

Synthesis and Antioxidant Activity of Mixed Ligand Complex of Quercetin and Aspartic Acid with Cobalt(II)

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Abstract

Mixed ligand complexes are widely used in the field of pharmaceutical science due to their curing nature to various diseases. The present study was aimed to synthesize and explore the antioxidant activity of cobalt-quercetin-aspartic acid mixed ligand complex using quercetin (Q) and aspartic acid (AA) as ligands. The complex was synthesized by applying a versatile approach and characterized with the help of available sophisticated analytical techniques, example: FT-IR, UV-VIS, ¹H NMR, DSC and elemental analysis. The antioxidant activity of the free ligands as well as mixed ligand complex was also explored well and observed that mixed ligand complex is more antioxidant than the free ligands.

Keywords: Quercetin; Aspartic acid; Cobalt; Mixed ligand complex; Antioxidant

Introduction

Most of the biomolecules (vitamins, enzymes, amino acids, lipids etc.) present in our body are in the form of mixed ligand complexes coordinated with biologically important metals such as Fe, Cu, Zn, Co and Mg etc. [1]. It has been reported that the synthetic mixed ligand complexes are biologically active against various pathogenic microorganisms and have increased their applications and activity *in vivo* processes [2-4]. Among these synthetic complexes, the hetero ligand complexes of flavonoids (quercetin) and amino acids (aspartic acid) with various biologically important metal ions (Cu, Zn, Co, Fe) can have promoting effects in body regulatory processes [5] (Figure 1).

Cobalt, as a biologically important metal, is an essential micronutrient for living organisms. It is involved in various biological processes in body [6]. It is essential to all animals, including humans, however excessive exposure can lead to tissue and cellular toxicity [7]. Cobalt has been shown to provide promising potential in clinical applications [8]. The ligands such as flavonoids (quercetin, rutin and morin) and amino acids (tryptophan, arginine, aspartic acid and alanine) have effective role in the field of biochemistry and pharmaceuticals [9]. Quercetin is polyphenolic natural compound found in plant based foods [10,11]. It is one of the effective chelator with three possible chelating sites. It is used in various biological and pharmacological processes that is antioxidant, anti-allergic, anti-cardiovascular diseases, anti-ulcer, anti-inflammatory and anti-viral potentials [12,13].

The aspartic acid, another constituent of complexes have also significant role in enzyme-metal ion processes *in vivo* [14]. These are useful as antibacterial agents and work against *Staphylococcus aureus*, *Escherichia coli* and nutritive agents in humans and animals [15,16]. Aspartic acid provides energy to cells; which burn it to generate adenosine triphosphate that is a cellular energy currency [17]. It is useful but not indispensable in the human diet, it has significant role in gluconeogenesis, which is the process of making glucose, or sugar, when your dietary supply is low [18] (Figure 2).

Literature reveals that the mix ligand complexes of transition metals with flavonoids and amino acids have not been synthesized so far. Keeping in view these facts and immaculate uses of amino acids and flavonoids, present work emphasis the synthesis of mixed ligand complex of biologically important metal Co(II) with Q and AA.

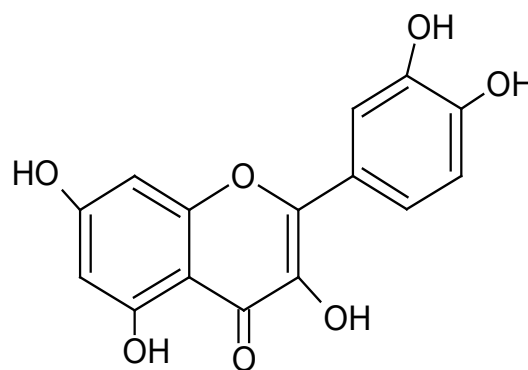


Figure 1: Quercetin.

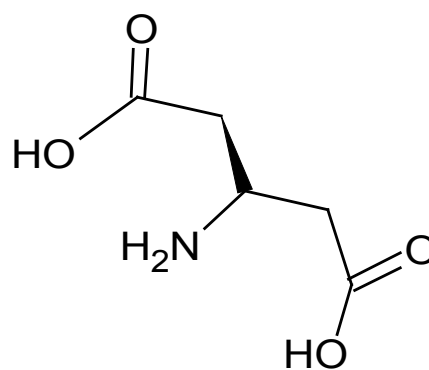


Figure 2: Aspartic acid.

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Experimental

Materials and methods

All the reagents and solvents were of analytical or chemically pure grade. Quercetin dihydrate (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one) was purchased from Sigma Aldrich and $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ from Fluka. HPLC grade methanol was obtained from Fisher scientific UK Ltd. KBr and DPPH were purchased from Aldrich Chemical Co. All reagents were weighed with an accuracy of ± 0.0001 g.

UV-VIS spectra were obtained on Perkins Elmer Lambda 35 UV-visible double beam spectrophotometer using standard 1.00 cm quartz cells in methanol solvent. $^1\text{H-NMR}$ spectra (in DMSO solvent) were obtained on a Bruker 500 MHz spectrometer using TMS as internal reference. The IR spectra were recorded by using KBr pellets in the spectral range 400-4000 cm^{-1} on a Nicolet 5700 FT-IR instrument. The DSC curve was obtained using DSC822 $^\circ$ Mettler Toledo (Heating rates of 20 $^\circ\text{C min}^{-1}$, aluminum crucible, mass 20 mg, in nitrogen atmosphere). The elemental analyses for Carbon, Hydrogen and Nitrogen were obtained on microanalyses Perkins-Elmer 240c.

Synthesis of cobalt (II) complex: 20 mL equimolar quantities of metal chloride, Q and AA (0.1 M) solutions were mixed in flask by equimolar ratios, that is 1:1:1 at cold temperature (-4 $^\circ\text{C}$). The pH of the contents was maintained at 6-8 in final mixture by using buffer. The mixture was kept on stirring for overnight at cold; the yellow green colored precipitates were obtained. The contents of the flask were evaporated in china dish to isolate the precipitates. The resulted product was washed and dried at room temperature. The precipitates weighed and the yield was calculated as 67% while melting point was measured by Gallenkamp apparatus. Elemental analysis found C, 46.25; H, 2.95; N, 2.50%. Anal. Calc. for $[\text{CoQ}(\text{AA})(\text{H}_2\text{O})_2]\text{Cl}$: C, 46.46; H, 2.87; N, 2.85%.

Results and Discussion

UV/VIS study

It has been determined from elemental analyses data that the Co(II) mixed ligand complex of Q and AA is in 1:1:1 stoichiometry. The synthesis of such complex is denoted by the general formula $L_1ML'_1$ given in equation (I):



For complexation, Q deprotonates at 3-OH functional group due to being more acidic and forms anionic specie then coordinates to metal ion to form metal complex. The OH group at position 5 is less acidic and is sterically hindered by first coordination at position 3. Similarly, the AA also forms anionic species by deprotonation at -COO functional group and complexation at -NH₂ group also. The complex formation was further supported by the insolubility of the complex in water, methanol and dilute alkali solution. The Q, AA and their complex, in general, were non-hygroscopic and stable solids. The Q was easily soluble in Acetonitrile, DMF, DMSO and methanol, whereas the AA is soluble in H₂O, DMF, DMSO, Acetonitrile and the complex was having limited solubility in organic solvents. The complex was yellow green in color and thermally stable (Table 1), representing a strong metal-ligand bond. The elemental analyses data of the metal complex was consistent with their general formula as presented in equation (1).

The electronic spectrum for the complex is purely ligand-field spectrum. Where ligand such as quercetin shows the dominant peaks and undergoes bathochromic shift in its peaks after metal complex

formation. The UV/Visible spectra for free Q, AA and complex is depicted in Figure 3, Q displays two major absorption bands band-I at 256 nm and band-II 375 nm, band-I correspond to cinnamoyl system (ring B) while band-II corresponds to benzoyl system (ring-A), after complex formation band-II shows negligible bathochromic shift due to presence of amino acid while band-I was clearly seen to be shifted to longer wavelength with appearance of new band-III at 384 nm. The band-I and new band-III show the bathochromic shift due to interaction of 3-OH and 4-CO oxygen with metal ion in Q. On other hand, -COO⁻, -NH₂ moieties of AA are also involved in complexation resulting in overall electronic redistribution between metal ion and both the ligand molecules to give extended pi bonding system. Due to more acidic nature of 3-OH, it is more probable chelating site in conjunction with oxo group in Q. In addition, -COO⁻ and -NH₂ sites of aspartic acid are more susceptible to be involved in complexation process. On the other hand, 5-OH group in Q is not involved in complexation process due to experiencing steric hindrance and having less acidic proton nature. Since, none of the single crystal has been isolated so far hence; crystallography is not possible here, however with the help of above data the tentative structure of metal complex has been given in Figure 4.

FT-IR study

The coordination sites and the binding properties of Q and AA were determined by using IR spectroscopy. The prominent bands with tentative assignments are listed in Table 2, some useful data can be

Complex	Color and texture	Melting point
$[\text{CoQ}(\text{AA})(\text{H}_2\text{O})_2]\text{Cl}$	Yellow-green precipitates	158 $^\circ\text{C}$

Table 1: Physicochemical properties of complex.

Quercetin	Complex	Peak Type
3251	3262	-OH
1660/1601	1667/1601	C=O
1611	1605	Ring A and B
1561	1557	C=C
	1510	Ring C, C ₄ =O ₂
	1431	Ring C, C ₃ =O ₃
1316	1361	Ring B, C ₂ -OH, C ₃ -OH
1257	1240	C-O-C
	541	Co-O
	592	δOH

Table 2: Relative FT-IR spectral assignments of quercetin and complex.

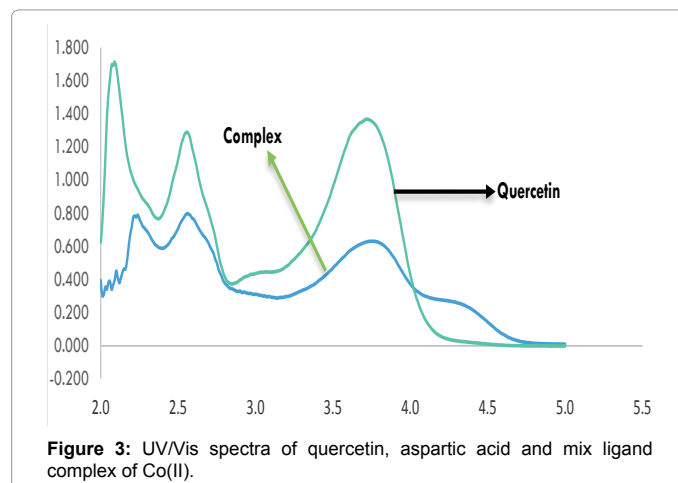


Figure 3: UV/Vis spectra of quercetin, aspartic acid and mix ligand complex of Co(II).

attained by comparing the IR spectra of free Q and AA with resulting complex (Figure 5). The C=O stretching mode of the free Q occurs at 1660 cm^{-1} , which has been shifted to 1667 cm^{-1} after complex formation. This shift proposes the coordination of carbonyl oxygen with metal ion.

The decrease in C=O bond order when coordinated to metal ion along with 3-OH, in complex may give rise to coupling of the vibrations of these two bands. The new bands at around 1610 and 1453 cm^{-1} are related with the anti-symmetric and symmetric stretching modes of the C-O group at the chelating site, respectively. The bands positioned at 1611 and 1257 cm^{-1} pertained to $\nu(\text{C}=\text{C})$ and $\nu(\text{C}-\text{O}-\text{C})$ frequencies are somewhat shifted by complex. The $\nu(\text{C}-\text{O}-\text{H})$ deformation mode

observed at 1319 cm^{-1} in the ligand is shifted to 1361 cm^{-1} in the complex demonstrating an increase in bond order, which is usually observed when metal coordination involves with the ortho-phenolic $\nu(\text{O}-\text{H})$ group on the Q (ring B). Additionally, the occurrence of $\nu(\text{M}-\text{O})$ stretching vibration at 541 cm^{-1} indicates the formation of metal complex, while the ligand displays no such band.

The AA is coordinated from other side of the plane to same metal ion is indicated from spectral change caused by N-H asymmetric and N-H symmetric vibrations observed at ~ 2955 and $\sim 2655\text{ cm}^{-1}$, respectively. These stretching vibrations have been shifted to higher value that is 3251 cm^{-1} in the spectrum of complex. It suggests the coordination of the two metal ions through nitrogen. The asymmetric $\nu(\text{COO}^-)$ band of the free AA that is $\sim 1581\text{ cm}^{-1}$ is shifted to higher wave number, that is 1601 cm^{-1} . In addition, the symmetric $\nu(\text{COO}^-)$ mode observed at $\sim 1420\text{ cm}^{-1}$ is shifted to lower wave number, that is 1350 cm^{-1} in the of complex spectrum. It indicates that the coordination of the carboxylic acid group to metal ion has taken place via oxygen. Besides this, some new bands of weak intensity were observed at 592 and 563 cm^{-1} attributed to M-O and M-N vibrations, respectively. It is seen that these vibrational bands are absent in the spectrum of free aspartic acid. Some important bands of complex and free ligands are shown in Table 3.

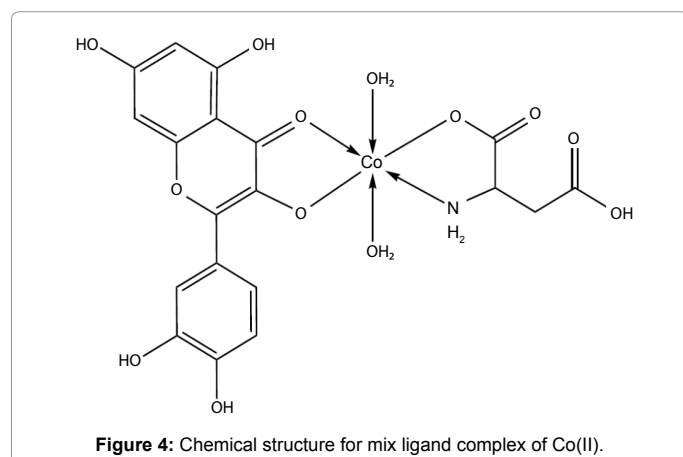


Figure 4: Chemical structure for mix ligand complex of Co(II).

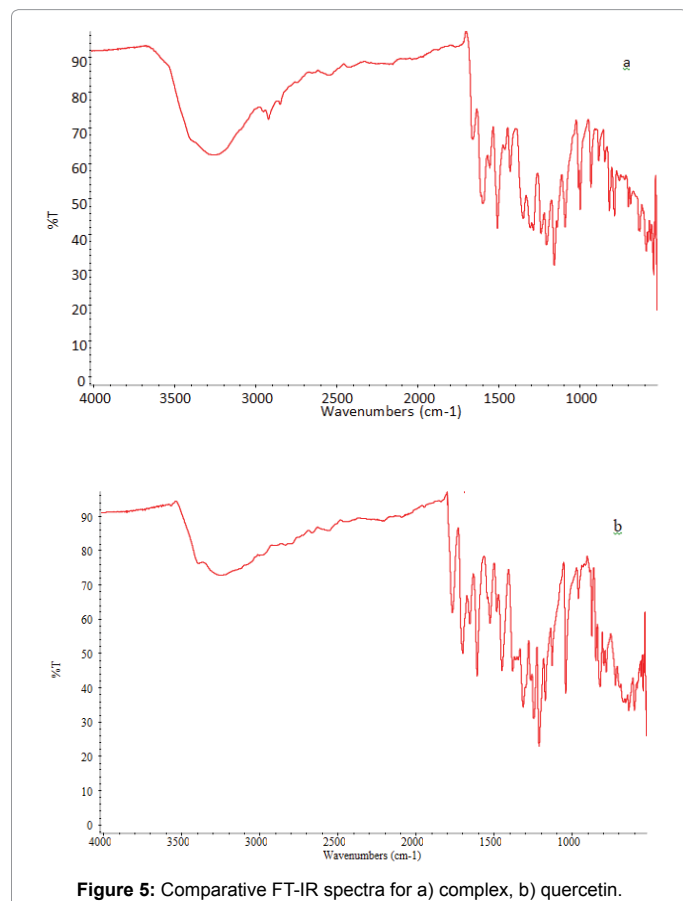


Figure 5: Comparative FT-IR spectra for a) complex, b) quercetin.

DSC study

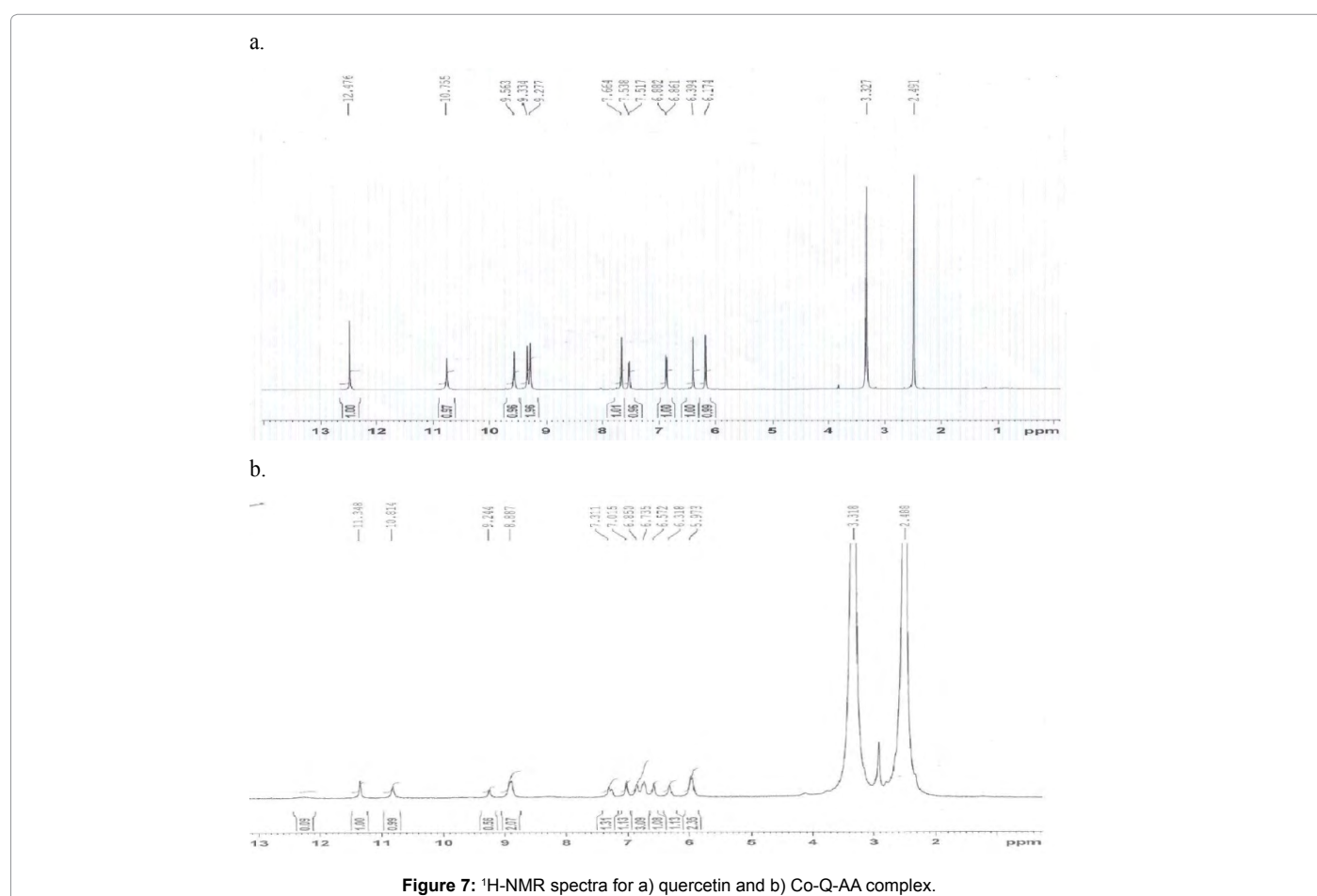
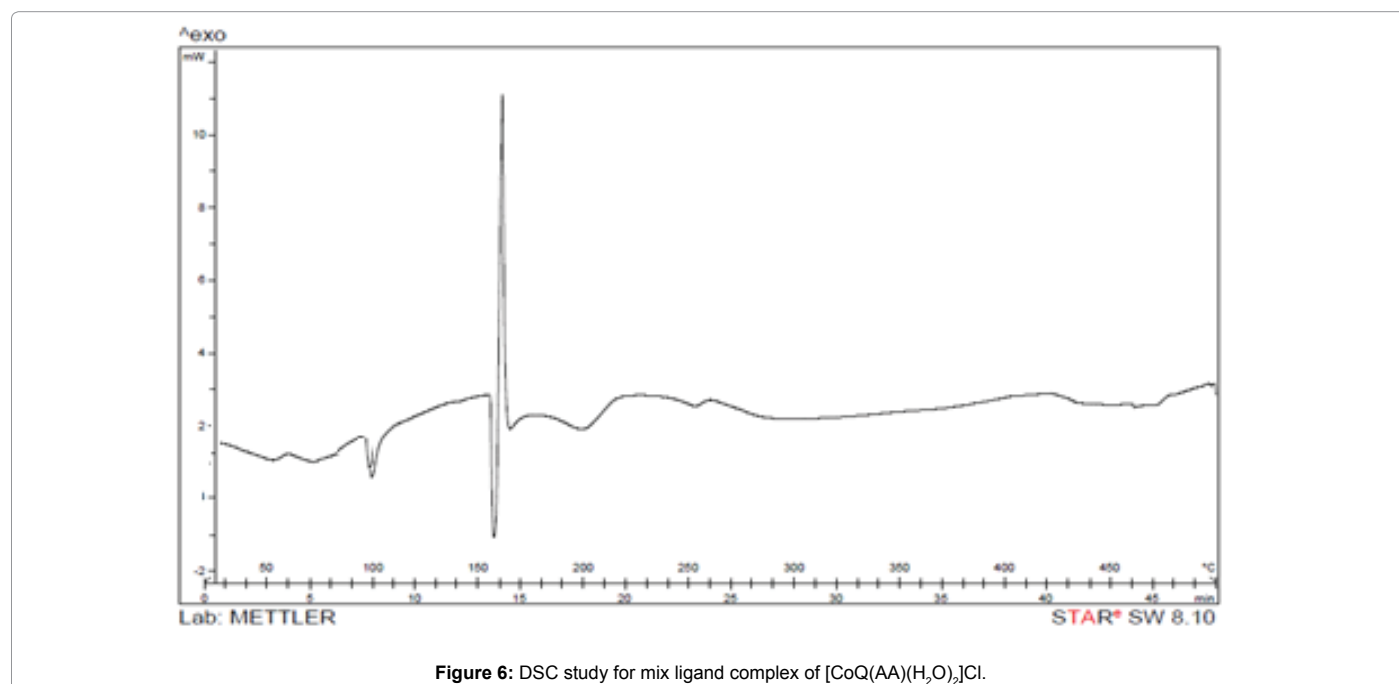
The DSC study shows characteristic thermal events at heating rate of $10^\circ\text{C min}^{-1}$. The DSC curve of complex shows some definite endothermic and exothermic changes and series of thermal events that show the dehydration, melting and series decomposition. It has been noted that thermal event observed at 45°C is due to dehydration consistent with mass loss. The endothermic peak at 158°C is representing the melting point of the complex as observed in thermogram (Figure 6). Whereas the series of small and very broad exothermic peaks indicate the sequential degradation of organic part of complex compound at the 161°C , 241°C and 250°C respectively. All the exothermic and endothermic peaks show agreement with relevant mass changes (Table 4).

$^1\text{H-NMR}$ study of complex

The $^1\text{H-NMR}$ data (Table 5) show the downfield chemical shift in Co-Q-AA complex as compared to free ligands due to their coordination with metal ion. The quercetin (Figure 7a) coordinates with metal by replacing 3-OH proton, which is evident from $^1\text{H-NMR}$ spectrum of complex (Figure 7b) where signal at 3-OH is missing which suggests the quercetin coordination position with metal. The coordination of aspartic acid with metal ion is confirmed by shift in $^1\text{H-NMR}$ signals in complex spectrum compared to free aspartic acid such as the N-H signal intensity is decreased due to coordination with metal, besides, all the $^1\text{H-NMR}$ signals related to aspartic experience downfield shift due to conjugation and coordination with metal ion.

Antioxidant activity of complex by DPPH radical scavenging method

In our body, number of biochemical processes going on every time, which results in formation of various biomolecules. Among these useful moieties some highly reactive and potentially damaging chemical substances are also formed, such as hydroxyl radicals, hydrogen peroxide and superoxide anions [19]. These reactive oxygen species (ROS) can cause serious chronic diseases that is, cancer, aging and coronary heart disease etc., by altering the structure of lipids, fats, proteins and various other biomolecules[20]. For healthy life these ROS



are trapped by some natural antioxidants in our body or artificially by incorporating synthetic drugs. There are vast numbers of metal complex based drugs synthesized in laboratory as antioxidants to

prevent these free radicals. The antioxidant activity of Co-Q-AA and free ligands was measured for their scavenging ability through UV-VIS spectrophotometer with the help of DPPH free radical (Figure 8). The

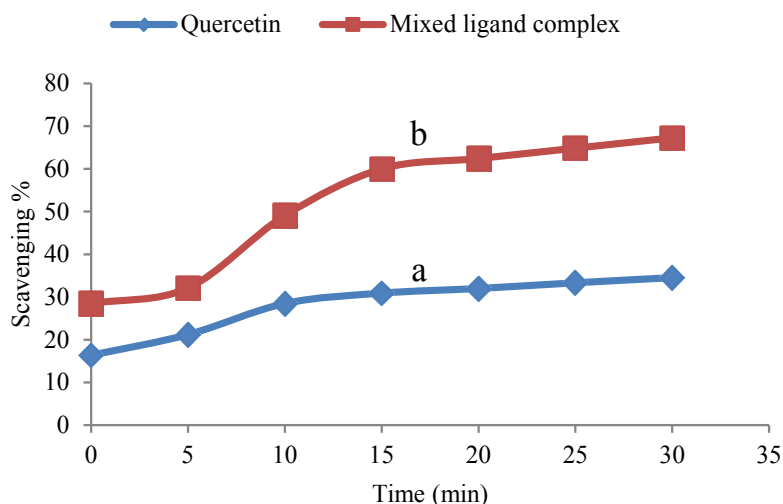


Figure 8: Antioxidant activity for a) quercetin and b) mixed ligand complex.

Aspartic acid	Complex	Peak type
2955/2655	3251	N-H asymmetric and symmetric
1686	1667	C=O
1581/1505	1601/1510	COO- (asymmetric)
1420	1350	COO- (symmetric)
989	930	C-N
663/598	592/563	M-N, M-O

Table 3: Relative FT-IR spectral assignments for aspartic acid and complex.

Complex and Ligands	Temperature	Type of event
Quercetin	124°C (Melting)	Endotherm
	331°C (Decomposition)	Endotherm
Aspartic Acid	227°C (Decomposition)	Endotherm
	384°C	Exothermic
Complex	158°C (Melting)	Endotherm
	161°C (Decomposition)	Exothermic

Table 4: Thermal data for characteristic thermal changes in ligands as well as their complex.

Type of H	Quercetin signals (in ppm)	Complex signals (in ppm)
5-OH	12.74	11.34
7-OH	10.75	10.81
3-OH	9.56	-
4'-OH	9.33	9.24
3'-OH	9.27	8.88
2'-H	7.66	7.31
6'-H	7.53	7.01
5'-H	6.88	6.73
8-H	6.39	6.57
6-H	6.17	6.31

Table 5: ¹H-NMR data for the various protons in quercetin and complex compound.

reaction between analyte and DPPH occurs in two steps: (I) DPPH absorbance (λ_{max} = 515 nm in methanol) decays quickly (typical time, 60-120 sec); and (II) DPPH absorbance decays slowly in ~1 h to reach a constant value. In fact the molecular structure of flavonoids plays an important role in their antioxidant activity. Similarly, the increase in antioxidant activity of this species is due to appreciable involvement

from 3-OH of C ring. Actually, H-atom transfer may take place from 3-OH of Quercetin to DPPH during reaction assisting the abstraction process as well as stabilizing corresponding radical formed later on. The mixed ligand complex $[CoQAA(H_2O)_2]$ exhibit higher antioxidant activity comparative to free ligand. Therefore, it indicated that chemical properties of considerable changed by the Co(II) metal ion. In reaction of quercetin to DPPH radical, a H atom is abstracted from the $[CoQAA(H_2O)_2]$ to give a semiquinone complex which is stabilized by the metallic center and by conjugation with the 3-OH group. This property is not only unique for the Co(II) ion but it is reported [21] that it is also showed by some other metals but some time less effective than the Co(II) ion, the oxidation potential is decreased on metal complexation with flavonoids, hence complexed quercetin becomes higher antioxidant compared to uncomplexed one.

Conclusion

It has been concluded from the study that synthesis of mixed ligand complex of Co with two ligands quercetin and aspartic acid has been confirmed from UV-VIS, IR, NMR as well as elemental analysis. The complex has the melting point 158°C. The UV/VIS study shows the bathochromic shift as well as a new peak appeared from a d-d transition of d^7 system in a Co metal ion. FT-IR study reveals the formation of new bonds between metal and ligands. In addition, NMR shows the replacement of specific protons to evidence the proper sites of coordination. While DSC indicates the melting point as well as oxidation degradation of compound. A tentative structure of the compound has also been given which is fully supported by results evidenced by various analytical techniques. The newly synthesized complex is observed to be more antioxidant than ligand molecules. It may be the best available agent against the carcinogens in future.

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