

Microwave-assisted synthesis and characterization of oxime derivative of substituted chalcone and its nickel(II) complex

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In this study, microwave-assisted green synthesis of a complex of Ni (II)-2',4'-dihydroxy 4-fluoro chalcone oxime has been carried out. The structure of the complex was characterized by elemental analysis, molar conductance, magnetic moment, IR, ¹H-NMR, ¹³C-NMR spectroscopic data and thermal studies. IR data showed that Ni(II) is coordinated to the ligand through the phenolic-O and azomethine-N of the latter. TGA studies confirmed the presence of coordinated water molecules. The 1 : 2 metal to ligands stoichiometry was indicated by mole ratio method, which was further confirmed by Job's method of continuous variation modified by Turner and Anderson. Stability constant (K_s) of the complex determined by mole ratio method was 8.5245. Standard free energy change (ΔG^0) for metal–ligand complex formation was found to be -11.7026 K cal/mol at $27 \pm 2^\circ\text{C}$. On the basis of analytical data, octahedral structure has been proposed for the complex. All compounds were tested for antibacterial activity and showed moderate to good activity against *Escherichia coli* and *Staphylococcus aureus* at different concentrations. In this study, we report synthesis of oxime derivative of chalcone and its Ni(II) complex in the hope of developing new compounds which might be used as analytical reagents or intermediates in the synthesis of various classes of bioactive compounds.

Keywords: Antibacterial activity, chalcone oxime, metal complex, mole ratio.

COMPOUNDS in which the carbonyl group is bonded to carbon–carbon double bond/s have gained considerable attention because of many pharmacological and biological activities due to presence of α,β -unsaturated carbonyl system¹. In this class, chalcone and its derivatives are found to possess broad-spectrum biological and multiprotecting biochemical activities with a number of commercial applications^{2–8}. Oxygen, nitrogen and sulphur atoms at the active sites of many biomolecules play a significant role in the coordination with metals⁹. Hydroxychalcones and their nitrogen- or sulphur-containing derivatives like oximes and thiosemicarbazones possess coordinating

sites for different metals for complex formation that are pharmacologically and biologically active and have been employed as useful analytical reagents for estimation of different metal ions^{10,11}. Experimental studies reveal that the metal complexes display more potent bactericidal and fungicidal properties than their corresponding ligands.

Synthesis by microwave irradiation (MWI) technique is definitely a step ahead towards green chemistry. It is an easy, eco-friendly and economic method in comparison to the conventional method. Here we report a quick, environmentally safe and clean MWI synthesis of complex of Ni(II) with oxime derivative of substituted chalcone.

Experimental

Material and methods

Reagents (AR-grade) were purchased from Sigma Aldrich and HiMedia Laboratories Ltd (Mumbai, India) and used without further purification. Elemental analysis was carried out using an elemental analyser (Perkin-Elmer 2400 Series II CHNS/O). Molar conductance of compounds (DMSO $\sim 10^{-3}$ M) at $27^\circ \pm 2^\circ\text{C}$ and pH measurements were done using an electronic μP -multiparameter analyser. Magnetic susceptibility was determined using a Gouy balance at room temperature. The IR spectra in KBr discs were recorded on a Perkin-Elmer Spectrum Version 10.4.3. The UV-Vis spectra of compounds (UV-2600–220 V, Shimadzu Spectrophotometer) and NMR spectra (Bruker Avance 400 NMR spectrometer) were recorded. Thermogravimetric analysis (TGA) of metal complex was performed in the range 0–1200°C in nitrogen atmosphere (Schimadzu DTG-50) with a heating rate $10^\circ\text{C min}^{-1}$. Syntheses of compounds were done in a round-bottom flask on a domestic microwave oven (model MC2881SUP) with rotating tray using on/off cycling at 230 V.

Synthesis of 2',4'-dihydroxy 4-fluoro chalcone

Conventional method: 2',4'-dihydroxy 4-fluoro chalcone (DHFC) was synthesized by taking equimolar quantities (0.002) of dihydroxy acetophenone and *p*-fluoro benzaldehyde in ethanol (15 ml) with dropwise

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Table 1. Physical and analytical data of the synthesized compounds

Compound	Solvent (ml)	Time (s)	Yield %		Experimentally found (calculated; %)			
			CM	MW	C	H	N	Metal
DHFC	4	120	66	85	69.10 (69.77)	4.29 (4.18)	–	–
DHFCO _x	3	150	55	80	67.10 (65.69)	4.80 (4.74)	5.25 (5.11)	–
(DHFCO _x) ₂ Ni(II)·(H ₂ O) ₂	5	180	–	80	52.12 (50.73)	3.76 (3.66)	4.16 (3.95)	18.50 (18.26)

Table 2. Stability constant and free energy change of Ni(II)–DHFCO_x complex

	E_m	E_s	α	$\log K_s$	$-\Delta G$ Kcal/mol
Mole ratio	0.77	0.72	0.088	8.5245	11.7026

addition of KOH (0.003 M) and continuous and constant stirring at 40°C for 1.5 h and then kept for 22–23 h.

Microwave method: Microwave-assisted synthesis of DHFC¹² was done by Claisen–Schmidt condensation of equimolar (0.002 M) quantities of 2',4'-dihydroxy acetophenone and 4-fluoro benzaldehyde with 3 ml of alcohol in a conical flask. To this, few drops of aqueous KOH (0.003 M) solution were added slowly and mixed. The flask was covered with a funnel and then the mixture was irradiated under 200 W for 120 sec in a domestic microwave oven.

Progress and completion of reaction was monitored by thin layer chromatography (TLC) on silica-G gel slides using iodine vapour for detection. On cooling, the resulting mixture was washed with ice-cold water and then neutralized by ice-cold HCl (10%), and then precipitated out as solid. The separated solid was filtered and washed with ice-cold water till the washing was neutral to litmus. The compound was then recrystallized with ethanol and dried at room temperature.

Synthesis of 2',4'-dihydroxy 4-fluoro chalcone oxime

Conventional method: 2',4'-dihydroxy 4-fluoro chalcone oxime (DHFCO_x) was synthesized by mixing an alcoholic solution of dihydroxy *p*-fluoro chalcone (DHFC; 0.001 M) and hydroxylamine hydrochloride (0.001 M) with 0.5 ml of glacial acetic acid and thin refluxed for about 3 h.

Microwave method: DHFCO_x was synthesized by taking equimolar amounts (0.001 M) of DHFC and hydroxylamine hydrochloride in ethanol (3 ml) with dropwise addition of glacial acetic acid (0.5 ml). The covered flask was irradiated with microwaves at 200 W for 150 sec. On cooling, the product was collected by filtration, washed with water and dried.

Synthesis of DHFCO_x-Ni(II)

Ligand (DHFCO_x, 0.002 M) and metal salt (NiCl₂, 0.001 M) mixture in 3–5 ml ethanol was irradiated in a microwave oven at 200 W for about 180–240 sec with occasional shaking. The resulting solid was recrystallized with ethanol and finally dried over anhydrous CaCl₂ in desiccators, and kept in an air-tight container.

Tables 1 and 2 provide analytical and physical data of the synthesized compounds.

Spectrophotometric study of Ni(II)-DHFCO_x complex to determine the metal–ligand ratio

For spectrophotometric studies, absorbance of chelate solution (5 mg metal in 25 ml alcohol) was measured between 340 and 800 nm. The spectrum showed a shoulder band at 590 nm. Study (between pH 6.0 and 9.0) showed maximum absorbance at pH 8.5. So wavelength of 590 nm and pH of 8.5 were taken for further experimental work. The composition of Ni(II) with the ligand (DHFCO_x) has been determined spectrophotometrically using the mole ratio method as described by Nardo and Dawson¹³ and by Job's¹⁴ method of continuous variation as modified by Turner and Anderson¹⁵.

Calculation of stability constant: The stability constant (K_s) was calculated from the modified mole ratio curve using the following relation

$$K_s = c(1 - \alpha)/\alpha c (n \alpha c)^n,$$

where c is the concentration and n is the number of ligand

$$\alpha = (E_m - E_s)/E_m,$$

E_m is the maximum absorbance obtained at the intersect of extrapolated lines and E_s is absorbance at the correct stoichiometric molar ratio of metal to reagents in the complex.

Standard free-energy change (ΔG^0) for reaction of complex formation was calculated at $27 \pm 2^\circ\text{C}$ using the formula

$$\Delta G^0 = -RT \ln K_s.$$

Table 3. Antimicrobial activity of chalcone derivatives and Ni(II) complex

Compound	Zone of inhibition (mm)*					
	<i>Escherichia coli</i>			<i>Staphylococcus aureus</i>		
	25%	50%	100%	25%	50%	100%
1	–	9	9	–	–	–
2	–	11	8	–	–	8
3	3	–	16	14	–	11
Amikacin	16	16	16	15	15	15
DMSO	–	–	–	–	–	–

*Zone of inhibition less than 3 mm is not shown.

Antibacterial activity

In this study, inhibitory activities of synthesized compounds were examined against *Escherichia coli* (Gram-negative bacteria) and *Staphylococcus aureus* (Gram-positive bacteria). *In vitro* antibacterial activity was determined by agar disc diffusion method¹⁶. The agar nutrient, i.e. Muller Hinton agar for bacterial activity was purchased from Ranbaxy Laboratory Ltd, India. The bacterial strains were procured from Department of Life Sciences, SGRITS, Dehradun. Dimethyl sulphoxide (DMSO) (100%) was used to give different concentrations of 25, 50 and 100 µg/ml of compounds. Amikacin (30 µg/ml) was used as reference standard (positive control) for both bacterial species under conditions similar to those of the test compounds. Any inhibition was not revealed by microbial culture with 100% DMSO-wetted disc (as negative control) for experiments. The zones of inhibition shown by the compounds were measured (mm) after 24 h at 37° ± 1°C. Table 3 gives the recorded observations.

Results and discussion

The synthesized chelate was dark brown and stable. The formation of complex of DHFCO_x with Ni(II) took place in the ratio 2:1. The modified mole ratio method was used to calculate the stability constant and free-energy change of the metal complex. The negative value of free-energy change shows the feasibility of complex formation. Molar conductance of 10⁻³ M solution of complex in DMSO corresponds to low value (31.29 S cm² mol⁻¹), suggesting its non-electrolytic nature^{17,18}. The magnetic moment values (2.9 BM) indicate the octahedral coordination of Ni(II) ion with ligand (DHFCO_x)^{19,20}.

Stoichiometry study

Metal ligand mole ratio (1:2) for the complex was obtained by mole ratio method and Job's method (Figures 1 and 2 respectively), and was in agreement with elemental analysis (Table 1).

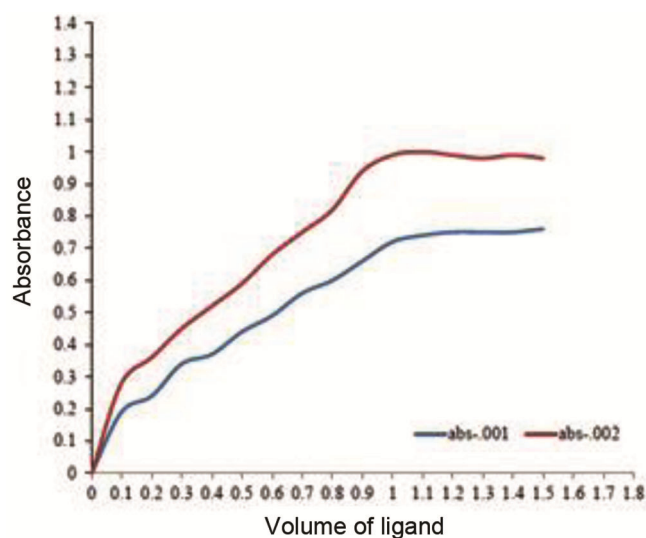


Figure 1. Mole ratio method.

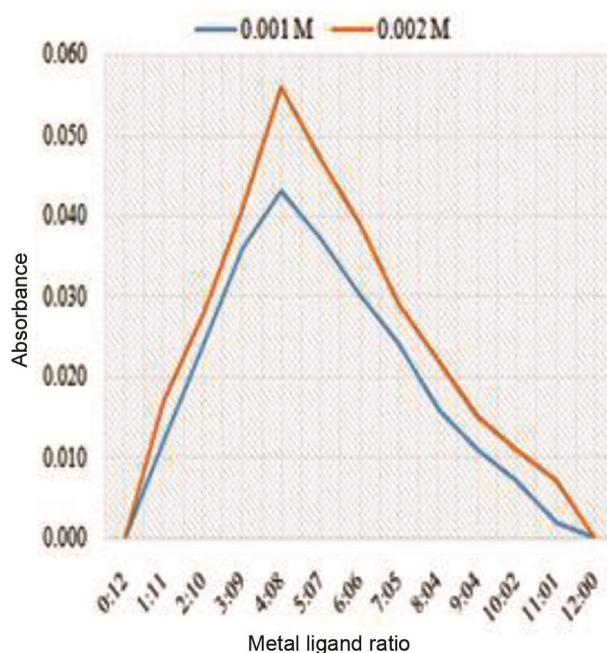


Figure 2. Job's ratio method.

IR spectral studies

The IR spectra of compounds were recorded within the range 4000–450 cm^{-1} . Assignments of IR spectral bands are based on the literature²¹. The IR spectrum of DHFCO_x showed broad band at 3252–3391 cm^{-1} , which may be due to phenolic –OH groups and –OH moiety of N–OH group. On comparing the spectra of the ligand with Ni(II)- DHFCO_x , the complex showed a weak band around 3209–3394 cm^{-1} , indicating that among two phenolic–OH groups, the hydrogen of one hydroxy group is lost and other unreacted hydroxy group is considered to be unaffected²². The shift of C–O phenolic bands at 2-position from 1281.68 cm^{-1} in the ligand to 1216.15 cm^{-1} in the spectra of metal complex supports the loss of hydrogen of 2-hydroxy group and coordination of the phenolic oxygen atom to the metal ion²³. The ketonic ($>\text{C}=\text{O}$) stretching frequency of DHFC at 1681.75 cm^{-1} had lowered to 1637.40 cm^{-1} in DHFCO_x due to the formation of ($>\text{C}=\text{N}$). Coordination of the azomethine nitrogen ($>\text{C}=\text{N}$) with metal shift frequency 20–30 cm^{-1} lower as band shift to 1606.67 cm^{-1} in metal chelate spectra²⁴. The shift of $>\text{C}=\text{N}$ bond indicates involvement of this group in complexation by formation of M–N band²⁵. The band appeared in the range 526–564 cm^{-1} was assigned to M–O bond in the complex²⁶. The band in the range 460–508 cm^{-1} in the spectra of the complex was assigned to M–N band²⁷. The appearance of these new bands indicates the coordination of the ligand to the central metal ion

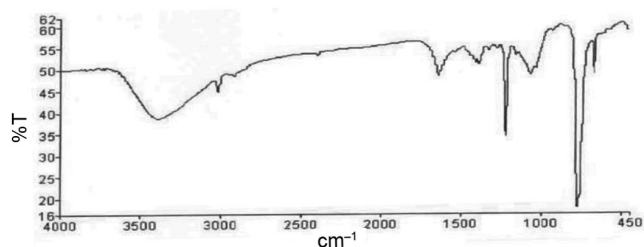


Figure 3. IR of Ni(II)- DHFCO_x complex.

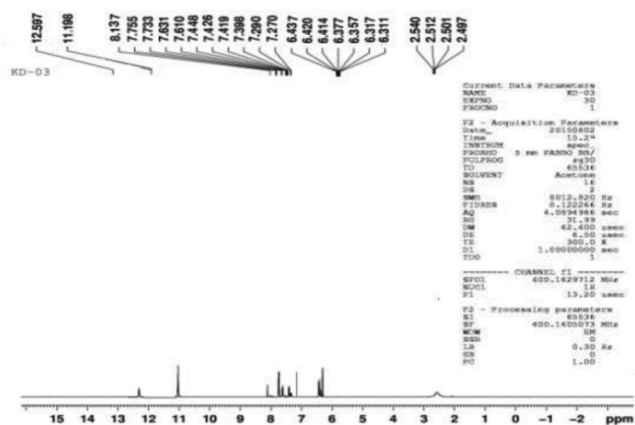


Figure 4. ^1H -NMR of Ni(II)- DHFCO_x complex.

through its phenolic-O and azomethine-N. Strong bands at 3019.62 cm^{-1} and 2923.91 cm^{-1} in the ligand and the complex were similar and may be assigned to aromatic C–H stretching and aliphatic C–H stretching respectively. Medium-intensity bands appearing around 1589.79–1493.87 cm^{-1} in the ligand and complex were assigned to C=C, aliphatic. Figure 3 shows the spectrum of compound 3.

^1H NMR- and ^{13}C -NMR spectra

^1H -NMR spectra of synthesized compounds were recorded at room temperature in $\text{CDCl}_3/\text{DMSO}-d_6$ in the range

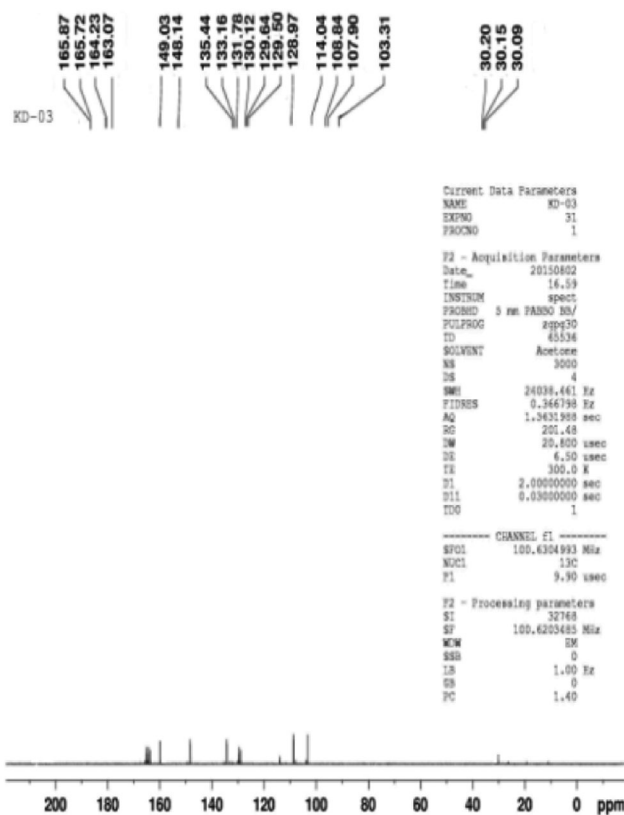


Figure 5. ^{13}C NMR of Ni(II)- DHFCO_x complex.

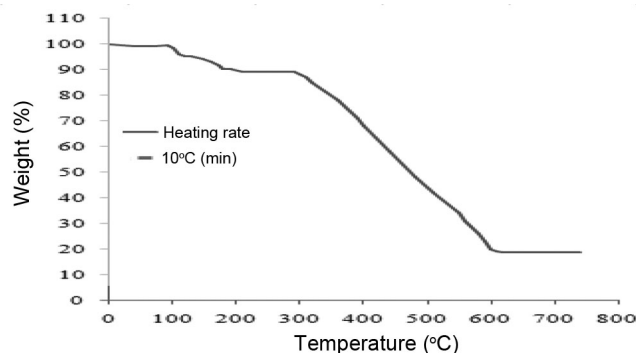


Figure 6. Thermogravimetric analysis of Ni(II)- DHFCO_x complex.

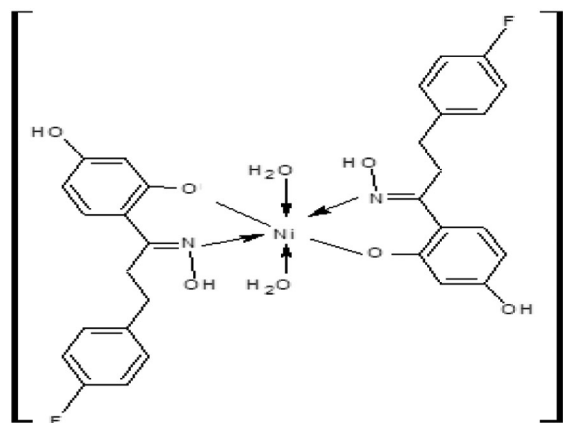


Figure 7. Ni(II)-DHFCO_x complex.

0–15 ppm. A doublet resonated at 7.41 ppm and a 7.75 were attributed to H- α and H- β respectively, which coupled in a trans-relationship with each other. A singlet resonated at 12.59 (s, 1H) due to proton of –OH group at 4'-position. Signal at 11.35 ppm due to 2-OH moiety proton observed in the ligands disappeared in the complex due to deprotonation, indicating the participation of phenolic oxygen in the coordination^{28,29}. The signal at 11.19 ppm (s, 1H) corresponding to the N-OH proton in ligand and metal complex, suggests that OH group does not participate in coordination with metal atom.

The ¹³C-NMR spectrum displayed a deshielded carbon signal at 163.0 ppm indicated for C=N of metal ligand complex. The peak at 157.34 ppm was attributable to C-2' in ligand shifted to 164.30 ppm in metal chelate due to coordination. The olefinic carbons C α and C β were observed at 128.9 and 148.1 ppm respectively. Aromatic carbons showed characteristic signals for the free ligand as well as metal complex. Figures 4 and 5 show ¹H-NMR and ¹³C-NMR spectra respectively, of compound 3.

Thermal analysis

The TGA curve of DHFCO_x-Ni(II) complex shows stability of the complex up to 85–90°C (Figure 6). Decomposition of the complex starts from 90°C up to 190°C gradually, corresponding to loss of lattice and coordinated moles of water. The horizontal portion (plateau) in the temperature range 190–280°C indicates the undissociated anhydrous chelate. After 290°C anhydrous chelate begins to decompose up to 610°C. A plateau in the temperature range 610–700°C indicates the region where there is formation of metal oxide NiO. Hence, the thermogram shows that chelate contains two water molecules which are coordinated to the metal ion³⁰.

Structure of the compounds: The newly synthesized compounds have been confirmed from elemental analysis, molar conductance, magnetic property, IR, ¹H-NMR,

¹³C-NMR and thermal analysis data. Figure 7 shows the structure proposed for DHFCO_x-Ni(II) complex.

The antibacterial data indicate the metal complex to be more potent compared to its parent ligands to both pathogenic microorganisms. Synthesized compounds possess moderate to good antibacterial activity. Bacterial strain *E. coli* was sensitive to all compounds with inhibition zones varying from 10 to 16 mm at different concentrations. *S. aureus* was found to be sensitive to compounds 2 and 3, with zones of inhibition from 10 to 14 mm, and completely resistant to compound 1.

Conclusion

The synthesis of chalcone derivatives and complex by microwave irradiation method showed that time of reaction reduced from hours to seconds with higher yields. The various physico-chemical data confirmed the formation and stoichiometry of the compounds. The IR and NMR spectra indicated that DHFCO_x behaves as monobasic bidentate ligand towards Ni(II) ion and the complex having octahedral geometry. The complexation of ligand with metal was spontaneous. The results of antibacterial activity of compounds 2 and 3 against *E. coli* and *S. aureus* in terms of minimum inhibitory concentration were quite promising at different concentrations. These findings might be useful in the optimization of DHFCO_x as a lead for future development of an agent for synthesis of new complexes and various pharmacological activities.

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