Synthesis, Spectroscopic Interpretations and Antioxidant Efficiency of Two Vital Selenium Complexes

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ABSTRACT

Synthesis of two new selenium complexes with nicotinamide (Nic) and riboflavin (RF) drug chelates. The speculated structures of the synthetic selenium complexes have been discussed using different tools of spectroscopic analyses like (infrared, Raman, electronic, 1H–NMR, and mass). Accordingly, FT–IR and 1H–NMR spectra the mode of complexation is supported as, four molecules of nicotinamide drug acts as a monodentate chelate through the N–atom of the pyridine ring with [Se(Nic)4]2H2O formula. The two riboflavin drug molecules coordinated to selenium metal as a bidentate chelate through azomethine nitrogen of pyrazine ring and O–atom of C=O pyrimidine-2,4-dione group with general formula [Se(RF)2]. Both of Nic and RF chelates acts as a neutral charge ligands. The conductivity measurements indicated that the selenium complexes are non-electrolytes behaviors. Thermal analyses (TG–DTG) of the studied complexes show that the decomposition process takes place in one broadening step with a wide temperature range. The surface morphology of the mentioned complexes was studied by scanning electron microscope (SEM) and the particle size is calculated using X-ray powder diffraction (XRD). Thermodynamic kinetic parameters are calculated by using Coats and Redfern equation. Screening of antioxidant activities of selenium complexes in vitro are assessment. The antioxidant activity is studied by three methods (DPPH assay; β-Carotene/linoleic acid a bleaching assay and ferric reducing power assay, the studied complexes have a significant antioxidant activity compared to synthetic antioxidants like trolox and BHT.

Keywords: Nicotinamide; Riboflavin; Selenium; Syntheses; Characterization; Antioxidant

INTRODUCTION

Selenium element and organoselenium play an essential role in the biological systems [1-7]. Generally, metal ions were required for many critical functions in humans [8-10]. Metal complexes have been played key role in the development of modern chemotherapy [11-14]; however, the study of metal–drug complexes is still in its early stages, thus representing a great challenge in current synthetic chemistry and coordination chemistry. Nicotinamide namely vitamin B3 (Figure 1A) has been demonstrated as anti-inflammatory actions and anticancer agent [15,16]. Some transition metal complexes of nicotinamide have been discussed both structurally and spectroscopically [17-24]. Riboflavin (Figure 1B) is a member of vitamins (B complex) that is an important antioxidant agent. There is little attention in the literature about the complexation of RF [25-27]. For the first time Malele et al. [26] Synthesized and characterization RF-Mo(V) complex in powder form using [Mo3O12(H2O)9]3+ complex as a precursor for the synthesis. To the best of our knowledge, little attentions have been made to discuss the interaction between drugs and selenium metal, that the literature survey is still poor in such spectroscopic characterizations. The interpretation is based on the ability of the cited two drugs to form complexes associate with selenium metal as an antioxidant agent. The spectroscopic characterizations and the thermal stability of the formed complexes were discussed. The present study is take place in vitro screening of antioxidant activity of new synthetic selenium complexes. Vitamins have a number of essential functions in the body, main role is as an antioxidant. It protects body cells from toxic compounds, heavy metals, such as lead and cadmium, and also from the side effects of drugs, radiation and free radical damage.
EXPERIMENTAL SECTION

Reagents
All chemicals used throughout this study were Analar or extra pure grade and received from Aldrich chemical company. The selenium metal, nicotinamide, and riboflavin used in this paper were of analytical grade and used without further purification. The solvents were used without distillation. Diphenylpicrylhydrazyl (DPPH), methanol, n-hexane, 2-deoxy-2-ribose, β-carotene and linoleic acid, were procured from Sigma (Sigma--Aldrich GmbH, Sternheim, Germany). Tween 40 and dimethyl sulphoxide (DMSO) were from Merck (Darmstadt, Germany).

Synthesis of Nic and RF Selenium Complexes
A mixture of solid powders of selenium metal (4 mmole) with nicotinamide (16 mmole) or riboflavin (8 mmole) in toluene solvent (50 mL) were refluxed for 24 hr at 60°C. The un-reacted of selenium metal powder was removed by filtration, and the color resultants solutions were reduced to ca. ⅓ of its volume and cooled to room temperature. The solid complexes obtained were then collected by filtration, washed with little amount of toluene and dried in vacuo over anhydrous calcium(II) chloride. The purity was checked by thin layer chromatography.

Instruments
The micro--analytical analyses of %C and %H percentages were calculated using a Perkin Elmer CHN 2400 (USA). The metal content was determined gravimetrically by converting the compounds to its corresponding stable form. The molar conductivity of the two selenium complexes with 10⁻³ mol/cm² concentration in DMSO solvent was measured using Jenway 4010 conductivity meter. The UV–Vis. absorption spectra were recorded in DMSO solvent within 800-200 nm range using a UV2–Unicam UV/Vis Spectrophotometer fitted with a quartz cell of 1.0 cm path length. The infrared spectra with KBr discs were recorded on Bruker FT–IR Spectrophotometer (4000–400 cm⁻¹), while while Raman laser spectra of samples were measured on the Bruker FT–Raman with laser 50 mW. The thermal studies TG/DTG–50H were carried out on a Shimadzu thermo gravimetric analyzer under nitrogen till 800°C. All experiments were performed using a single loose top loading platinum sample pan under nitrogen atmosphere at a flow rate of 30 mL/min and a 10°C/min heating rate for the temperature range 25-800°C. ¹H-NMR spectra were recorded as DMSO solutions on a Bruker 600 MHz spectrometer using TMS as the internal standard. SEM images were obtained using a Jeol Jem–1200 EX II Electron microscope at an acceleration voltage of 25 kV. X-ray diffraction (XRD) patterns of the sample was recorded on X Pert Philips X-ray diffractometer. All the diffraction patterns were obtained by using CuKα1 radiation, with a graphite monochromator at 0.02°/min scanning rate.
Anti-oxidative Assays
The antioxidant activity of selenium complexes were measured in terms of hydrogen-donating or radical-scavenging ability, using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent [28,29]. The β-Carotene/linoleic acid bleaching assay was determined by measuring the inhibition of selenium complexes and conjugated diene hydroperoxides arising from linoleic acid oxidation by described method [30,31]. The reductive potential based on the ferric reducing antioxidant power of the studied complexes and the standards positive controls (BHT and trolox) was determined [32]. Each of the measurements described was carried out in three replicate experiments and the results are recorded as mean ± standard deviation. The significantly different calculated at level of p ≤ 0.05.

RESULTS AND DISCUSSION

Micro-Analytical and Physical Study
The analytical, physicochemical results and spectroscopic outcome are in a good agreement with the speculated structures of mentioned two selenium complexes. The elemental analyses data of the selenium complexes of nicotinamide and riboflavin reveal that the two complexes have 1:4 and 1:2 stoichiometry (metal:ligand), respectively. The resulted selenium complexes are soluble in DMSO and DMF with gently warming but insoluble with alcohols and other organic solvents. The physicochemical results like color, yield, melting point as well as molar conductance values of these complexes are listed in Table 1. At room temperature, the conductance data of the selenium complexes which dissolved in DMSO are existed within 12-14 Ω⁻¹ cm² mole⁻¹ range; these data confirm that the two prepared selenium complexes are non-electrolytes [33]. The speculated structures of both selenium complexes with Nic and RF are represented in Figures 2A and 2B.

Table 1: Elemental analysis and physical data of Se (IV) folate complex

<table>
<thead>
<tr>
<th>Empirical formula</th>
<th>Color</th>
<th>M.P (°C)</th>
<th>Am (Ω⁻¹ cm² mole⁻¹)</th>
<th>Yield</th>
<th>Elemental analysis, % Found % (Calcd.)</th>
</tr>
</thead>
</table>
| [Se(Nic)₄]H₂O     | Light pink | 279      | 12                  | 84    | C  49.27  4.4  13.43  
                           |          |          |                     |       | H  4.48  4.85  9.44  
                           |          |          |                     |       | Se 49.1  4.85  9.49  |
| [Se(RF)₂]        | Yellow   | 320      | 14                  | 88    | C  49.27  4.4  13.43  
                           |          |          |                     |       | H  4.48  4.85  9.44  
                           |          |          |                     |       | Se 49.1  4.85  9.49  |

Infrared and Raman Spectra
The infrared spectra of the nicotinamide and riboflavin selenium complexes (Figures 3 and 4) are compared with that of the Nic and RF as a free ligands to notified the changes that might have taken place during the complexation (Tables 2 and 3). The FT-IR spectrum of [Se(Nic)₄]H₂O complex is shown in Figure 3 and its spectral data (Table 2) are assigned to give an idea about the place of the coordination between the selenium metal and Nic ligand. The infrared spectrum of Nic selenium complex shows bands at 3345 cm⁻¹ which is not existed in the free chelat, that this band is assigned to the ν(O−H) stretching vibration motion of uncoordinated water molecule. The stretching vibration motion ν(N−H) of −NH₂ group is presented at 3150 cm⁻¹ in case of complex form, this supported that
it's not participated in the coordination process. The shifted in the wavenumbers of the pyridine ring gave an indication about the formation of bond between the nitrogen of pyridine ring and selenium metal [34].

![Diagram](image)

**Figure 2B:** The speculated formula of selenium riboflavin complex

**Table 2:** Assignment of spectral data of Nic and [Se(Nic)₄].H₂O complex

<table>
<thead>
<tr>
<th>Compound</th>
<th>ν(OH)</th>
<th>ν(NH)</th>
<th>ν(CH)</th>
<th>ν(C=O) + ν(C=C) + ν(C=N)</th>
<th>Se-N</th>
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<tbody>
<tr>
<td>Nic</td>
<td>3200</td>
<td>3060</td>
<td>1699</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2787</td>
<td>1681</td>
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<td>2777</td>
<td>1621</td>
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<td>2765</td>
<td>1593</td>
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<td>1486</td>
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<td></td>
<td>1341</td>
<td>1254</td>
<td>1232</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Se(Nic)₄].H₂O</td>
<td>3345</td>
<td>3221</td>
<td>3068</td>
<td>1715</td>
<td>539</td>
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<tr>
<td></td>
<td>3250</td>
<td>2972</td>
<td>1666</td>
<td>415</td>
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<td>2904</td>
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The infrared spectrum of the [Se(RF)₂] complex (Figure 4) is compared with its RF free ligand to notify the changes occurs during the complexation (Table 3). In case of free RF ligand, the bands at (1732 and 1716 cm⁻¹) and 1548 cm⁻¹ are corresponded to the stretching vibrations of C=O amide group and C=N of conjugated system, respectively. These frequencies are shifted or absence in complexation state, due to the involvement of the C=O and C=N in coordination process [34]. The free RF ligand and its selenium complex have an intense peak at 3172 cm⁻¹, which is assigned to stretching frequency of −NH group attached with the heterocyclic ring. This interpretation confirms that the −NH group free from the sharing in the complexation. The IR spectrum of the RF selenium complex has a broadening band at 3304 cm⁻¹, which is assigned to the stretching band of O−H group. The Raman spectra of Nic and RF selenium complexes are assigned and illustrated in (Figures 5 and 6). The Raman spectra are found to be a complementary with infrared spectroscopic techniques. The two new bands...
which are exhibited in both infrared and Raman spectra at around 600 and 400 cm\(^{-1}\), respectively are assigned to \(\nu(M-O)\) and \(\nu(M-N)\), that refer to binding of selenium ions with oxygen and nitrogen atoms.

Table 3: Assignment of spectral data of RF and [Se(RF)]\(_2\) complex

<table>
<thead>
<tr>
<th>Compound</th>
<th>Assignments</th>
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<tbody>
<tr>
<td></td>
<td>(\nu(OH)+\nu(NH))</td>
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<tr>
<td>RF</td>
<td>3372</td>
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<td></td>
<td>3362</td>
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<tr>
<td></td>
<td>3180</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>[Se(RF)](_2)</td>
<td>3304</td>
</tr>
<tr>
<td></td>
<td>3172</td>
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<td></td>
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</table>

Figure 3: FT-IR spectrum of [Se(Nic)]\(_3\).H\(_2\)O complex

Figure 4: FT-IR spectrum of [Se(RF)]\(_2\) complex
Figure 5: Raman spectrum of [Se(Nic)₄]H₂O complex

Figure 6: Raman spectrum of [Se(RF)₂] complex

Electronic Spectra
Figures 7 and 8 refer to the electronic (UV-vis) spectra of the Nic and RF selenium complexes, respectively. The nicotinamide chelat has absorption spectra in the ultraviolet region in the region of the 200–400 nm and in some cases these bands extends over to higher wavelength region due to conjugation. But upon complexation with selenium metal, due to interaction with the metal ion there will be an interesting change in the electronic properties of the system. New bands in the visible region due to charge transfer spectra from metal to ligand (M–L) or ligand to metal (L–M) can be observed and this data can be processed to obtain information regarding the structure of the complexes [35]. Electronic spectrum of [Se(Nic)₄]H₂O complex was recorded in DMSO with 10⁻³ mol/cm³. UV-visible peaks corresponding to the π→π* transition in the Nic complex was observed at 284 nm [36]. The peak belonging to n→π* transition is recorded a wavelength 378 nm [37]. The first range can be assigned to π→π* transitions in the aromaticity of pyridine ring while the second range is most probably due to the n→π* transitions of NH₂ and carbonyl of amide group beside to nitrogen atom of pyridine ring [38]. The third type of transition in visible region located at 466 nm can be attributed to the ligand-to-metal charge transfer bands LM⁰CT from the electronic lone pairs of pyridine nitrogen to the metal ions [39]. The absorption spectrum of RF free chelat exhibits four peaks [40] at 223, 267, 375 and 444 nm, respectively. The first two bands are due to π→π* transitions but the other two bands at 375 and 444 nm are assigned to n→π* transitions. After complexation, the spectrum of [Se(RF)₂] complex has four absorption bands at 276, 384, 398 and 444 nm with a red shift due to coordination towards selenium metal.

¹H-NMR Spectra
The ¹H–NMR spectrum of free nicotinamide chelat displays four chemical shifts at δ = 9.081, 8.735, 8.249 and 7.527 ppm due to 4H of pyridine ring and two other peaks at δ = 8.22 and 7.67 ppm which are corresponded to 2H of –NH₂ amido group. The ¹H–NMR spectrum of [Se(Nic)₄]H₂O complex (Figure 9) has some peaks at (δ = 8.528, 8.702, 8.717, 8.905, 8.914, and 9.212 ppm), (δ = 7.909, 7.921, and 7.936 ppm), δ = 5.831 ppm due to 4H of pyridine ring, 2H of –NH₂ and 2H of H₂O, respectively. This results indicates that the coordination take place through –N atom of pyridine ring and faraway of both –N and –O atoms of amido group. The presence of new chemical shift at 5.831 ppm due to 2H of water molecule support the located of uncoordinated water molecule outside the coordination sphere. ¹H–NMR spectrum of riboflavin free chelat in DMSO-d₆ refer to distinguish peaks for the imide proton that is observed at 11.34 ppm, aromatic protons are at 7.92 ppm, methyl groups are
observed at 3.64 ppm and 2.41 ppm, protons of the side chain including the O–H protons are observed in the range from 2.50–5.14 ppm. In case of 1H–NMR spectrum of [Se(RF)₂] complex (Figure 10), the protons of aromatic rings, methyl groups, O–H protons are observed with small chemical shifts and the proton of –NH imide group is still unshifted which exhibited at δ = 11.32, these results confirm that the RF coordinated to selenium metal through –N and –O atom of pyrimidine-2,4-dione moiety and the –N of –NH imide group far away of chelation. The large peak at 2.50 ppm belongs to DMSO.

Figure 7: Electronic spectrum of Se(IV) Nic complex

Figure 8: Electronic spectrum of Se(IV) RF complex

**Thermal Analyses**

The thermogravimetric (TG) curves for the [Se(Nic)₄]H₂O and [Se(RF)₂] complexes are shown in Figures 11 and 12. Based on the TG curve, the following mass loss sequences can be proposed concerning [Se(Nic)₄]H₂O complex. The first mass loss is associated with the release of one uncoordinated water molecule with (calc. 3.07%; found 2.66% at DTG max = 76°C). The release of uncoordinated water molecule is followed by the release of four nicotinamide (Nic) molecules with (calc. 83.44%; found 83.93% at DTG max = 300°C). The selenium metal is a final degradation with (calc. 13.49%; found 13.41%). It is clearly obvious that the TG curve of the [Se(RF)₂] complex do not exist any mass loss up to 200°C, which interpretive the thermal stability. At 320°C, this complex was loss two riboflavin (RF) molecules with (calc. 84.74%; found 84.10%). The final decomposition product is selenium metal contaminated with four carbon atoms as a solid residual with (calc. 15.26%; found 15.90%). The thermodynamic activation parameters of decomposition processes of Nic and RF selenium complexes namely activation energy (E'), enthalpy (ΔH'), entropy (ΔS') and Gibbs free energy change of the decomposition (ΔG') were evaluated graphically by employing the Coats-Redfern relation [41].

The entropy of activation (ΔS'), enthalpy of activation (ΔH') and the free energy change of activation (ΔG') were calculated using the following equations:

\[ ΔS' = 2.303 \log(A\text{h/kT})R \ldots (1) \]

\[ ΔH' = E' - RT \ldots (2) \]

\[ ΔG' = ΔH' - TΔS' \ldots (3) \]

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The thermodynamic activation data are summarized in Table 4. The activation energies of decomposition were found to be in the range 125-181 kJmol\(^{-1}\). The high values of the activation energies reflect the thermal stability of the complexes [42–46]. The entropy of activation was found to have negative values in all the complexes which indicate that the decomposition reactions proceed with a lower rate than the normal ones.
Table 4: Thermodynamic parameters of the A: [Se(Nic)_4]H_2O and B: [Se(RF)_2] complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>Parameter</th>
<th>$E$ (kJ mol$^{-1}$)</th>
<th>$A$ (s$^{-1}$)</th>
<th>$\Delta S$ (J mol$^{-1}$ K$^{-1}$)</th>
<th>$\Delta H$ (kJ mol$^{-1}$)</th>
<th>$\Delta G$ (kJ mol$^{-1}$)</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>125</td>
<td>1.62x10$^{10}$</td>
<td>-54</td>
<td>121</td>
<td>149</td>
<td>0.9904</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>181</td>
<td>4.97x10$^{13}$</td>
<td>-12</td>
<td>175</td>
<td>168</td>
<td>0.9957</td>
</tr>
</tbody>
</table>

Figure 11: TGA diagram of Se(IV) Nic complex

Figure 12: TGA diagram of Se(IV) RF complex

X-Ray Powder Diffraction and SEM Studies
The XRD diffraction patterns within the 0°<2θ<80° range for the [Se(Nic)$_4$]H$_2$O and [Se(RF)$_2$] complexes were carried out in order to obtain an idea about the lattice dynamics of these complexes (Figures 13 and 14). All definite peaks of Se metal, Nic and RF are indexed, which are matched and compared with the standard data. The grain size for nano compounds were calculated according to Scherrer’s formula [47].

Purity and morphology of the nicotinamide and riboflavin selenium complexes obtained were studied using SEM to confirm the fact that each solid represents a definite compound of a definite structure which is not contaminated with starting materials. The obtained SEM micrographs, shown in (Figures 15 and 16), is allowed to verify that these complexes are the ones with the well-formed crystalline shapes. Such facts are in agreement with the formation of new complexes and were supported the XRD data.

Antioxidant Activity
The potential antioxidant activity of the tested samples was determined on the basis of three methods, the scavenging activity of the stable free radical DPPH (EC$_{50}$ value); inhibition of the coupled oxidation of linoleic acid and beta-carotene (AA % value) and Ferric reducing antioxidant power (EC$_1$ value). Since the reaction followed a concentration-dependent pattern, only values of EC$_{50}$; AA% and EC$_1$ of each sample; BHT and Trolox are presented in (Table 5). In general the lower the EC$_{50}$ value the higher them free radical scavenging activity of a sample. The selenium complexes of Nic and RF had significantly lower EC$_{50}$ value compared to
trolox and BHT. Regarding the EC₁ values, the lower EC₁ value the higher the ferric reducing activity of the sample.

Figure 13: XRD patterns of [Se(Nic)₄]H₂O complex

Figure 14: XRD patterns of [Se(RF)₂] complex
In present study, the Nic and RF selenium samples had significantly higher activity and lower EC\textsubscript{1} than trolox (8.35 ± 0.22 µg/ml) and BHT (4.61 ± 0.35 µg/ml). In the β-carotene linoleic acid system assay, Nic and RF complexes also possessed better antioxidant activity than trolox (54.31 ± 2.51%) and BHT (90.20 ± 1.81%). The efficiency of an antioxidant component to reduce DPPH essentially depends on its hydrogen donating ability, which is directly related to the chemical composition of each compound. In the β-carotene linoleic acid system assay, selenium complexes have ability to block the chain reaction of lipid peroxidation mainly by scavenging the intermediate lipid peroxyl radicals which are generated [30]. Antioxidant activities of studied vitamins, like any other vitamins were never intended for the prevention of chronic disease and mortality. They are not magic bullets. They are intended for health maintenance this does, in appropriate amounts, include a protective antioxidant effect in the body’s tissues.

Table 5: Antioxidants activities of the control, selenium complexes, BHT and Trolox samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>EC50 (µg/ml)</th>
<th>EC1(µg/ml)</th>
<th>AA %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.91 ± 0.09</td>
<td>1.01 ± 0.15</td>
<td>16.26 ± 2.31</td>
</tr>
<tr>
<td>Nicotine amide</td>
<td>8.11 ± 0.09</td>
<td>2.91 ± 0.25</td>
<td>25.73 ± 1.91</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>4.11 ± 0.17</td>
<td>3.91 ± 0.23</td>
<td>41.73 ± 3.01</td>
</tr>
<tr>
<td>BHT</td>
<td>21.51 ± 1.61</td>
<td>4.61 ± 0.35</td>
<td>90.20 ± 1.81</td>
</tr>
<tr>
<td>Trolox</td>
<td>6.75 ± 0.22</td>
<td>8.35 ± 0.12</td>
<td>54.31 ± 2.51</td>
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</table>

\[a,b,c,d\] data bearing different superscript letters in the same raw were significantly different (P < 0.05). Each value in presented as means ± standard deviation (n=3).

CONCLUSION

Preparation and characterization of new selenium(IV) nicotinamide and riboflavin were discussed. The coordinated behavior of both chelate drugs and its Se(IV) complexes have been described based on
spectroscopic (infrared, Raman, electronic, ^1^H–NMR, and mass) analytical techniques. The infrared, Raman, electronic, and ^1^H–NMR spectra measurement were used to identify the structure and to refer to the coordination place. Thermal analyses are used to detect the crystallization and coordinated water molecules. The surface morphology and particle size of Se(IV) complexes were examined using X-ray powder diffraction (XRD) and scanning electron microscope (SEM) analyzer. Screening of antioxidant activities of selenium(IV) complexes in vitro were investigated. The antioxidant activity was studied by three methods (DPPH assay; β-Carotene/linoleic acid a bleaching assay and ferric reducing power assay. The studied Se(IV) complexes have a significant antioxidant activity compared to synthetic antioxidants like trolox and BHT.

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