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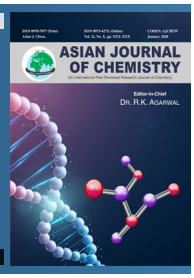

Antineoplastic Activity of Alkylaminolapachol Analogues and their Copper Complexes against MDA-MB-231 Human Breast Cancer Cell Lines

O.B. PAWAR[1], K.V. SANAS[1], R.B. PATWARDHAN[2], S.A. WAGHMODE[3] and M.Y. KHALADKAR[1,*]

[1]Department of Applied Science, College of Engineering Pune, Pune-411005, India
[2]Department of Microbiology, Haribhai V. Desai College, Pune-411002, India
[3]Department of Chemistry, M.E.S. Abasaheb Garware College, Pune-411004, India

*Corresponding author: Fax: +91 20 25507299; E-mail: myk.appsci@coep.ac.in

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In present study, three alkylamino substituted hydroxynaphthoquinone analogues of naturally occurring bioactive lapachol are synthesized. Further, copper(II) complexes of these ligands have been synthesized using hassle free modified grindstone method. Both ligands and copper(II) complexes were characterized using UV-visible spectroscopy, FTIR, elemental analysis and NMR spectra. Cell viability assay of the compound was carried out against growth of MDA-MB-231 human breast cancer cell lines. A better tumorocidal activity was observed for ligands and their copper(II) complexes in concentration of 2.95 to 0.11 mmol/L. Antineoplastic activity of copper(II) complexes was found to be more than parent ligands. Antimicrobial assay results showed that compound exhibit better activity against organisms under study. Antimicrobial susceptibility of compounds was carried out against culture of *E. coli* K12 (RP4), *B. subtilis* (pUB110), *K. pneumoniae* and *S. paratyphi*. The lowest MIC of < 0.20 mg/mL was obtained for L-3 against *S. paratyphi*. Cyclic voltammetry of ligand and complexes performed in non-aqueous system shows that ligand exhibits classical two step redox couple corresponds to the formation of semiquinone and catechole moieties in solution state at characteristic potential values. Whereas cyclic voltammogram of copper(II) complexes exhibit additional peak at characteristic potential corresponds to reversible redox reaction of central Cu(II) ion in complexes.

Keywords: Antineoplastic activity, Lapachol, Antimicrobial activity, Copper(II) complexes.

INTRODUCTION

Naphthoquinones constitutes a class of secondary metabolites of plants and microorganisms, where by it shows key role in many living oxidative process and also serves as a part of defense system in many plants. Plumbagin, juglone, lawsone, lapachol, naphthazarin, shikone and mopain together form class of naturally occurring hydroxylated 1,4-naphthoquinones with essential biological functions. These members of hydroxylated naphthoquinones were reported for their applications in traditional Indian medicines [1-6]. In past decades, naphthoquinone and its hydroxyl derivatives have gained key importance due its structural, chemical and biological applications. It has been more interesting to study the effect of various substituents on the naphthoquinone skeleton and the bioactivity of resulting molecule [7,8]. The principle mode of action suggested for antitumor activity of naphthoquinone manly involves its reaction with higher levels of cytochrome P450 reductase found in some tumour cells than the normal ones [9]. Coordination of metal ion with bioactive ligand moiety usually alters its bacteriostatic and/or carcinostatic properties [10-14]. Naphthoquinone moiety is found to be effective chelator for divalent and trivalent metal ions. 1,4-Naphthoquinones with at least one phenolic (OH) group are strong inhibitor of topoisomerase enzymes [15]. The capacity of these naphthoquinones to complex with divalent metal ions such as Cu^{2+} parallels their topoisomerase inhibition characteristics. It also considered as the fundamental mechanism of their anticancer activity [15,16].

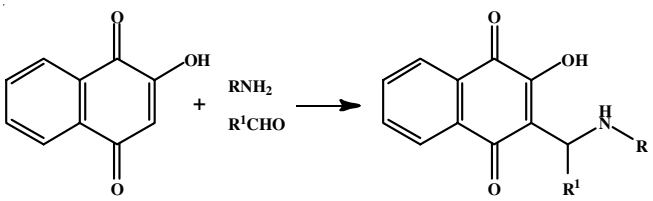
With continuous increase in drug resistance characteristic of several bacteria, it is necessary to develop new compounds with enhanced antibacterial potency [17-19]. Particularly, an infection caused by cultures of *E. coli*, *B. subtilis*, *K. pneumoniea* and *S. paratyphi* need more attention in this regards. In present work, we are reporting anticancer activity of newly

synthesized 2-hydroxy-3[(alkyl)(alkylamino)]-1,4-naphthoquinone and their copper(II) complexes against an epithelial human breast cancer cell line MDA-MB-231. The antibacterial assay of these compounds was also carried out against pathogenic colonies of *E. coli*, *B. subtilis*, *K. pneumoniea* and *S. paratyphi*.

EXPERIMENTAL

2-Hydroxy-1,4-napahthoquinone, copper(II) perchlorate and triethylamine were purchased from Sigma-Aldrich (Purity 99 %) and used without further purification. Solvents used were of spectroscopic grade with purity 99.5 % (Merck). Amines and aldehyde used for synthesis were purchased from Merck (AR grade) and used as received. UV-Visible spectra of compounds were recorded in methanol from 200 to 800 nm on Thermo Scientific UV-Visible spectrophotometer. FTIR spectra were recorded in KBr matrix on Vertex 80 FTIR system in range 4000-400 cm^{-1}. NMR spectrum of ligand was recorded on high resolution Bruker Avance III HD 500 MHz instrument in DMSO-d_6 solvent. Elemental analysis was done on Thermon finniagn, FLASH EA1112 CHN analyzer.

Synthesis: One pot synthesis of ligands (**L 1-3**) was carried out as follows: 2-Hydroxy-1,4-naphthoquinone (5 mM) was dissolved in about 10 mL of ethanol, to this equimolar amount of amine was added. The resultant solution was stirred for 5 min at room temperature. It was then mixed with ethanolic solution of 5.5 mM of respective aldehyde over continuous stirring. The final mixture was stirred (1000 rpm) at room temperature (27-29 ºC) for 24 h (**Scheme-I**). The progress of reaction was monitored using thin layer chromatography. Wherever required the crude product was purified by column chromatography over silica gel (60 × 120 mesh size) as stationary phase and hexane:ethyl acetate as eluent.



where: R = Methyl for **L-1** R^1 = Methyl; **L-2**, R^1 = Benzyl; **L-3**, R^1 = Phenyl

Scheme-I

2-Hydroxy-3-[(methylamino)(methyl)]-1,4-naphthoquinone (L-1): Orange coloured solid; yield: 76.3 %; m.p.: 180-181 ºC; FTIR (KBr, ν_{max} cm^{-1}): 3441, 3006, 2969, 2789, 1680, 1650, 1611, 1591, 1538, 1478, 1439, 1460, 1397, 1375, 1347, 1336, 1319, 1283, 1229, 1169, 1023, 955, 920, 860, 799, 738, 706, 695, 548, 551, 515, 482, 451 and 422; ^{1}H NMR (500 MHz, DMSO) δ ppm: 3.34 (3H, s, *J* = 7.5 Hz), 2.50 (2H, d, *J* = 1.5 Hz), 7.57 (1H, t, *J* = 7.5 Hz), 7.71 (1H, t, *J* = 7.5 Hz), 7.83 (1H, d, *J* = 7.5 Hz), 7.94 (1H, d, *J* = 7.5 Hz), 8.15 (br, s, 2H); ^{13}C NMR (500 MHz, DMSO) δ ppm: 32.62, 43.76, 107.95, 125.55, 125.82, 131.05, 132.11, 134.10, 135.63, 172.21, 178.89, 185.16; Elemental analysis: calcd. (found) % for $C_{12}H_{11}NO_3$: C, 66.35 (66.19); H, 5.10 (5.12); N, 6.44 (6.19); UV-visible λ_{max} (MeOH): 217, 270 and 441 nm.

2-Hydroxy-3-[(methylamino)(phenyl)]-1,4-naphthoquinone (L-2): Orange-red coloured solid, yield: 89.2 %; m.p.: 178 ºC; FTIR (KBr, ν_{max} cm^{-1}): 3567, 3463, 3147, 3060, 3029, 2960, 1679, 1589, 1526, 1474, 1456, 1373, 1271, 1229, 1154, 1048, 893, 739, 702, 561, 488, 434; ^{1}H NMR (500 MHz, DMSO) δ ppm: 2.52 (3H, s), 5.42 (H, s), 7.29 (H, dt, *J* = 7.0 Hz), 7.35 (2H, dd, *J* = 7.5 Hz), 7.59 (2H, dt, *J* = 7.5 Hz), 7.57 (1H, t, *J* = 7.5 Hz), 7.72 (1H, t, *J* = 7.5 Hz), 7.84 (1H, d, *J* = 7.5 Hz), 7.92 (1H, d, *J* = 7.5 Hz), 9.25 (br.s, 2H); ^{13}C NMR (500 MHz, DMSO) δ ppm: 32.23, 60.553, 111.51, 125.52, 125.78, 128.27, 128.06, 128.79, 131.24, 131.98, 134.18, 139.06, 139.06, 178.64, 170.99, 184.90; Elemental analysis: calcd. (found) % for $C_{18}H_{15}NO_3$: C, 73.70 (72.10); H, 5.15 (5.03); N, 4.77 (4.25); UV-visible λ_{max} (MeOH): 209, 272 and 450 nm.

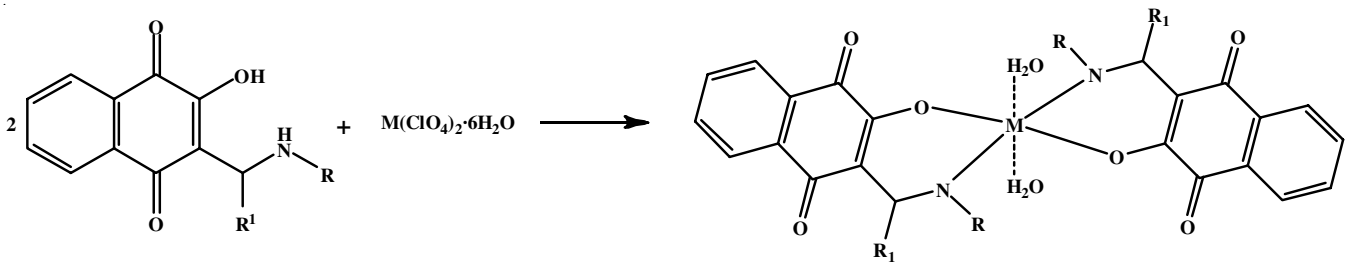
2-Hydroxy-3-(methylamino)(2-hydroxyphenyl)]-1,4-naphthoquinone (L-3): Dark orange coloured solid; yield: 87.4 %; m.p.: 187 ºC; FTIR (KBr, ν_{max} cm^{-1}): 3397, 3313, 3007, 2969, 1679, 1647, 1610, 1590, 1535, 1478, 1460, 1397, 1347, 1320, 1283, 1229, 1023, 955, 737, 695, 585, 851, 421; ^{1}H NMR (500 MHz, DMSO) δ ppm: 3.33 (3H, t), 5.61 (H, s), 6.73 (H, dd, *J* = 6.8Hz), 6.87 (1H, dt, *J* = 7.2 Hz), 7.11 (1H, dt, *J* = 7.0), 7.29 (1H, dd, *J* = 7.0), 7.60 (1H, t, *J* = 7.5 Hz), 7.72 (1H, t, *J* = 7.5 Hz), 7.84 (1H, d, *J* = 7.5 Hz), 7.93 (1H, d, *J* = 7.5 Hz). 8.14 (br.s, 2H); ^{13}C NMR (500 MHz, DMSO) δ ppm: 184.46 179.85, 171.91, 155.75, 134.92, 134.28, 131.96, 131.55, 129.86, 129.03, 125.92, 125.57, 124.16, 119.49, 116.40, 110.54, 43.76, 18.11; Elemental analysis: calcd. (found) % for $C_{18}H_{15}NO_4$: C, 69.89 (69.50); H, 4.88 (4.65); N, 4.52 (4.43); UV-visible λ_{max} (MeOH): 217, 270 and 449 nm.

Synthesis of metal complexes (M 1-3): A 6 mM of ligand and 3 mM copper(II) perchlorate was mixed and grinded in clean mortar pastel for 30 min. To the resultant mixture, 850 µL of triethylamine was added where colour becomes dark. A mixture was then extracted with 10 mL of methanol solution (10 %) and stirred for 30 min. Upon cooling green to brown color solid starts to separate, it was then kept in refrigerator for overnight. Solid thus formed was filtered and several times washed with equal volumes of alcohol water mixture. A product then dried in vacuum over calcium chloride. The dried product further recrystallized through distilled methanol (**Scheme-II**).

[Cu(L1)$_2$·2H$_2$O] (M-1): Olive green colour solid; yield: 76.2 %; FTIR (KBr, ν_{max}, cm^{-1}): 3398, 3217, 2980, 2927, 1674, 1593, 1545, 1459, 1410, 1383, 1374, 1329, 1281, 1255, 1228, 1083, 935, 734, 684, 549, 502, 479, 432 and 412; Elemental analysis: calcd. (found) % for $C_{24}H_{18}N_2O_6Cu·2H_2O$: C, 53.97 (52.47); H, 4.81 (5.24); N, 3.81 (4.15); Cu, 11.90 (8.97); UV-visible λ_{max} (MeOH): 212, 271 and 433 nm.

[Cu(L2)$_2$·2H$_2$O] (M-2): Pale Brown colour solid; yield: 85.3 %; FTIR (KBr, ν_{max}, cm^{-1}): 3457, 3242, 1668, 1629, 1592, 1371, 1343, 1281, 1227, 1109, 1090, 1055, 943, 835, 755, 733, 706, 627, 519, 492 and 437; Elemental analysis: calcd. (found) % for $C_{36}H_{26}N_2O_6Cu·2H_2O$: C, 63.38 (58.26); H, 3.84 (4.29); N, 4.11 (3.91); Cu, 9.32 (8.01); UV-visible λ_{max} (MeOH): 212, 260 and 431 nm.

[Cu(L3)$_2$·2H$_2$O] (M-3): Brown colour solid; yield: 79.6 %; FTIR (KBr, ν_{max}, cm^{-1}): 3264, 3067, 2970, 1675, 1591, 1524, 1476, 1456, 1373, 1281, 1253, 1159, 1121, 1093, 1048, 948, 870, 790, 717, 733, 696, 665, 584, 491, 473, 447 and 425;



where, **M-1**, **M-2** and **M-3** are copper(II) complexes of **L-1**, **L-2** and **L-3**, respectively

Scheme-II

Elemental analysis: calcd. (found) % for $C_{36}H_{26}N_2O_8Cu·2H_2O$: C, 60.54 (57.19); H, 3.67 (4.64); N, 3.92 (3.74); Cu, 8.90 (9.15); UV-visible λ_{max} (MeOH): 210, 272 and 447 nm.

Cyclic voltammetry: The cyclic voltammetry of compounds was performed on BASi-Epsilon instrument. The solution of each compound was prepared in distilled DMSO by dissolving 30 mg of compound in 25 mL of solvent. Three electrode system comprising of glassy carbon as working electrode, platinum wire as an auxiliary electrode and silver-silver chloride as a reference electrode were used. The glassy carbon electrode was activated using the procedure given by Thorp *et al*. [20]. Tetrabutylammonium perchlorate (0.1 M) was used as supporting electrolyte. The peak corresponds to electrochemical conversion of NQ (naphthoquinone) to NSQ (naphtho-semiquinone) and NSQ to CAT (catechol) were found to be shifted in potential. A reversibility of these peaks was also observed to be affected upon complexation. In voltammogram characteristic peak for oxidation/reduction of central copper were observed at potential in accordance of coordinated ligand.

Antimicrobial assay: The synthesised ligands and copper complexes were tested for the antimicrobial activity using 96-well plate microdilution method. In the present investigations, standard cultures of *E. coli* K12 (RP4) MTCC 391, *B. subtilis* (pUB110) MTCC 1558, *Klebsiella pneumoniae* MTCC 109 and *S. paratyphi* MTCC 3220 were procured from MTCC culture collection. The working bacterial cultures were prepared on Luria-Bertani agar plates at 37 ºC Müeller Hinton broth (MHB) (Difco) was used for the microdilution MIC assay [21].

Determination of minimal inhibitory concentration (MIC): The synthesized ligands and copper(II) complexes were tested for the antimicrobial activity (minimum inhibitory concentration assay) using 96-well plate microdilution method. Single bacterial colonies from the bacterial strains were grown in Müeller Hinton broth medium at 37 ºC overnight. These starter cultures were diluted and grown in Müeller Hinton broth medium until OD (600 nm) reaches in the range 0.5-1.0 (MacFarland standard). The number of colony-forming units was determined, and the bacterial cultures were adjusted to 5 × 10^6 cfu/mL. Plates were prepared under aseptic conditions. 100 µL of Müeller Hinton broth was dispensed into wells of the microtitre plate. Test compound solution (100 µL) was added in the wells of column 1. From column 1 (100 µL) was transferred into column 2, mixed by autosucking and then transferred to column 3, the procedure was repeated up to column 8. Bacterial culture (10 µL) was added into wells in columns 1 to 8. The plates were incubated at 37 ºC for 12-18 h. After incubation, plates were subjected to ELISA plate reader with necessary blank correction. Absorbance of content in each well was measured at standard wavelength of 620 nm. Cefotaxime was used as control drug against Gram-negative bacteria *E. coli*, *K. pneumoniae* and *S. paratyphi* while Erythromycin was used as control drug against Gram-positive bacteria *B. subtilis*.

Antineoplastic activity: MDA-MB-231 cells were seeded at a density of 1 × 10^5 cells/mL density in 96-well micro plates [22]. An untreated group was kept as a negative control. The samples were added at concentrations: 0, 20, 40, 80, 160, 320, 640 and 1280 µg/mL, in wells in triplicates. The MTT solution (5 mg/mL) was added to each well and the cells were cultured for another 4 h at 37 ºC in 5 % CO_2 incubator. The formazan crystals formed were dissolved by addition 100 µL of DMSO. The amount of coloured formazan derivative was determined by measuring optical density (OD) using the ELISA microplate reader. Standard doxorubicin drug was used as positive control and all reagents expect sample was treated as negative control.

RESULTS AND DISCUSSION

The reaction pathway used for the synthesis of ligands is amino alkylation of an acidic proton (at β-carbon) placed next to a carbonyl group by aldehyde and primary or secondary amine. The final product is β-amino-carbonyl compound also known as Mannich base. Copper(II) complexes of this ligand were synthesized in better yield using cold press grindstone methodology [23,24].

UV-visible analysis: A maximum absorption wavelength for all compounds in ultraviolet and visible region is summarized in Table-1. A π→π* was observed at its characteristic value. For L-1, L-2 and L-3, maximum absorption was observed in visible region at wavelength 441, 450 and 449 nm, respectively (Figs. 1-3). An effect of complexation was observed as shift in wavelength on shorter values. Among three complexes, maximum shift of 19 nm was observed for complex **M-2** while it's minimum for complex **M-3** that is of 2 nm than corresponding ligands (Figs. 4-6).

TABLE-1
UV-VISIBLE DATA FOR SYNTHESIZED ALKYLAMINO SUBSTITUTED HYDROXYNAPHTHOQUINONE (**L 1-3**) AND ITS COPPER(II) COMPLEXES (**M 1-3**)

	λ_1	λ_2	λ_3
L-1	217	270	441
L-2	209	272	450
L-3	217	270	449
M-1	212	271	433
M-2	212	260	431
M-3	210	272	447

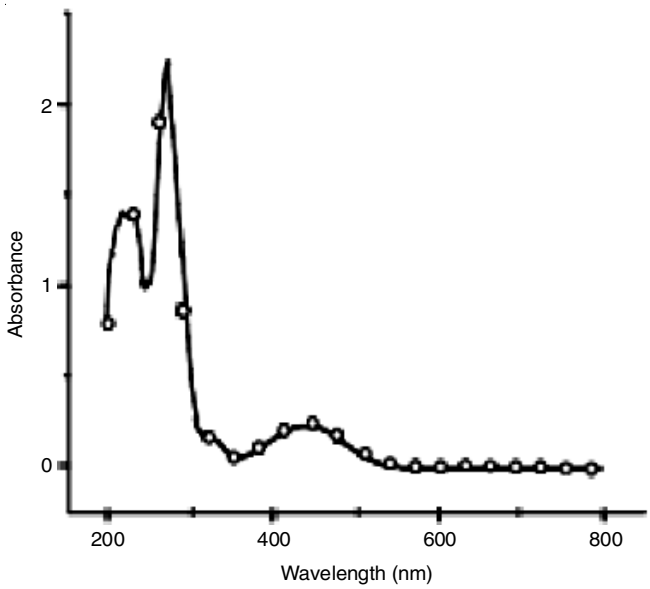


Fig. 1. UV-visible spectrum of ligand **L-1**

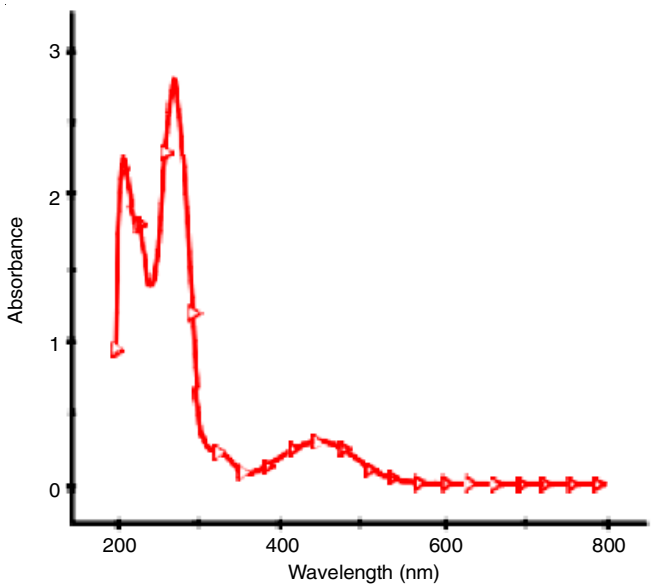


Fig. 2. UV-visible spectrum of ligand **L-2**

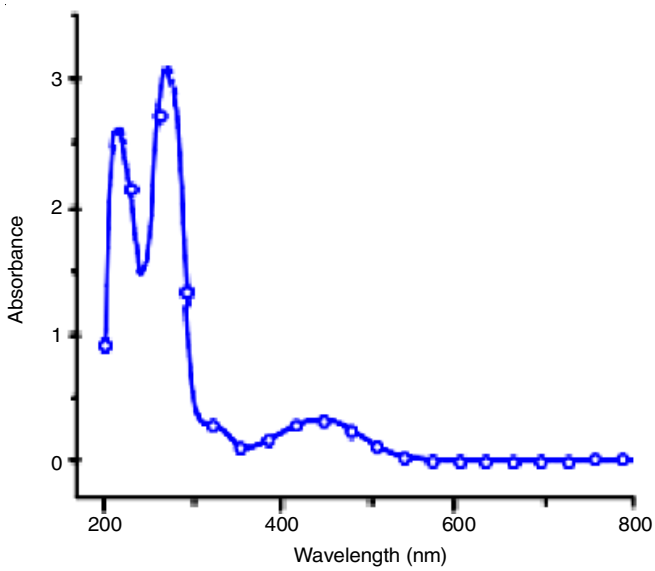


Fig. 3. UV-visible spectrum of ligand **L-3**

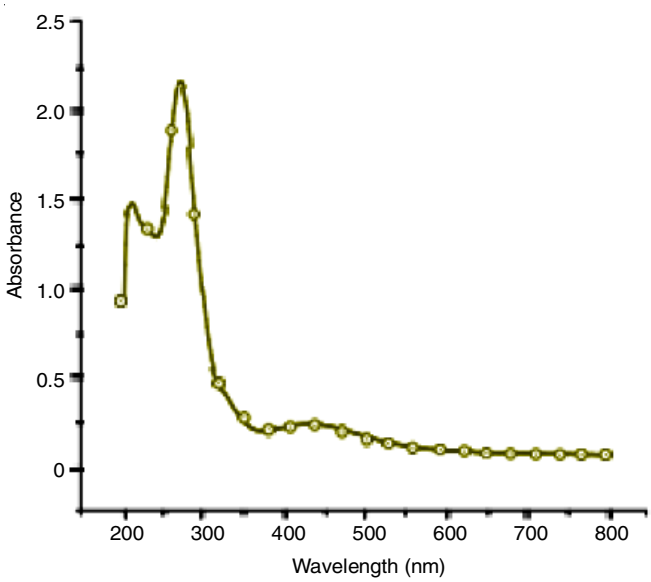


Fig. 4. UV-visible spectra of copper(II) complex **M-1**

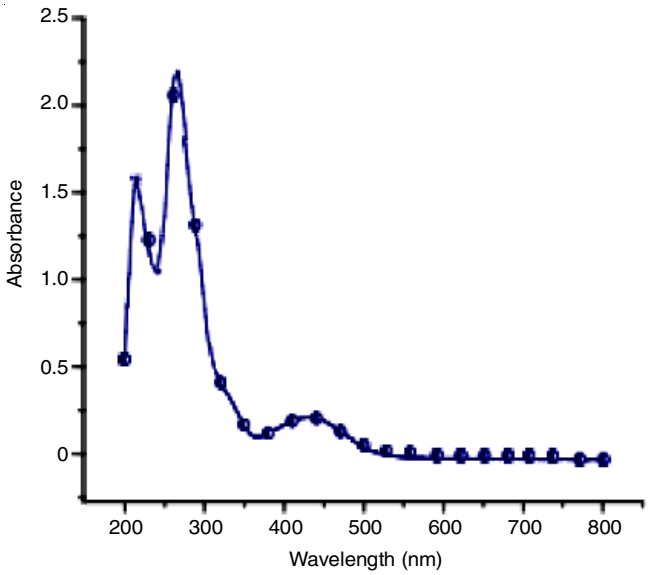


Fig. 5. UV-visible spectra of copper(II) complex **M-2**

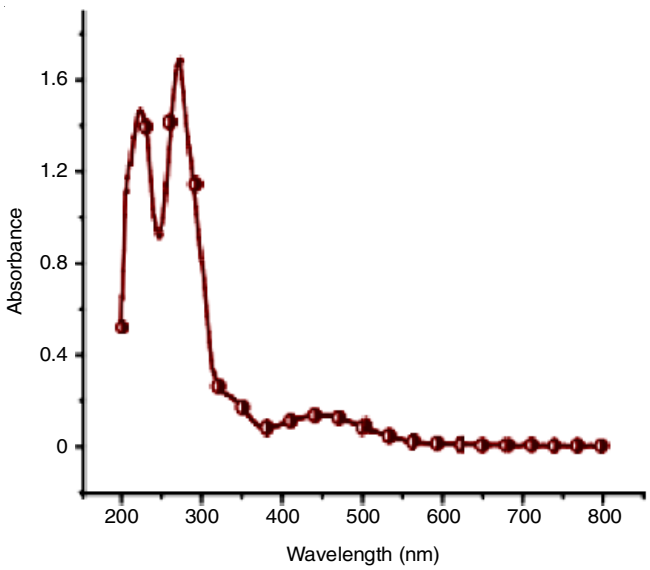


Fig. 6. UV-visible spectra of copper(II) complex **M-3**

FTIR analysis: The characteristic FTIR frequencies for precursor 2-hydroxy-1,4-naphthoquinone, synthesized ligands and complexes are tabulated in Table-2. Naphthoquinone motif can be assigned in the spectra for vibration of C=O, C=C (Ar), C-H(Ar) and C-O bonds. Additionally, a band corresponds to C-N stretching was observed in the spectra of synthesized compounds, which was absent in 2-hydroxy-1,4-naphthoquinone. In case of hydroxyl group, the vibrations observed were ν(OH), δ(OH), γ(OH) and the out of plane deformation vibration.

A moderately sharp peak at 3165 cm^{-1} observed in the precursor 2-hydroxy-1,4-naphthoquinone was due to O-H stretching vibration. FTIR spectra of all the synthesized ligands (**L 1-3**) shows peak corresponding to ν(OH) which appears over wide range of frequency as high as 3629 cm^{-1}. A shift is observed in OH stretching frequency for synthesized ligands mainly towards the higher value. A sharp to broad nature of corresponding peaks specifies the existence of intra- and intermolecular hydrogen bonding interactions. The FTIR spectra of all metal complexes show narrow to broad band in a range 3600-3200 cm^{-1} corresponds to hydrogen bonded OH stretching in coordinated water molecule in their structure. A nature of band varies from narrow to broad due to its involvement in hydrogen bonding to form polymeric network. FTIR spectra comprises of bands at 3150 ± 50 cm^{-1} is due to hydrogen bonded NH stretch in secondary amine. These bands are associated with several other bands over wide range of 3100-2800 cm^{-1}. These bands arised due to stretching vibration corresponds to Ar-CH and CH stretch in alkyl group.

In quinones, carbonyl group stretching frequency was observed from 1690-1675 cm^{-1} and the peak was associated with another lower frequency band (1650-1600 cm^{-1}) which results from stretching vibration of conjugated double bonds (C=C). A band centered at 1679 cm^{-1} corresponds to characteristic of carbonyl stretching vibration was observed in FTIR spectra of all ligands, same was observed at 1677 cm^{-1} for 2-hydroxy-1,4-naphthoquinone. A shift in carbonyl stretching frequency was observed for complexes than corresponding ligands, it is as much as 11 cm^{-1} for complex **M-2**. Frequency corresponds to N-H vibration is another one, which changed upon comple-xation was found to be decreased by 86 to 45 cm^{-1} from complex **M-1** to complex **M-3**.

Cyclic voltametric study in non-aqueous media: Naphthoquinone usually undergoes two classical one electron transfer processes. In step I, it forms one electron reduced form: semiquinone (SQ), which was further reduced to form catechole (CAT). Substitution on naphthoquinone ring shows notable effect on its redox potential values. The substituent on naphthoquinone also limits the reversibility of system in many cases [25]. Peak potential values for anodic and cathodic processes are given in Table-3. The nature of cyclic voltammograms of all the synthesized showed that the compounds undergoes characteristic redox process of naphthoquinone motif. Two electron transfer processes were slow and clearly observed in non-aqueous medium, since it was difficult to add an electron to a species itself with negative charge. Along with peak I and II, additional smaller peaks were observed due to self-protonation reaction and/or hydrogen bonding processes. These additional peaks almost disappearing on higher scan rates (Figs. 7-12). The extent of hydrogen bonding greatly affects the peak potential and additional peak appearance. The half wave potential for the first step was reported from -51 to -77 mV for 3-alkylamino-2-chloro-1,4-naphthoquinnones [26]. In case of lapachol, a shift in oxidation peak was noted on positive value as compared other naphthoquinone derivatives [27]. The reverse reaction of first step-I was observed to be slow and gave as hump rather than a sharp peak. On the other hand, further reduction into

TABLE-2
KEY FTIR BANDS (cm^{-1}) SYNTHESIZED ALKYLAMINO SUBSTITUTED HYDROXYNAPHTHOQUINONE (**L 1-3**) AND ITS COPPER(II) COMPLEXES (**M 1-3**)

	L	L-1	L-2	L-3	M-1	M-2	M-3
ν(O-H)	3165	3441	3567, 3463	3397, 3313	3398, 3217	3457, 3242	3264
ν(C-C)	3074	3006, 2969	3060, 3029, 2960	3007, 2969	2980, 2927	3061, 2998, 2924	3067, 2970
ν(C=O)	1677	1680	1679	1679, 1647 (sh)	1674	1668	1675
ν(C=C)	1640	1611	1613 (sh)	1610	1593	1629	1624 (sh)
ν(NO*)	1578	1538	1526	1535	1545	1553	1545 (sh)
ν(PNQ)	1284	1283	1271	1283	1281	1281	1281
ν(C-O)	1223	1229	1229	1229	1228	1227	1226
ν(C-N)	–	1169	1154	1166	1083	1109	1121

*sh = shoulder peak

TABLE-3
PEAK POTENTIAL VALUES OF SYNTHESIZED ALKYLAMINO SUBSTITUTED HYDROXYNAPHTHOQUINONE (**L 1-3**) AND ITS COPPER(II) COMPLEXES (**M 1-3**)

	L-1	L-2	L-3	M-1	M-2	M-3
Epc_1	-1.2333	-1.1761	-1.3945	-1.5143	-1.9116	-1.2846
Epc_2	-0.2718	-0.0284	-1.0388	-1.1487	-1.4651	-0.9026
Epc_3	0.7018	-0.4031	-0.3883	-0.5368	-0.6368	-0.392
Epc_4	0.8890	–	–	0.0224	–	0.09
Epa_1	-1.4827	-1.6945	-1.2010	-1.2319	-1.2245	-1.0486
Epa_2	-0.9672	-1.4125	-0.3237	-0.5988	-0.5693	-0.461
Epa_3	-0.3123	-1.0495	0.4165	0.2656	0.7906	-0.2263
Epa_4	0.6166	–	–	0.9406	–	–

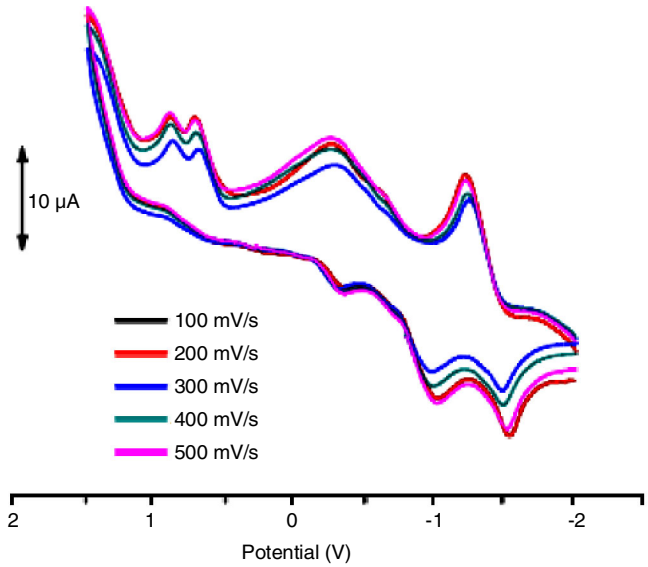


Fig. 7. Cyclic voltammogram of ligand **L-1** with scan rate variation

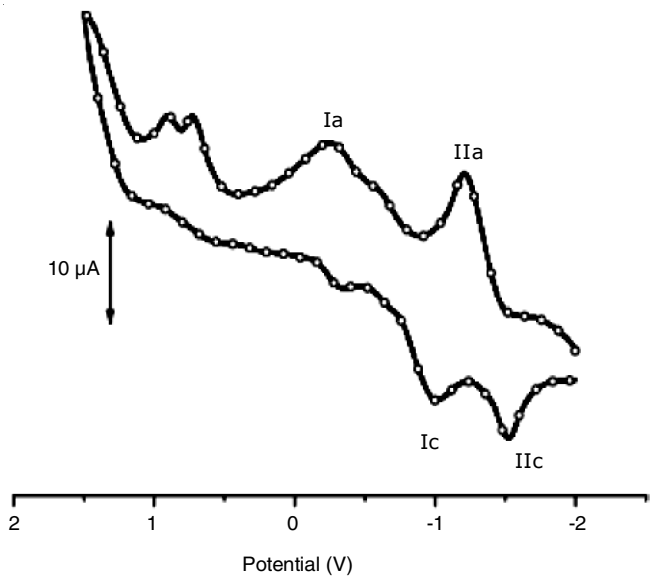


Fig. 8. Cyclic voltammogram of ligand **L-1**

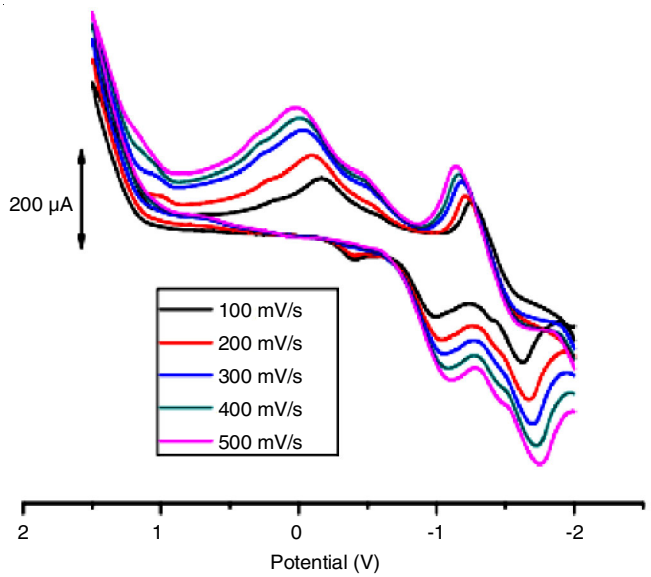


Fig. 9. Cyclic voltammogram of ligand **L-2** with scan rate variation

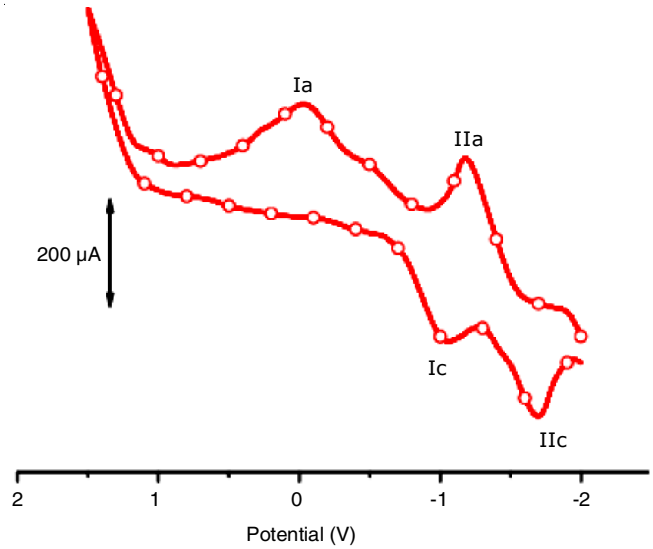


Fig. 10. Cyclic voltammogram of ligand **L-2**

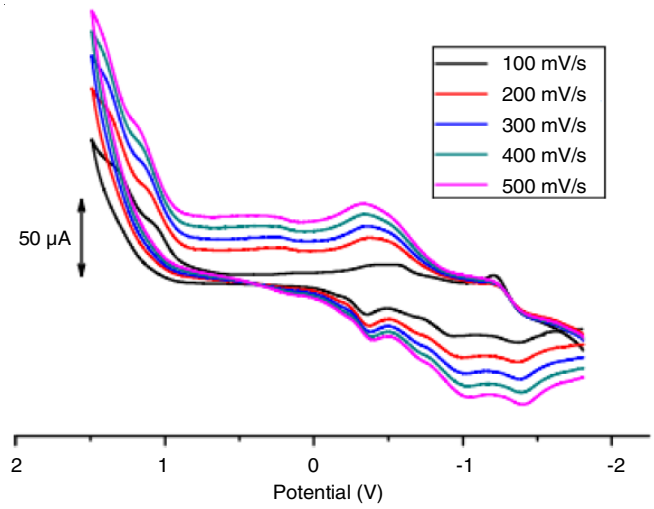


Fig. 11. Cyclic voltammogram of ligand **L-3** with scan rate variation

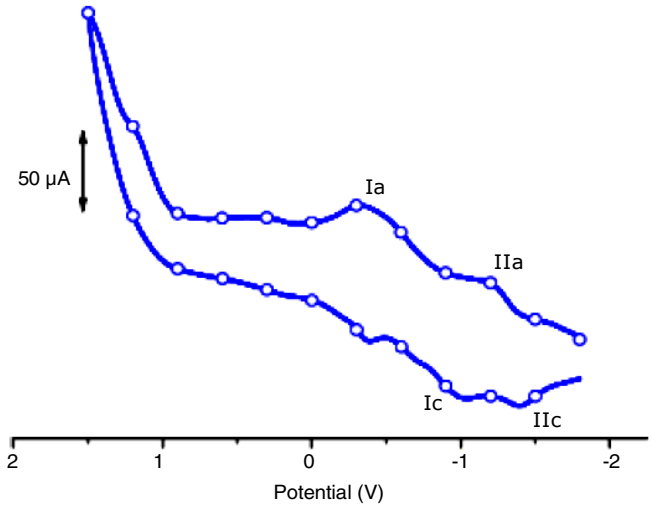


Fig. 12. Cyclic voltammogram of ligand **L-3**

catechol give rise to sharp peak. The half wave potential values are in good correlation with that reported for the naphthoquinone derivatives [28-30].

The presence of benzene ring at C-11 dramatically shifts the second reduction potential of **L-2** on more negative side by 20 mV than its **L-1** analogue. In cyclic voltammogram of the copper(II) complexes, peak corresponds to electrochemical conversion of NQ to SQ and SQ to CAT were found to be shifted in potential. A reversibility of these peaks was also observed to be affected upon complexation. In voltammogram, characteristic peaks for oxidation/reduction of central copper were observed at potential in accordance of coordinated ligand. In complex **M-1** peak potentials for ligand are slightly shifted towards more negative value as compared to corresponding ligand (Fig. 13). The nature of cyclic voltammogram for complex **M-2** seems to be different than complex **M-1**. Complex **M-1** shows characteristic peak for conversion of naphthoquinone to catechol in single step, while in complex **M-2** ligand moiety shows distinctive two step redox process (Fig. 14). In both complexes, peaks at -0.21 V and -0.64 V corresponds to electrochemical reduction of Cu(II) to Cu(0) from Cu(II) and Cu(I) were observed, out of which peak at -0.64 V was dominant one. A distinct oxidation peak in a range 0.64 to 0.82V present stands for oxidation of Cu(I) to Cu(II). For complex **M-3**, a reversible peak was obtained for reduction of Cu(I) to Cu(0). Complex **M-3** also showed a peak at ± 0.03 V to correspond to reduction of complex with either naphthoquinone ligand in semiquinone form (Fig. 15). The cathodic peak was observed at potential -0.7 to -0.9 V accounts for simultaneous one electron reduction of two copper(II) ions to form Cu(I) and process was irreversible (Table-4).

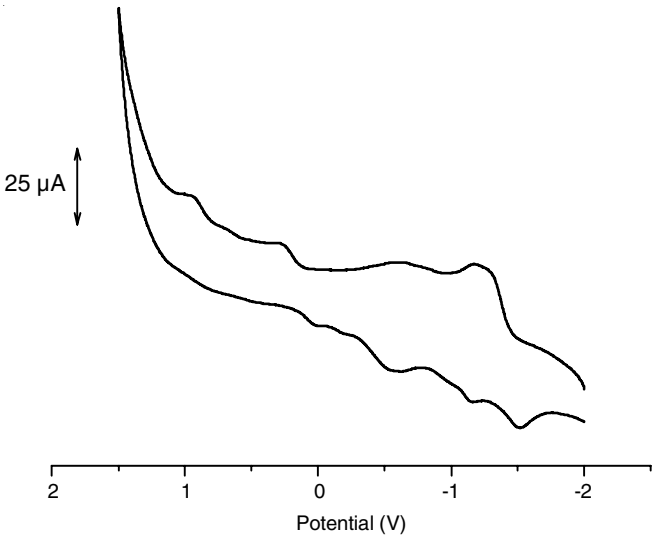


Fig. 13. Cyclic voltammogram of copper(II) complex **M-1**

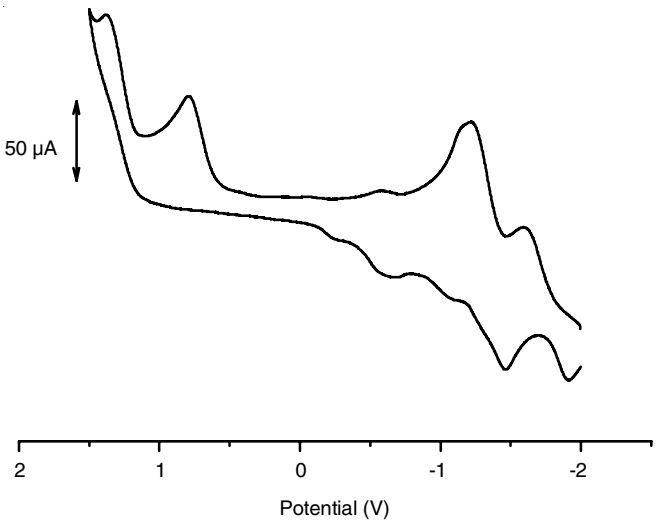


Fig. 14. Cyclic voltammogram of copper(II) complex **M-2**

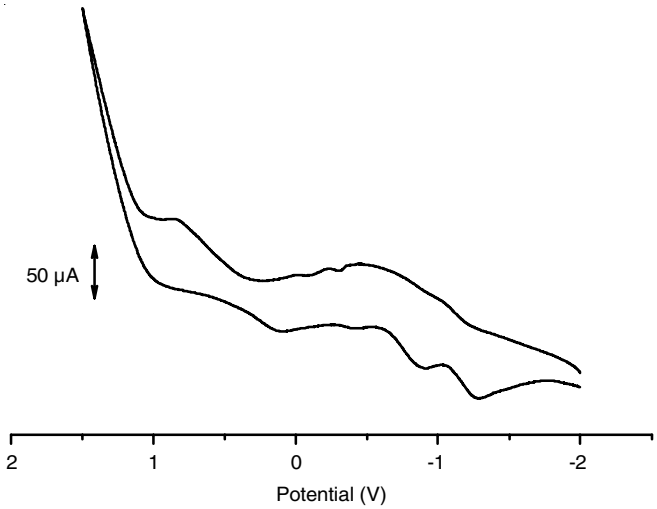


Fig. 15. Cyclic voltammogram of copper(II) complex **M-3**

TABLE-4
ASSIGNMENT OF POTENTIALS FOR ELECTROCHEMICAL REACTION IN COPPER(II) COMPLEXES (**M 1-3**)

Electrochemical reaction			Potential (V) Forward	Reverse
Complex **M-1**				
NQ	⇌	CAT	-1.5143	-1.2319
NQ	⇌	SNQ	-1.1487	-0.5988
Cu(II)+ 2e⁻	⇌	Cu(0)	–	0.2656
Cu(I)+ 1e⁻	⇌	Cu(0)	-0.5381	-0.5988
Cu(II) + Cu(II) + 2e⁻	⇌	Cu(I) + Cu(I)	-1.1487	0.9406
Cu(II)NQNSQ + 1e⁻	→	Cu(0) + 2NQ	0.02	–
Complex **M-2**				
NQ	⇌	CAT	1.91	1.22
Cu(I)+ 1e⁻	⇌	Cu(0)	-0.6368	-0.5821
Cu(II) + Cu(II) + 2e⁻	⇌	Cu(I) + Cu(I)	-1.06	–
Cu(II)NQNSQ + 1e⁻	→	Cu(0) + 2NQ	–	–
Cu(I)	→	Cu(II) + 1e⁻	–	0.7906
Complex **M-3**				
NQ	⇌	CAT	-1.2845	-1.0486
NQ	⇌	NSQ	-0.9026	-0.461
Cu(I) + 1e⁻	⇌	Cu(0)	-0.392	-0.2263
Cu(II) + Cu(II) + 2e⁻	⇌	Cu(I) + Cu(I)	-0.9026	–
Cu(II)NQNSQ + 1e⁻	⇌	Cu(0) + 2NQ	0.09	–
Cu(I)	→	Cu(II) + 1e⁻	–	0.8516

Thermal analysis: Table-5 comprises TGA-DTA data for the synthesized complexes (**M 1-3**). Thermogram of complex **M-1** shows a decomposition of ligand over wide range of temperature in two steps. It shows formation of copper oxide residue at 577 ºC. Exothermic DTA peak at 215 ºC is sharped and reflect as weight loss in thermogram due to oxidative decomposition of ligand part. Another exothermic DTA peak at 383°C is comparatively broad and indicative of decomposition of remainder ligand. Complex **M-2** and **M-3** are more stable than complex **M-1**. Complex **M-1** shows a loss in weight of ligand part over

TABLE-5
TG-DTA DATA OF SYNTHESIZED Cu(II) COMPLEXES OF ALKYLAMINO SUBSTITUTED HYDROXYNAPHTHOQUINONE (**M 1-3**)

	m.f.	Stage	Temp. (°C)	DTA (°C)	Process	Weight loss (%)		Proposed composition
						Obs.	Calcd.	
M-1	$[Cu(L_1)_2 \cdot 2H_2O]$	I	45-163	137	Endo	11.4	12.47	$2H_2O \cdot C_2H_6$
		II	207-577	215	Exo	73.78	72.51	$C_{22}H_{12}N_2O_6$
		Residue	> 577	383		14.82	14.90	Leaving CuO
M-2	$[Cu(L_7)_2 \cdot 2H_2O]$	I	45-207	66	Endo	5.88	5.25	$2H_2O$
		II	208-420	227, 237	Exo	36.79	34.72	$C_{16}H_{18}N_2$
		III	421-539	468	Exo	46.53	50.16	$C_{20}H_8O_6$
		Residue	> 539			10.80	10.42	Leaving Cu_2O
M-3	$[Cu(L_{13})_2 \cdot 2H_2O]$	I	41-106	–	–	5.15	5.01	$2H_2O$
		II	147-196	165	Exo	9.81	9.75	$O_2C_2H_8$
		III	196-611	303-471	Exo	72.8	72.84	$C_{34}H_{20}N_2O_6$
		Residue	> 641			12.26	12.40	Leaving CuO

wide range of temperature in a single step from 207 to 577 ºC. However, complex **M-2** and **M-3** shows a decomposition of ligand in two steps. A DTA curves of complex **M-2** and **M-3** exhibited more than two exothermic broader peaks correspond to sequential oxidative decomposition of ligand. A good agreement was observed between experimental and calculated percentage of copper oxide residue (Figs. 16-18).

Antimicrobial activity: Minimum inhibitory concentration for all the copper(II) compounds has been summarized in Table-6. Ligands and complexes both exhibited excellent inhibitory action against standard bacterial cultures used in the present investigation. Among all ligands, **L-3** exhibited lowest MIC of

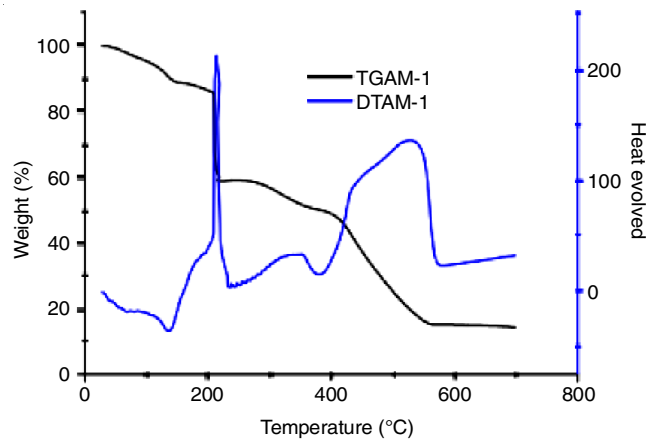


Fig. 16. TGA-DTA thermogram of copper(II) complex **M-1**

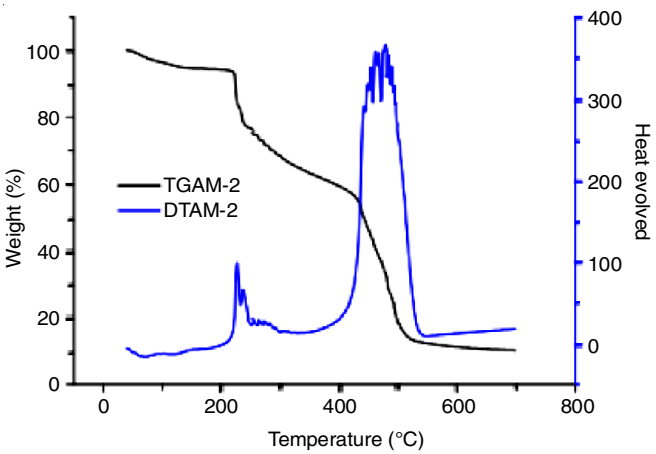


Fig. 17. TGA-DTA thermogram of copper(II) complex **M-2**

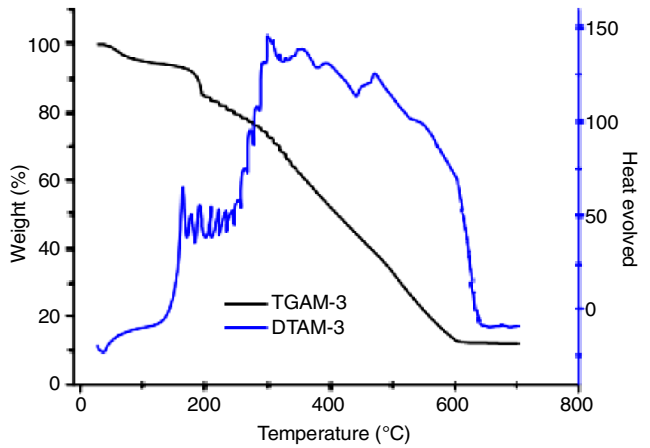


Fig. 18. TGA-DTA thermogram of copper(II) complex **M-3**

TABLE-6
MIC'S DATA OF SYNTHESIZED ALKYLAMINO SUBSTITUTED HYDROXYNAPHTHOQUINONE (**L 1-3**) AND ITS COPPER(II) COMPLEXES (**M 1-3**)

	MIC (mg/mL)			
	B. cereus	*K. pneumoniae*	*S. typhi*	*E. coli*
L-1	1.56	1.56	0.20	0.20
L-2	3.13	1.56	0.78	0.78
L-3	0.78	1.56	< 0.20	0.20
M-1	0.78	0.78	0.20	1.56
M-2	1.56	1.56	1.56	1.56
M-3	0.39	0.39	0.39	0.39
Control drug	3.96	2.75	2.32	2. 58

< 0.20 mg/mL against *S. paratyphi*. The activity of all the ligands was higher against *S. paratyphi* and *E. coli* than *B. subtilis* and *K. pneumoniae*. Upon complexation, lowering of MIC has been observed for *B. subtilis* and *K. pneumoniae*. Complex **M-3** was found to exhibit two-fold and four-fold MIC than its ligand against *B. subtilis* and *K. pneumoniae*. However, activity of complexes was less than their ligand against *S. paratyphi* and *E. coli*. Among three complexes, complex **M-1** showed lowest MIC of 0.20 mg/mL against *S. paratyphi* culture. While complex **M-3** displayed lower MIC of 0.39 mg/mL against all the cultures under investigation than its ligand. MIC values of control drug were found to be higher than the ligands and complexes against the respective bacteria.

Antineoplastic activity: Compounds were found to be effective against MDA-MB-231 cell line (Table-7). Assay shows

TABLE-7
IC_{50} VALUES OF SYNTHESIZED ALKYLAMINO SUBSTITUTED HYDROXYNAPHTHOQUINONE (**L 1-3**) AND ITS COPPER(II) COMPLEXES (**M 1-3**) AGAINST MDA-MB-231

		L-1	L-2	L-3	M-1	M-2	M-3
IC_{50}	µg/mL	1280	320	160	160	160	80
	mol/L	2.95×10^{-3}	1.09×10^{-3}	5.10×10^{-4}	3.07×10^{-4}	2.30×10^{-4}	1.12×10^{-4}

that **L-3** was more effective in controlling cell viability than **L-1** and **L-2**. The lowest IC_{50} of 160 µg/mL was recorded for **L-3** against MDA-MB-231 human breast cancer cell line. Interesting results were obtained with MTT assay of complexes **M-1**, **M-2** and **M-3**. The inhibition of tumor cells was observed at lower IC_{50} values for complexes than their ligands. It required 160 µg/mL of complex **M-1** to shows 50 % inhibition while its corresponding ligand showed concentration of 1280 µg/mL to produce same extent of inhibition. The IC_{50} value of complex **M-1** and **M-2** was found to be identical while least value was noticed for complex **M-3**.

Conclusion

The most efficient and economical solvent free cold press grind stone method was successfully used for the synthesis of three copper(II) complexes of 2-hydroxy-3-[(methylamino)-(alkyl/aryl)]-1,4-naphthoquinone. Modification was performed by changing group substituted at C-11 position as methyl, phenyl and hydroxyphenyl. The effect of substituent in ligands and respective complexes were studied. The ligands **L-1** and **L-3** substituted with methyl and hydroxyphenyl group were found to be more active in inhibiting bacterial growth than its phenyl substituent. Similar observation was observed for the corresponding complexes. As in hydroxy phenyl derivatives, presence of hydroxyl group provides additional polar bondig site which may allows its penetration into bacterial cell. Antibacterial activity of complex **M-3** against *S. paratyphi* was better among all listed compounds.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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