^{2}\text{mol}^{-1}$, respectively [22].

**$^1$H NMR and IR spectra studies**

The $^1$H NMR spectrum of H$_2$BHNH in DMSO is displayed in Figure 1 have two signals at 11.98
and 11.30 ppm assignable to the protons of (OH)$_{\text{naphthoic}}$ & (NH) correspondingly. The intramolecular hydrogen bonding was suggested to be due to the appearance of (OH)$_{\text{naphthoic}}$ signal at high value downfield from TMS. Also, the aromatic and (-N = C-H) protons have multiplet signals displayed in 7.34–8.47 ppm range.

In the IR spectrum of H$_2$BHNH, the bands of $\nu$ (C = N) & $\nu$(C = O) were displayed at 1622 & 1646 vibrations [23], correspondingly (Table 2). The bands of $\nu$(OH)$_{\text{naphthoic}}$ & $\nu$(NH) were observed at 3449 & 3271 cm$^{-1}$ respectively [23]. The intramolecular hydrogen bonding was suggested to be due to the existence of $\nu$ (OH) band at 3449 cm$^{-1}$. Also, there is another confirmation for the existence of intramolecular hydrogen bonding (O-H·-O) which is the presence of broad weak bands in the 1900–2050 and 2100–2230 cm$^{-1}$ regions [23].

In IR spectra of Ni(II) as well as Co(II) complexes the H$_2$BHNH behaved as a mononegative tridentate ligand coordinating via (C = N)$_{\text{azo}}$, (C = O) & (OH)$_{\text{naphthoic}}$ with the displacement of the hydrogen atom from it. This manner of complexation was recommended depending on; (i) the absence (OH)$_{\text{naphthoic}}$ group; (ii) the lower shift of $\nu$ (C = O) as well as $\nu$(C = N) and (iii) the appearance of new bands at (508, 511) & (416, 430) cm$^{-1}$ that ascribed to $\nu$(M-O) & $\nu$(M-N) [23], correspondingly.

**Electronic spectra, magnetic properties and ligand field parameters**

The UV-visible data in DMSO, magnetic moments and ligand field parameters of Ni(II) & Co(II) complexes are collected in Table 3 and represented graphically in Figure S1. The outcomes results suggested octahedral configuration for Ni(II) & Co(II) complexes [24].

**XRD**

The diffraction patterns of investigated Ni(II) & Co(II) complexes as shown in Figure 2 were

| Compound | $\nu$(OH)$_{\text{naphthoic}}$ | $\nu$(NH) | $\nu$(C = O) | $\nu$(C = N) | $\nu$(C-O)$_{\text{naphthoic}}$ |
|----------|-------------------------------|-----------|--------------|--------------|-------------------------------|
| 1        | 3449                          | 3271      | 1646         | 1622         | 1268                          |
| 2        | -                             | 3207      | 1630         | 1608         | 1282                          |
| 3        | -                             | 3116      | 1631         | 1602         | 1290                          |
Table 3. Magnetic moments, electronic spectra and ligand field parameters of H₂BHNH metal complexes.

| Compound | μ_{eff} (B.M.) | Band position (cm\(^{-1}\)) | D_{q} (cm\(^{-1}\)) | B (cm\(^{-1}\)) | β | u_{2}/u_{1} | Transition |
|----------|----------------|-----------------------------|----------------|----------------|---|--------------|------------|
| 2        | 3.14           | 13341, 23812                | 815            | 857            | 0.82 | 1.62        | \(3^{A_{2}} \rightarrow 3^{T_{1g}}\) (F) |
| 3        | 5.21           | 13793, 18182               | 728            | 856            | 0.88 | 2.15        | \(3^{A_{2g}} \rightarrow 3^{T_{1g}}\) (P) |

Figure 2. XRD of (a) [Co(HBHNH)\(_{2}\)].2H\(_{2}\)O, (b) [Ni(HBHNH)\(_{2}\)].2H\(_{2}\)O complexes.

determined in of 10° < 2θ < 80° region at RT by X-ray diffraction using (Cu, K\(_{α}\)) radiation [25]. The patterns displayed sharp peaks in two investigated complexes reflecting crystalline nature [25]. The crystallite size was determined through FWHM for characteristic peaks by applying the equation of Deby–Scherrer:
The Thermal irregular images agglomerated played. The 26. Scanning Table are 0.741 plexes. values The θ:

\[ B = 0.94\lambda / (S \cos \theta) \]

θ: The diffraction angle, Cu/Kα (λ) = 1.5406 Å
S: The crystallite size
B: The line width at half maximum height
Also, by applying Bragg equation, the values of inner crystal plane d-spacing are calculated as follows:

\[ n\lambda = 2dsin(\theta) at \ n = 1 \]

The particle size as well as lattice parameter values are displayed in Table 4 for examined complexes. The particle size appeared within 0.740 & 0.741 nm for Ni(II) & Co(II) complexes, respectively.

**Scanning electron microscope studies**

The SEM of the Co(II) & Ni(II) complexes displayed that the presence of small grains of non-uniform size and the particles were agglomerated with controlled morphological structure as shown in Figure 3. Also, the SEM images of Co(II) & Ni(II) complexes reveal irregular shaped grains [26].

**Thermal studies of Ni(II) complex**

The TG curves of [Ni(HBHNH)₂].2H₂O complex are thermally stable up to 137°C, above that point the dehydration of complex begins [27,28]. This low temperature indicated that the two molecules of water are not coordinated with the nickel ion. In the temperature range 137°C–198°C, the TG curve displayed 5.3% weight loss that related to two water molecules as shown in Figure S2. The degradation of organic moiety started >198°C. In the TG curve, the temperature ranges 198°C–329°C, the weight loss 23.0% was ascribed to the partial decomposition of the chelating agent with the removal of the two loosely bound phenyl moieties (2C₆H₅). The second weight loss stage (329°C–410°C) which could be correlated with the elimination of the two (C₂H₂N₂) portions. Finally, at 618°C the weight loss of 44.4% was mainly ascribed to complete decomposition of the remaining more tightly bound fragment of the organic moiety, besides breaking the chelate bond, leaving nickel oxide (11.2%) of the complex initial mass.

**Thermodynamic and kinetic studies**

**Kinetic parameters**

Horowitz–Metzger and Coats–Redfern equations [29,30] were applied to estimate the kinetic as well as thermodynamic parameters of the decomposition steps for Ni(II) complex as shown in Figure 4 and Table 5. The outcomes data from the two applied methods were nearly similar to a certain extent, so the results can be concluded as follows:

![Figure 3. SEM views of (a) [Co(HBHNH)₂].2H₂O, (b) [Ni(HBHNH)₂].2H₂O complexes.](image-url)
The higher values of $\Delta G$ demonstrated the lower elimination rate of $H_2BHNH$ from one step to another. The decomposition step was non-spontaneous process due to positive sign of $\Delta G$ referred to the higher quantity of residue than the beginning.

The positive values of $\Delta S$ referred to the disordered reaction, while the negative values indicated ordered reaction. DTG curves referred to high activation energies that indicated the high stability of Ni(II) complex. The decomposition steps are endothermic processes due to positive sign of $\Delta H$.

**Figure 4.** Coats–Redfern and Horowitz–Metzger plots for $[Ni(HBHNH)_2].2H_2O$ (a) first step, (b) second step, (c) third step and (d) fourth step.
Table 5. Kinetic parameters evaluated by Coats–Redfern and Horowitz–Metzger equations for Ni(II) complex.

| Compound | step | Method | $E_a$ ($kJ/mol$) | A ($S^{-1}$) | $\Delta H^\ne$ ($kJ/mol$) | $\Delta S^\ne$ ($J/Kmol$) | $\Delta G^\ne$ ($kJ/mol$) |
|----------|------|--------|-----------------|-------------|-------------------|-----------------|-----------------|
| 2        | 1st  | HM     | 131             | $1.2 \times 10^{18}$ | 127              | 99.8            | 92.8            |
|          |      | CR     | 122             | $5.9 \times 10^{16}$ | 119              | 74.9            | 92.9            |
| 2        | 2nd  | HM     | 66              | $1376592$      | 63               | $-130.4$        | 119.3           |
|          |      | CR     | 59              | $168312$       | 56               | $-146.1$        | 106.9           |
| 3rd      |      | HM     | 204             | $9.6 \times 10^{13}$ | 199              | 15.9            | 187.8           |
|          |      | CR     | 193             | $1.2 \times 10^{13}$ | 190              | 4.1             | 188.3           |
| 4th      |      | HM     | 115             | $7.3 \times 10^{2}$ | 108              | $-160.1$        | 237.5           |
|          |      | CR     | 108             | 27927          | 105              | $-161.1$        | 160.9           |

**Computational studies**

**Optimized molecular structure**

The optimized molecular structures for H$_2$BHNH and its Ni(II) & Co(II) complexes are represented in Figure 5. The bond length as well as bond angle are renowned for the following explanations:

- On complexation the bond angles of H$_2$BHNH were altered where the major change observed through ‘C(14)-C(8)-O(11), O(13)-C (14)-N(15), N(15)-C(17)-H(31)’ angles.
- The bond angles of the isolated solid complexes were referred to an octahedral geometry with d$^2$sp$^3$ or sp$^3$d$^2$ [31].
- The bond angles of the investigated complexes were longer than free ligand due to donor atoms shared in coordination [32].
- On chelation, a large difference in bond lengths observed through C(8)-O(11), O (11)-H(12), C(14)-O(13), N(15)-H(30), N(15)-N(16) and C(17)-H(31) atoms of free ligand than complexes. Anywhere, the bond length of investigated complexes became more longer than the ligand [33].
- In addition, DFT theory was applied to calculate the dipole moment, sum of atomic energies, electrostatic energy, spin polarization energy, binding energy and kinetic energy, total energy, LUMO, HOMO as represented in Figure 6 and Table 6.

**MEP**

Both electrophilic and nucleophilic attack points were resolute by electrostatic potential.

The MEP of H$_2$BHNH and its Ni(II) & Co(II) complexes that were shown in Figure 7 displayed three characteristic colors: blue color that referred to electron-poor area which prefers the nucleophilic attack. On the other hand, red color referred to electron rich which preferred electrophilic attack. While, the green color referred to electrostatic potential area [34].

**Biological studies**

**Antimicrobial studies**

A lot of studies were established an increase in antimicrobial activity following the interaction of active organic compounds with metal ions. The current study used a methodology modified for antimicrobial bioassays against Gram-positive (+)ve as well as Gram (-)ve bacteria. The results obtained were considered appropriate for determining MIC for performing antimicrobial sensitivity testing with good quality reproducibility as well as efficiency.

The antimicrobial activities of H$_2$BHNH and its Ni(II) & Co(II) complexes (0.2 ml of 10 μg/ml DMSO) versus P. Aeurginosa & B. Subtillis are represented in Table 7. Generally, the studies ligand showed variable antibacterial activity against either B. Subtillis & P. Aeurginosa. Moreover, the activities were dependent upon the type of the organic ligand used. On the other hand, the antimicrobial study of the isolated metal complexes versus the tested bacteria which arranged in the subsequent order: Ni(II) > Co(II) > H$_2$BHNH. The data also showed the dependence of the antimicrobial activity on the size of metal cation used.
where the higher the size of the metal ion, the higher the antibacterial activity.

**Cytotoxicity**

The cytotoxicity of H$_2$BHNH and its Ni(II) & Co(II) complexes assayed at various concentrations (50, 150, 200 and 400 μg/mL) on RD (ATCC) human muscle cell line was shown to have cell inhibition in range of 51–68%, 41–56% and 46–58%, correspondingly (note that: the number of observed viable cells was decreased). The cytotoxic potential of Ni(II) is greater than H$_2$BHNH as shown in (Figure 8 and Table 8). The cytotoxicity was observed to increase with the increase in concentration.
Various researchers have used scavenging effect of synthesized compounds on DPPH radical as a quick and reliable parameter to measure the in vitro antioxidant activity. DPPH is stable nitrogen radical that bears no similarity to the highly reactive and temporary peroxyl radicals concerned with the peroxidation of lipids. A lot of antioxidants that react quickly to peroxyl radicals

![Figure 6. The HOMO and LUMO of (a) H_2BHNH, (b) [Ni(HBHNH)_2].2H_2O, (c) [Co(HBHNH)_2].2H_2O.](image)

| Compounds | Total Energy (Ha) | Binding Energy (Ha) | Dipole moment (debye) | HOMO (eV) | LUMO (eV) | Spin polarization Energy (Ha) | Exchange-correlation Energy (Ha) | Electrostatic Energy (Ha) | Kinetic Energy (Ha) | Sum of atomic Energies (Ha) |
|-----------|------------------|--------------------|-----------------------|-----------|-----------|-------------------------------|-------------------------------|------------------------|-------------------|--------------------------|
| 1         | −954             | −6.874             | 2.857                 | −4.975    | −2.624    | 2.021                         | 2.724                        | −2.255                 | −9.363           | −947                     |
| 2         | −2102            | −13.872            | 2.296                 | −4.991    | −2.925    | 3.579                         | 6.408                        | −6.716                 | −17.144          | −2088                    |
| 3         | −2075            | −14.027            | 0.397                 | −4.201    | −3.118    | 3.596                         | 4.557                        | −8.557                 | −13.624          | −2061                    |

**Antioxidant studies**

| Compounds | Total Energy (Ha) | Binding Energy (Ha) | Dipole moment (debye) | HOMO (eV) | LUMO (eV) | Spin polarization Energy (Ha) | Exchange-correlation Energy (Ha) | Electrostatic Energy (Ha) | Kinetic Energy (Ha) | Sum of atomic Energies (Ha) |
|-----------|------------------|--------------------|-----------------------|-----------|-----------|-------------------------------|-------------------------------|------------------------|-------------------|--------------------------|
| 1         | −954             | −6.874             | 2.857                 | −4.975    | −2.624    | 2.021                         | 2.724                        | −2.255                 | −9.363           | −947                     |
| 2         | −2102            | −13.872            | 2.296                 | −4.991    | −2.925    | 3.579                         | 6.408                        | −6.716                 | −17.144          | −2088                    |
| 3         | −2075            | −14.027            | 0.397                 | −4.201    | −3.118    | 3.596                         | 4.557                        | −8.557                 | −13.624          | −2061                    |
may even be inert to DPPH owing to steric seclusion. Also, DPPH was decolorized by reducing agents as well as H transfer that contributes to inaccurate interpretations of antioxidant capacity.

The antioxidant activity of the H₂BHNH and its Ni(II) & Co(II) complexes studied by DPPH method at 20 μL – 140 μL showed scavenging potential (~19-43%), (~23-48%) and (~27-51%), correspondingly (Figure 9 and Table 9) substantiating the fact that complexation leads to enhanced antioxidant activity [35–37]. The discoloration from purple DPPH radical solution to yellow may be ascribed to hydrogen donation from the (O-H) and (N-H) groups in line with earlier reports on different metals [38]. The resultant free radical formed in complexes through the exchange mechanism was more stabilized due to charge delocalization than that in case of the ligand thereby affording increased antioxidant activity of complexes over ligand.

Cyclic voltammetry of CoCl₂

The cyclic voltammetry technique was applied within the potential window of (0.8 to −0.8 V) using scan rate 0.1 V/sec at 298.15 K to study the electrochemical redox mechanism of Co²⁺ in 0.1 M KCl as a supporting electrolyte and GC as working electrode (Figure 10). In the forward scan, (Co³⁺/Co²⁺) cathodic potential peak (Epc1) and (Co²⁺/Co¹⁺) cathodic potential peak (Epc2) come into view at 0.121 V and −0.325 V, correspondingly. While, in the reverse scan the (Co¹⁺/Co²⁺) anodic potential peak (Epa2) exists at 0.035 V followed by (Co²⁺/Co³⁺) anodic potential peak (Epa1) at 0.212 V. Thus, the redox mechanism of the reaction can be represented as follows:

\[
Co³⁺ + e^{-} \text{Red.} \rightarrow Co²⁺ \text{at } 0.121 V \\
Co²⁺ + e^{-} \text{Red.} \rightarrow Co¹⁺ \text{at } -0.325 V \\
Co¹⁺ \text{Oxid.} \rightarrow Co²⁺ + e^- \text{at } 0.036 V
\]
After that, the peak current is given by the Randles-Sevcik equation [39].

\[ I_p = (2.69 \times 10^5) n^{3/2} AC V^{1/2} D^{1/2} \]

**Figure 8.** The cytotoxicity of H$_2$BHNH and its Ni(II) & Co(II) complexes.

**Table 8.** % Cell viability.

| Compounds | Dose Concentration (µg/mL) |
|-----------|-----------------------------|
|           | 50  | 150 | 200 | 400 |
| 1         | 68  | 61  | 54  | 51  |
| 2         | 56  | 52  | 47  | 41  |
| 3         | 58  | 57  | 51  | 46  |

**Figure 9.** Cyclic voltammogram of different concentration CoCl$_2$ in absence of H$_2$BHNH at 298.15 K.
Table 9. % DPPH scavenging activity data at T = 60 min.

| Compounds | 20 µL | 40 µL | 60 µL | 80 µL | 100 µL | 120 µL | 140 µL |
|-----------|-------|-------|-------|-------|--------|--------|--------|
| 1         | 19    | 24    | 26    | 27    | 33     | 38     | 43     |
| 2         | 23    | 28    | 31    | 32    | 36     | 43     | 48     |
| 3         | 27    | 33    | 37    | 43    | 50     | 47     | 51     |

Figure 10. Cyclic voltammogram of CoCl₂ in the presence of different concentration of H₂BHNH at 298.15 K.

\[ i_P = \text{the current in ampere} \]
\[ D = \text{diffusion coefficient in cm}^2/\text{s} \]
\[ A = \text{surface electrode area (cm}^2) \]
\[ C = \text{molar concentration of metal ion (mol/cm}^3) \]
\[ \nu = \text{scan rate in volts/sec} \]

Furthermore, the difference potential (\( \Delta E_P \)) was determined from the following equation [40]

\[ \Delta E_P = E_{p,a} - E_{p,c} \]

The above quantities (\( E_{pa1}, E_{pa2}, E_{pc1}, E_{pc2}, i_P \)) can be used to estimate numerous cyclic voltammetric parameters such as \( Q, \Gamma, an_a \) & \( k_s \). The surface concentration of the redox ion was determined by applying the following equation [41]

\[ \Gamma = i_P 4RT/n^2F^2A\nu \]

\( i_P \): the peak current
\( F \): faraday constant (96485.33 coulomb)
\( T \): temperature in K
\( N \): the number of e\(^-\) in redox reactions
\( R \): gas constant (8.314 J.mol\(^{-1}\).K\(^{-1}\))
\( A \): the surface area of working electrode in cm\(^2\)
\( \nu \): the scan rate in Vsec\(^{-1}\)

The charge transfer coefficient of electrons was evaluated by applying the following equation [42]

\[ an_a = 1.857RT/(E_{pc} - E_{pc/2}) \]

In case of cathodic peak, \( E_{pc/2} \) is the half-wave potential.

The charge consumed during the redox reaction was determined by applying the following equation [43].

\[ Q = nFA\Gamma \]
The heterogeneous charge transfer rate constant \(k_s\) was estimated by applying the following equation:

\[
k_s = 2.18 \times \left[ D c an_\alpha F \nu/RT \right]^{1/2} \times \exp \left[ a^2 nF \left( E_{p,c} - E_{p,a} \right)/RT \right]
\]

\(D_c\): the diffusion coefficient of oxidized particles  
\(N\): electrons number in redox reactions  
\(R\): the gas constant  
\(F\): Faraday constant  
\(T\): the temperature in K  
\(A\): charge transfer coefficient  
\(\Delta E_p\): the difference potential  
\(n_\alpha\): scan rate and the number of electrons transfer in the rate determining step

The previous parameters were estimated for the anodic as well as cathodic peak and tabulated in Table 10. Most of the evaluated parameters for the anodic and cathodic peaks increase as the concentration of CoCl\(_2\) increases. The increase in all peak currents supported the suggested diffusion mechanism [44].

### Cyclic voltammetry response of Co(II) in presence of H\(_2\)BHNH

The electrochemical behavior was studied for complexation between H\(_2\)BHNH and CoCl\(_2\) in 0.1 M KCl solution at GCE within (+0.8 to −0.8 V) potential window (Figure 11). The result current redox peaks are decreased by increasing the ligand concentration that favoring the covalency between the cobalt ions and H\(_2\)BHNH. Furthermore, the cathodic & anodic potential peaks were shifted. These outcomes results recommend a strong interaction between cobalt and H\(_2\)BHNH.

In addition to the previous variations in the shape of voltammogram, the calculated values

| M x10^(-3) | Ep,a (volt) | Ep,c (volt) | AEp (volt) | Ip,a x10^(-5) | Ip,c x10^(-5) | Ip,a/Ip,c | Ea x10^-11 | Dc x10^-11 | ana | Ks x10^(-4) | Gamma x10^(-8) | +Qc x10^(-5) | Gamma x10^(-8) | -Qa x10^(-5) |
|-------------|-------------|-------------|------------|----------------|----------------|-----------|-------------|-------------|-----|-------------|----------------|----------------|----------------|-------------|
| 0.66        | −0.006      | −0.324      | 0.318      | 4.37           | 1.23           | 3.55      | −0.165      | 61.11       |     | 1.646       | 8.47           | 0.417          | 1.26           | 1.481        | 4.49         |
| 1.32        | −0.008      | −0.322      | 0.314      | 4.28           | 1.15           | 3.73      | −0.165      | 14.90       |     | 1.908       | 4.13           | 0.390          | 1.18           | 1.453        | 4.40         |
| 1.96        | 0.008       | −0.292      | 0.300      | 4.77           | 1.86           | 4.72      | −0.142      | 28.12       |     | 2.168       | 4.17           | 0.629          | 1.91           | 2.975        | 9.01         |
| 2.60        | 0.035       | −0.264      | 0.299      | 17.8           | 4.22           | 4.22      | −0.115      | 66.02       |     | 2.074       | 6.92           | 1.431          | 4.34           | 6.040        | 18.3         |
| 3.23        | 0.037       | −0.255      | 0.292      | 17.7           | 4.01           | 4.41      | −0.109      | 42.36       |     | 2.168       | 5.06           | 1.362          | 4.13           | 6.007        | 18.2         |

**Figure 11.** Cyclic voltammogram of CoCl\(_2\) at different scan rates at final adding in absence of H\(_2\)BHNH at 298.15 K.
of $E_{pc}$, $E_{pa}$, $\Delta E_{pc}$, $i_{pa}$, $i_{pc}$ ($i_{pa}/i_{pc}$), $E^\circ$, $k_a$, $\alpha$, $\Gamma$ and $Q$ for CoCl$_2$ in the presence of H$_2$BHNH (Table 11).

**Effect of scan rate in (absence/presence) of H$_2$BHNH**

The cyclic voltammograms of Co$^{2+}$ in (absence/presence) for H$_2$BHNH at different scan rates 10, 20, 50 & 100 mV/s in 0.1 M KCl at 298.15 K are displayed in Figures 12 and 13. The outcomes data showed that both cathodic & anodic peak currents increase in linear manner with the increasing in the scan rate. Also, the cathodic peak was slightly shifted toward the negative potential while, the anodic peak was slightly shifted toward positive direction with the extent of the scan rate. These remarks suggest the diffusion controlled redox mechanism and supported by the linear relation between square root of scan rate and peak currents as displayed in Figures 14 and 15. The ratio of peak current is >1 that continue a correspondence to a quasi reversible system [45]. The solvation and kinetic parameters for CoCl$_2$ in absence/presence of H$_2$BHNH at various scan rates were shown in Tables 12 and 13.

**Table 11.** Solvation and kinetic parameters D, $k_a$, $\Gamma$ and Q of CoCl$_2$ at 298.15 K for wave (B) in presence of H$_2$BHNH.

| [M] x10$^{-3}$ | [L] x10$^{-3}$ | $E_{pa}$ (volt) | $E_{pc}$ (volt) | $\Delta E_{pc}$ (volt) | $-i_{pa}$ x10$^{-5}$ (Amp) | $i_{pc}$ x10$^{-5}$ (Amp) | $i_{pa}/i_{pc}$ | $E^\circ$ | $D_a$ x10$^{-11}$ | $D_c$ x10$^{-11}$ | $k_s$ x10$^{-4}$ (cm$^2$/sec) | $\Gamma_a$ x10$^{-6}$ (mol/cm$^3$) | $+Q_a$ x10$^{-5}$ (mol/cm$^3$) | $\Gamma_s$ x10$^{-6}$ (mol/cm$^3$) | $-Q_s$ x10$^{-5}$ |
|----------------|----------------|----------------|----------------|---------------------|-------------------|----------------|----------------|-------|--------------------|--------------------|-----------------------------|--------------------------|-----------------------|-----------------------------|---------------------|
| 3.20           | 0.65           | 0.04           | -0.25          | 0.29               | 15.2              | 4.65           | 3.23           | -0.10 | 31.2               | 2.96               | 2.39                         | 6.09                     | 1.579                 | 5.117                       | 145.5               |
| 3.18           | 1.28           | 0.03           | -0.26          | 0.29               | 12.8              | 4.13           | 3.01           | -0.11 | 22.4               | 2.33               | 2.28                         | 5.33                     | 1.394                 | 4.22                        | 13.1                |
| 3.16           | 1.91           | 0.04           | -0.30          | 0.34               | 11.4              | 3.87           | 2.91           | -0.13 | 17.8               | 2.12               | 1.33                         | 6.61                     | 1.314                 | 3.98                        | 11.6                |
| 3.14           | 2.53           | 0.07           | -0.35          | 0.42               | 8.89              | 3.76           | 2.36           | -0.14 | 11.3               | 2.01               | 0.95                         | 12.2                     | 1.274                 | 3.86                        | 9.12                |
| 3.12           | 3.14           | 0.08           | -0.40          | 0.48               | 7.34              | 3.52           | 2.08           | -0.16 | 7.76               | 1.81               | 0.73                         | 17.3                     | 1.196                 | 3.62                        | 7.54                |
| 3.10           | 4.61           | 0.04           | -0.41          | 0.45               | 2.97              | 1.67           | 1.78           | -0.18 | 1.34               | 0.43               | 1.26                         | 8.25                     | 0.568                 | 1.72                        | 3.07                |
| 3.06           | 6.07           | 0.03           | -0.44          | 0.47               | 1.52              | 1.34           | 1.13           | -0.20 | 0.35               | 0.29               | 1.16                         | 7.36                     | 0.449                 | 1.36                        | 0.508               |
| 2.96           | 8.83           | 0.04           | -0.47          | 0.47               | 0.51              | 1.47           | 0.33           | -0.23 | 0.05               | 0.38               | 0.86                         | 7.74                     | 0.502                 | 1.52                        | 0.170               |

**Figure 12.** Cyclic voltammogram of CoCl$_2$ at different scan rates at final adding in presence of H$_2$BHNH at 298.15 K.
**Figure 13.** Relation (ip vs. $\nu^{1/2}$) for CoCl$_2$ at final adding in absence of H$_2$BHNH at 298.15 K and different scan rate.

**Figure 14.** Relation (ip vs. $\nu^{1/2}$) for CoCl$_2$ in presence of H$_2$BHNH by molar ratio (1:1) at 298.15 K and different scan rate.

**Table 12.** Effect of scan rate on CoCl$_2$ [3.23x10$^{-3}$ M] for wave (B).

| $\nu$ | Ep,a (volt) | Ep,c (volt) | $\Delta$Ep (volt) | -Ip,a x10$^{-5}$ (Amp) | Ip,c x10$^{-5}$ (Amp) | Ip,c/Ip | E° x10$^{-11}$ (cm$^2$/sec) | Da x10$^{-11}$ (mol/cm$^2$) | Dc x10$^{-11}$ (mol/cm$^2$) | an | $K_s$ x10$^{-4}$ (mol/cm) | $\Gamma_c$ x10$^{-8}$ (mol/cm$^2$) | $\Gamma_a$ x10$^{-8}$ (mol/cm$^2$) | $-Q_a$ x10$^{-5}$ |
|-------|-------------|-------------|-------------------|--------------------------|-----------------------|--------|------------------------------|-----------------------------|-----------------------------|----|-------------------|--------------------------|--------------------------|-------------------|
| 0.1   | 0.038       | -0.25       | 0.292             | 17.7                     | 4.01                  | 4.411  | -0.109                       | 24.2                        | 2.177                       | 2.169 | 5.06              | 1.3618                   | 4.13                     | 6.007             |
| 0.05  | 0.034       | -0.24       | 0.267             | 18.1                     | 3.54                  | 5.117  | -0.098                       | 88.6                        | 3.383                       | 3.407 | 4.39              | 2.4010                   | 7.27                     | 12.29             |
| 0.02  | 0.024       | -0.23       | 0.213             | 9.26                     | 1.51                  | 6.151  | -0.129                       | 57.9                        | 1.530                       | 3.66  | 1.15              | 2.5535                   | 7.74                     | 15.70             |
| 0.01  | 0.020       | -0.22       | 0.244             | 7.81                     | 1.16                  | 6.711  | -0.101                       | 82.4                        | 1.829                       | 3.976 | 1.25              | 3.9480                   | 12.0                     | 26.50             |
Table 13. Effect of scan rate on co-complex for wave (B).

| v  | Ep,a (volt) | Ep,c (volt) | ΔEp | -Ip,a x10^5 (Amp) | Ip,c x10^5 (Amp) | Ip,a/Ip,c | E⁺ | Da x10^11 | Dc x10^11 | an,sec | Ks x10^-4 (mol/cm^2) | Γ⁺ | Γ⁻ x10^-8 (mol/cm^2) | -Qa x10^-8 (mol/cm^2) |
|----|-------------|-------------|------|-------------------|-----------------|----------|---|-----------|-----------|-------|----------------------|----|---------------------|----------------------|
| 0.1 | 0.08        | -0.34       | 0.42 | 7.34              | 3.52            | 2.09     | -0.128 | 7.74      | 1.787     | 7.952 | 31.6                 | 1.194 | 3.61                | 2.49                 | 7.56                |
| 0.05 | 0.06        | -0.39       | 0.45 | 5.58              | 1.94            | 2.87     | -0.167 | 8.92      | 1.075     | 0.723 | 6.98                 | 1.313 | 3.98                | 3.78                 | 11.6                |
| 0.02 | 0.03        | -0.36       | 0.39 | 3.20              | 1.42            | 2.27     | -0.168 | 7.27      | 1.403     | 0.824 | 3.18                 | 2.366 | 7.16                | 5.39                 | 16.5                |
| 0.01 | 0.01        | -0.33       | 0.35 | 2.85              | 0.94            | 3.07     | -0.163 | 11.48     | 1.216     | 1.194 | 1.59                 | 3.122 | 9.44                | 9.58                 | 29.2                |

**Conclusion**

The bivalent Ni (II) and Co (II) complexes of N'-benzylidene-3-hydroxy-2-naphthohydrazide ligand were synthesized by ball milling as an efficient technique for quantitative solvent-free synthesis. The isolated solid compounds will be characterized using several spectroscopic techniques. The theoretical studies represented in the molecular modeling of isolated solid compounds were investigated using the DFT method which showed the compatibility with the practical outcomes data. Moreover, the in vitro antimicrobial activity assay versus fungi and bacteria via MIC technique has higher potency of Ni (II) than Co (II). The in vitro cytotoxicity on human muscle rhabdomyosarcoma (RD) via MTT technique and the antioxidant by DPPH free radical scavenging have higher inhibitory effect of complexes than free ligand. Voltammetric studies explained the effect of Co(II) in absence/presence of H₂BHNH on the electrochemical behavior of cobalt solution through the change in the values of (i_p, E, Γ, a_n, rate k_s, and Q).

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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