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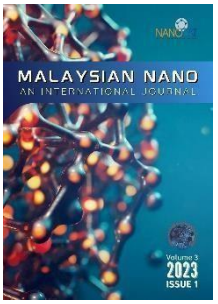

Research article

Synthesis and characterization of nanoscale level mono/bi-ligated metal(II) complexes

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Abstract

A series of nanoscale level metal complexes with bidentate (N_2MCl_2) and tetradentate (MN) chromophores as cisplatin analogues and intercalating agents of, [M(phen)Cl_2] (1- 3), [M(bpy)Cl_2] (4-6), [M(phen)$_2$] (7-9) and [M(bpy)$_2$] (10-12) (where respectively M = Cu(II), Ni(II) or Zn(II); phen = 1,10-phenanthroline and bpy = 2,2'-bipyridine) respectively were prepared in the present investigation. These complexes were prepared by sonochemical technique and characterized using elemental, spectral, and electrochemical techniques. Their nanorod or stick morphologies and crystalline nature were evaluated with scanning electron microscopy (SEM). Metal complexes, 1-6 are existing with two labile chlorides similar to the chemotherapeutic inorganic drug cisplatin, which may anticipate binding covalently with herring sperm DNA, While, complexes, 7-12 may undergo an intercalative mode of binding due to their structural differences relative to 1-6.

Keywords: Nanometal complexes, Mono/bi-ligation, Cisplatin, Intercalators, Covalent binding models

1. Introduction

Inorganic materials are gaining much attention in the medicinal chemistry community owing to their diverse molecular and unique spectral features. Recent literature has shown that the metal-based inorganic architecture has promise in myriad medical applications from synthetic scaffolds for small molecule therapeutics to gene delivery vectors in the field of molecular biotechnology. Such newly developed nanostructured materials could be able to gain access to cells which can enhance their mechanical properties compared to conventional materials due to their ability to interface with living cells. Nanostructured materials with varying structures play vital roles in cancer therapeutics due to their passive tumor-targeting properties.[1] In this circumstance, inorganic metal complexes occupy a pioneering role as they deal with a multipurpose platform for drug design and development. The pharmacological target of most metal-based cancer chemotherapeutics is the N7 atom of purines located in the major grooves of the double helix since these are the most accessible and reactive nucleophilic sites for binding DNA.[2]

Cisplatin, a well-known antitumor metallodrug has been used for the treatment of ovarian and testicular cancers since its invention.[3] It covalently binds with the nitrogen bases of guanine in the double strands of DNA in aqueous media under physiological conditions.[4-10] This coordination produces intra-strand crosslinks that result in the distortion of the DNA structure in the form of unwinding, bending, and shortening.[11] Owing to the toxicity of cisplatin towards healthy cells and the resistance developed by some tumors towards the drug, many new Pt(II) complexes have been prepared. However, these platinum-based anticancer medications, which are clinically utilized to treat 50% of malignant tumors, have a number of drawbacks, including poor stability, significant side effects, and low absorption.[10,12,13] They were tested for DNA-binding ability and anti-tumor efficacy in order to circumvent the problems that plagued cisplatin. Also, very recently, researchers focused on the development of non-platinated metal complexes based particularly on Cu, Ru, or Os due to their improved biocompatibility and negligible toxicity, which resulted in minimal side effects.[14-17]

Nanostructured metal complexes have attracted much attention in the biomedical field due to their diminutive size and good aqueous solubility. These properties enabled the development of a nontherapeutic approach for the effective administration of anticancer drugs to the target tumor with minimal collateral damage to surrounding healthy tissues. Nanoparticles, being smaller than the cancer cells themselves, can easily penetrate through cellular membranes, resulting in proper biodistribution, a prolonged circulation time after treatment, and reduced systemic toxicity.

Numerous studies have been reported on the bulk synthesis of several distinct nanostructured metal complexes for various applications.[18-20] A number of interesting observations have been made when comparing nanostructured complexes to a micro- or macroscopic form of the same materials.[19,21]

In view of this, we have developed some nanostructured transition metal complexes as cisplatin analogous by simple sonochemical method containing the bidentate 1,10-phenonthroline or 2,2'-bipyridine ligand systems having two labile chloride atoms with N_2MCl_2 [M = Cu(II), Ni(II), or Zn(II)] chromophore. They were characterized by spectral and electrochemical methods, and their individual morphologies and particle sizes were analyzed by scanning electron microscopy (SEM) and X-ray diffraction (XRD) methods.

2. Materials and Methods

1,10-phenanthroline monohydrate (herein referred to as "phen") and 2,2'-bipyridine (referred to as "bpy") with purities of 99.5% and hydrated salts of metal (II) chloride [M = Cu(II), Ni(II) and Zn(II)] were purchased from Merck. Spectroscopic-grade ethanol was purchased from Merck and was used without further purification. Other chemicals used were of reagent grade.

2.1 Physical measurements

Infrared spectra for the ligands and complexes were recorded on a Shimadzu FT-IR-8400s (4000−400 cm^{-1}) spectrophotometer by embedding them in KBr discs. Electronic absorption spectral measurements were recorded using a JASCO V-550 UV-vis spectrophotometer. The FAB mass spectra were recorded on a JEOL SX 102/DA-600 Mass spectrometer/Data system using Argon/Xenon (6 kV, 10 mV) as the FAB gas. The SEM images were obtained using a JEOL JSM-6390 electron microscope. The powder samples were placed over carbon tape and a thin layer of platinum was coated over the samples to avoid charging. Electrochemical measurements (cyclic voltammetry and differential pulse voltammetry) were made in MeCN: Tris HCl buffer (1:9 ratio of mixture) using a BAS CV-50W electrochemical analyzer. A three-electrode cell comprising a reference Ag/AgCl, auxiliary Pt, and a working glassy carbon electrode (working electrode surface area of 0.07 cm^2) was used for the measurement. The surface of the GC electrode was cleaned with alumina powder before every measurement.

2.2 Preparation of nano metal complexes

The nanostructured metal complexes, 1-6 (Figure 1a) were synthesized using the following procedure: 1,10-phenonthroline or 2,2′-bipyridine in ethanol (1 mmol, 60 ml) was added dropwise to an ethanol-based solution of $MCl_2.xH_2O$ [M = Cu(II), Ni(II), or Zn(II)] (1 mmol, 60 ml) under

ultrasonic agitation. Sonication continued with a high-intensity ultrasonic probe (Sonics VCX 750) for about 40 min. The solution was kept at room temperature overnight, and the precipitate thus formed was centrifuged at 10,000 rpm. It was purified further by repeated cycles of centrifuging and dispersing the nanoscale-level complex in an aqueous solution. Finally, it was filtered and dried under a vacuum at 60 ºC for 12 h. The final product was re-dispersed in water for further use. A similar procedure was also adopted to prepare the later six complexes, 7-12 (Figure 1b) with a slight modification: 2 mmol of phen or bpy was treated with 1 mmol of respective metal salts instead 1 mmol of phen or bpy.

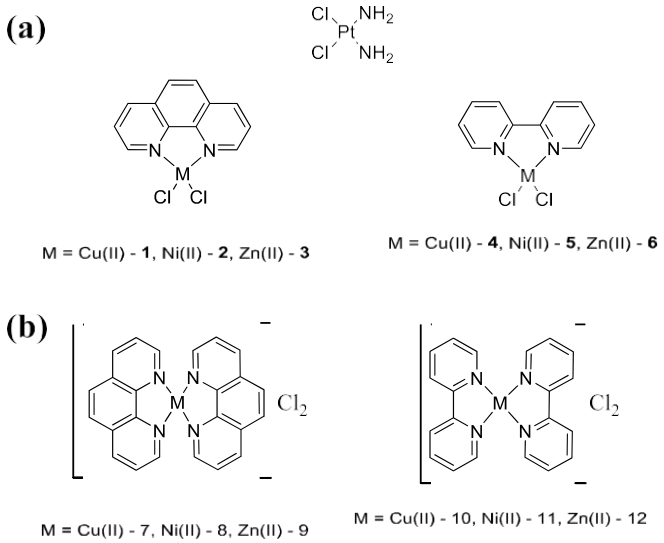

Figure 1: The chemical structure of (a) mono(phen/bpy)Cl_2 and (b) bis(phen/bpy) nanostructured metal complexes.

3. Results and discussion

3.1 Infrared spectroscopy

The IR spectral data of free ligands (phen and bpy) and their nano metal complexes (1-12) recorded using KBr discs are summarized in Tables 1 & 2. The IR spectra of phen, complexes 4 and7 are shown in Figure 2. Two strong bands that appeared at ~1625 and 1506 cm^{-1} were assigned respectively to υ(-C=N-) and υ(-C=C-) of phen and bpy and were shifted to lower frequencies by 20−32 cm^{-1} during the metalation. The υ(C-C) stretching vibration at 1160 cm^{-1} also underwent a coordination-induced shift towards its lower frequencies (15-20 cm^{-1}). These observations indicated that the nitrogen atoms in phen and bpy were strongly coordinated with the metal center.[23,24] The band observed in the 457−417 cm^{-1} region might be assigned to the ν(M-N)[24] stretching frequency, due to the formation of a metal-nitrogen bond. These results indicated

that these ligands are coordinated to the respective metal atoms as proposed in the structure (Figure 1). The ν(M-Cl) stretching vibrations for complexes 1-6 are expected below 350 cm^{-1}. Unfortunately, they were hidden due to the scale measurement begins at 400 cm^{-1}. The ν(M-Cl) vibration may not be expected for complexes, 7-12. Infrared spectral data of all these nanostructured metal complexes are comparable to their macroscale crystalline counterparts, indicating that there was no fundamental change in their chemical bonding and structure.

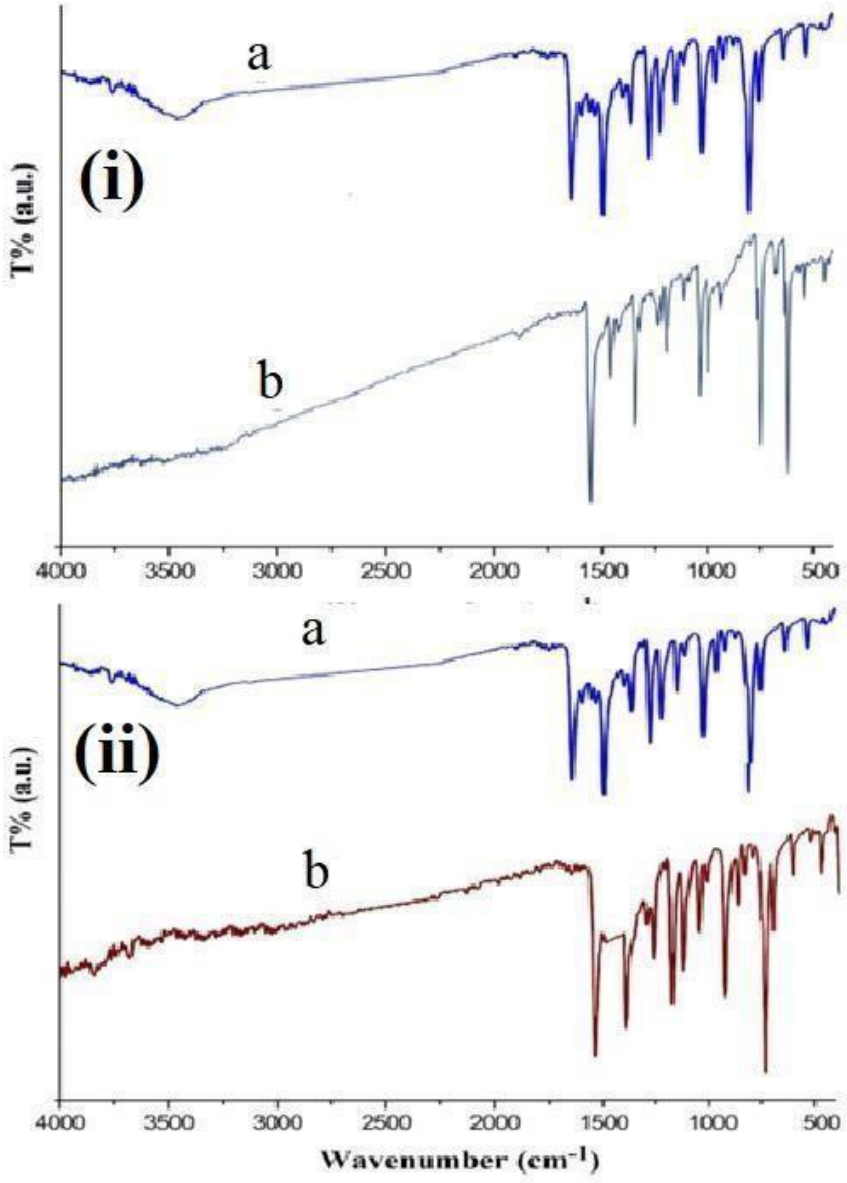

Figure 2: IR spectra of (i)a 1,10-phenanthroline and b $[Cu(phen)_2]Cl_2$ (1) and (ii)a 2-2'-bipyridile and$[Cu(bpy)_2]Cl_2$ (7)

Table 1: IR spectral assignments of phen, bpy and their metal(II) complexes in KBr disc (in cm^{-1})

Complexes		(C=N) (cm^{-1})	(C=C) (cm^{-1})	(C-C) (cm^{-1})	(M-N) (cm^{-1})
Phen		1625	1506	1160	---
$[Cu(phen)Cl_2]$	Nanocrystalline (Complex **1**)	1598	1491	1150	457
	Macrocrystalline	1597	1491	1149	458
$[Ni(phen)Cl_2]$	Nanocrystalline (Complex **2**)	1610	1478	1148	417
	Macrocrystalline	1610	1478	1147	418
$[Zn(phen)Cl_2]$	Nanocrystalline (Complex **3**)	1605	1494	1150	427
	Macrocrystalline	1605	1495	1150	426
Bpy		1621	1503	1161	---
$[Cu(bpy)Cl_2]$	Nanocrystalline (Complex **4**)	1615	1485	1149	428
	Macrocrystalline	1615	1485	1149	428
$[Ni(bpy)Cl_2]$	Nanocrystalline (Complex **5**)	1606	1493	1147	434
	Macrocrystalline	1607	1494	1147	435
$[Zn(bpy)Cl_2]$	Nanocrystalline (Complex **6**)	1609	1482	1151	437
	Macrocrystalline	1610	1483	1150	438

Table 2: IR spectral assignments of phen, bpy and their metal(II) complexes in KBr disc.

Complexes		(C=N) (cm^{-1})	(C=C) (cm^{-1})	(C-C) (cm^{-1})	(M-N) (cm^{-1})
1,10-Phenanthroline		1625	1506	1160	---
$[Cu(phen)_2]Cl_2$	Nanocrystalline **(7)**	1586	1490	1150	479
	Macrocrystalline	1587	1490	1149	476
$[Ni(phen)_2]Cl_2$	Nanocrystalline **(8)**	1608	1477	1147	448
	Macrocrystalline	1609	1477	1145	449
$[Zn(phen)_2]Cl_2$	Nanocrystalline **(9)**	1605	1494	1151	452
	Macrocrystalline	1605	1495	1150	453
2-2'-Bipyridine		1621	1503	1161	---
$[Cu(bpy)_2]Cl_2$	Nanocrystalline **(10)**	1617	1485	1149	446
	Macrocrystalline	1616	1485	1149	446
$[Ni(bpy)_2]Cl_2$	Nanocrystalline **(11)**	1606	1493	1147	461
	Macrocrystalline	1607	1494	1147	460
$[Zn(bpy)_2]Cl_2$	Nanocrystalline **(12)**	1609	1482	1151	449
	Macrocrystalline	1609	1483	1150	448

3.2 Electronic spectra

Electronic spectral data for the nanostructured metal complexes (1-6) in H_2O are listed in Table 3. They showed two strong absorption bands in the 225-236 and 269-304 nm (Figure 3) regions, which may be attributed to a combination of $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions. A relatively weak ligand-

metal charge transfer (LMCT) band was also observed in the region of 350-375 nm. All of these nano metal complexes, except for Zn(II), exhibited a d-d band in the 610-670 nm region.[25]

Table 3: Electronic spectral data of [M(phen/bpy)Cl_2] complexes in water

Complexes	Absorption bands			Element analysis Found (Calcd. %)		
	IL (nm)	LMCT (nm)	d-d (nm)	C	H	N
1	224, 267	375	650	45.77(45.80)	2.51(2.56)	8.88(8.90)
2	222, 270	368	624	46.50(46.52)	2.58(2.60)	9.01(9.04)
3	224, 268	375	---	45.52(45.54)	2.51(2.55)	8.82(8.85)
4	240, 304	357	670	41.30(41.33)	2.72(2.77)	9.62(9.64)
5	242, 297	350	610	42.00(42.03)	2.80(2.82)	9.78(9.80)
6	240, 302	353	---	41.02(45.06)	2.71(2.76)	9.55(9.58)

Table 4: Electronic spectral data of [M(phen/bpy)$_2$]Cl_2 in water.

Complexes	Absorption bands			Element analysis Found (Calcd. %)		
	IL (nm)	LMCT (nm)	d-d (nm)	C	H	N
7	223, 274	380	648	67.87(67.99)	3.76(3.80)	13.18(13.22)
8	214, 269	361	656	65.85(68.78)	3.78(3.85)	13.21(13.37)
9	218, 263	368	---	67.60 (67.69)	3.71(3.79)	13.12(13.16)
10	240, 306	376	667	63.85(63.90)	4.25(4.29)	14.85(14.90)
11	242, 300	361	609	64.70(64.74)	4.30(4.35)	15.05(15.10)
12	240, 304	358	---	63.56(63.59)	4.25(4.27)	14.80(14.83)

Electronic spectra of the bis-phen/bpy nanorod, [M(phen/bpy)$_2$] metal complexes (7-12) werealso recorded in H_2O, and the data are summarized in Table 4. The complexes exhibited their corresponding d-d band in the 609−667 nm region25 except, again, for Zn(II) complexes (9 & 12). In addition, they showed two strong absorption bands around 214−242 and 263−306 nm (Figure 5), which were assignable to n→π* and π→π* transitions. A weak LMCT band was observed in the 358−380 nm region. All these nanostructured metal complexes showed significant absorption intensity enhancement relative to their macrocrystalline counterparts, presumably due to their smaller size (Figure 4).

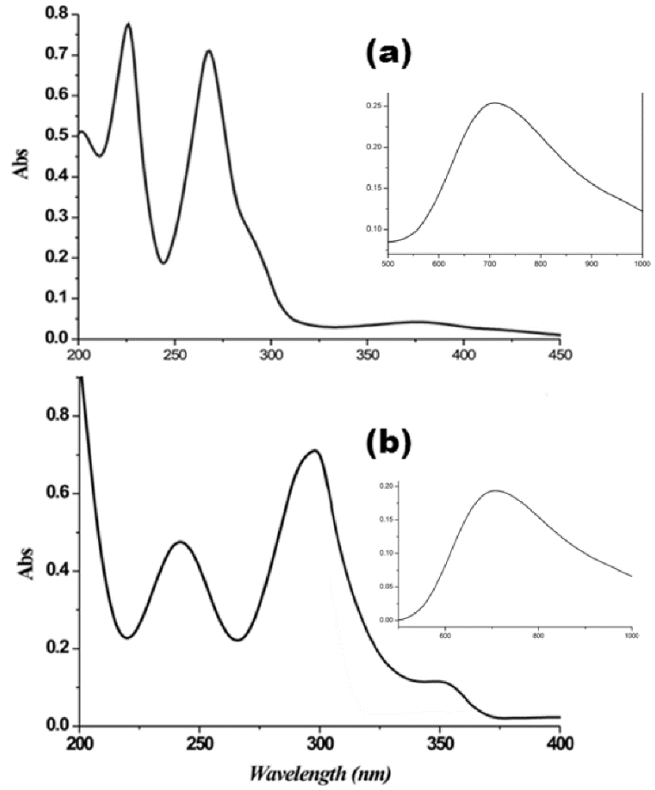

Figure 3: UV-vis spectra of nano-complexes (a) **1** and (b) **7** (inset d-d bands for the respective complexes).

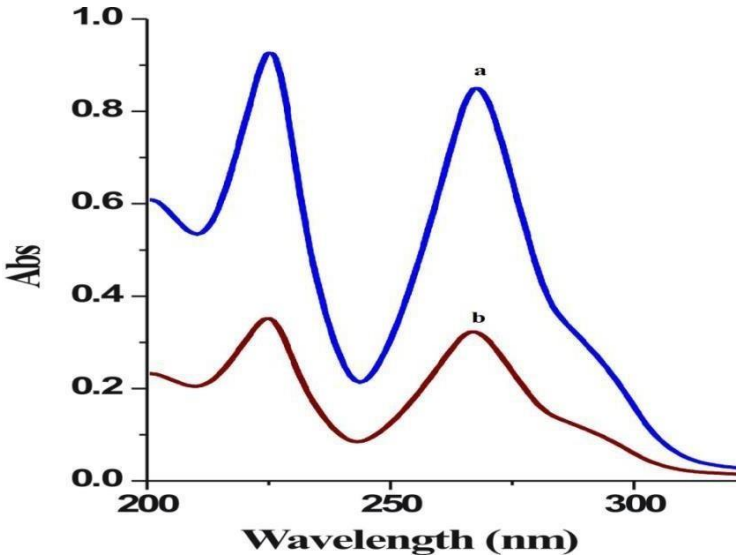

Figure 4: UV-vis absorption spectra of complex [Cu(phen)Cl_2] (4) (a) nano and (b) macro form.

3.2 Mass spectra

The FAB-mass spectroscopy (Fig. 4a) of the nano stick complex 4, showed a peak at m/z = 253 due to the formation of a molecular ion corresponding to $[CuII(bpy)Cl]^+$.[26] The most intense peak at m/z = 278 exhibited by complex 1 $[CuII(phen)Cl]^+$, due to the loss of one chloride unit upon fragmentations from [Cu(phen)Cl_2].[27] The FAB-mass spectra of complexes 7 and 10 showed a molecular ion peak consistent with their respective calculated molecular weights (complex 7, m/z = 423; complex 10, m/z = 375). The observed fragmentation patterns for complexes 7 and 10 were also in accordance with the molecular formula. The peak observed at m/z = 423 in the mass spectrum of complex 7 (Figure 2) is due to the loss of two Cl^- ions and the peak at m/z 243 is due to the loss of one phenanthroline.[28]

The FAB mass spectra acquired for all nanostructured complexes support the formation of complexes as illustrated in Figure 1.

3.3 Morphology analysis

The morphologies and sizes of the nanostructured metal complexes were studied by SEM. Metal complexes 1-6 were self-assembled during metalation in the presence of ultrasound waves. The resulting constructs formed nano-rod or -stick structures, shown in Figure 2.

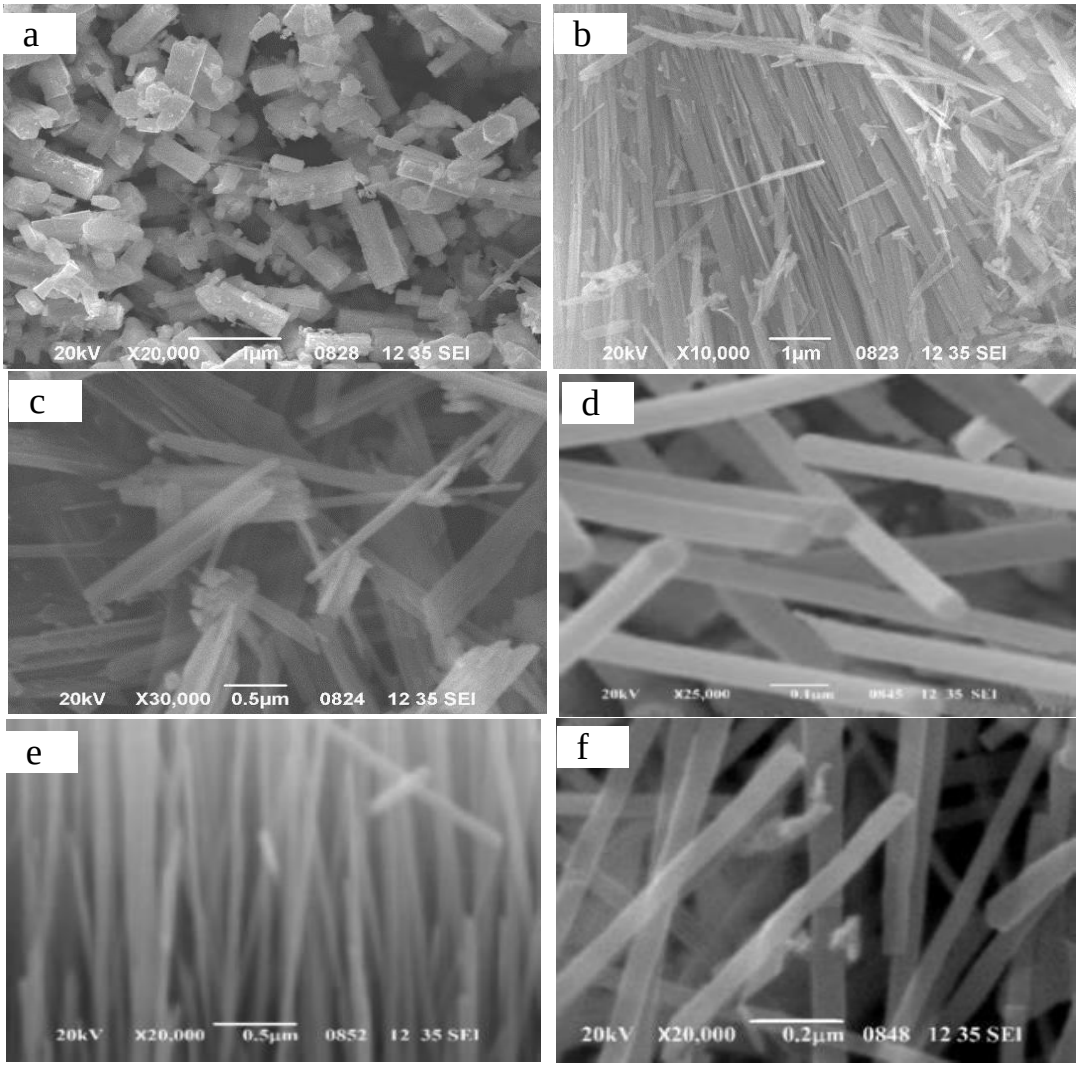

Figure 5: SEM images of nanostructured cisplatin analogy complexes (a-c) 1 - 3 (d-f) 7-9

The SEM images of phenanthroline nano complexes (1-6) revealed that they are in rod or stick-like morphology with diameters of 80−250 nm and lengths of about 0.5−3 μm (Figure 5a-c). Similarly, bipyridine complexes (7-12) also exhibited a high-aspect ratio stick shape with diameters of 50−100 nm and lengths between 500 nm and 3.5 μm. The surface morphologies and sizes of the latter nanostructured intercalating analogy metal complexes (7-12), $[ML_2]^{2+}Cl_2$, also appeared as nanoscale stick- or rod-like structures. The SEM images of complexes 7-9 revealed nano-sticks with possessed diameters of 90−250 nm, lengths of about 0.5−3 μm (Figure 5d-f), and 95−300 nm, lengths between 1 and 3.5 μm respectively.

3.5 Cyclic volumetric analysis

The cyclic voltammogram of nanoscale level metal complexes 1-6 showed a well-defined quasi-reversible redox couple (Ep = 96 mV, Figure 5) in DMSO. However, the voltammogram underwent a dramatic shift towards negative potential when Tris-HCl (pH 7.1) buffer media was used as the solvent instead of DMSO (Figure 7). This negative potential shift might be due to the labile nature of the weaker ligands (chloride ions in complexes 1-6) may replace with diaqua/hydroxide ligands (H_2O/OH^-) in buffer solution (diaquation or hydrolysis)[27-28] as depicted in Figure 6. While this did not occur in complex 7 and its counterparts (complexes, 8-12), confirmed that they may not undergo hydrolysis reaction during the electrochemical analysis in a buffer instead of DMSO solvent, due to their bi-ligated architecture. Such intriguing behavior of these complexes have inspired us to investigate how they interact with DNA, which they may do in a variety of ways. Due to the structural differences, complexes 1-6 may interact via covalent bonds while complexes 7–12 will interact via intercalating modes. These evaluations are currently in process in our laboratory using spectral and electrochemical techniques, subsequently, their anticancer activities also may carry out.

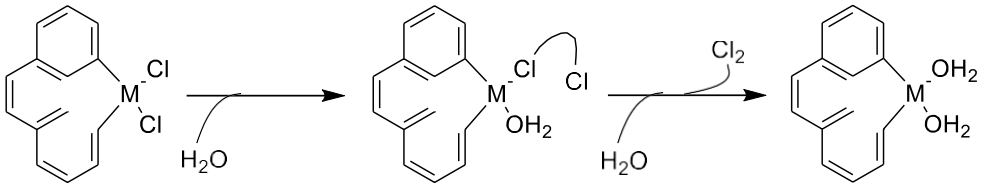

Figure 6: The proposed hydrolysis reaction occurred in complexes 1-6.

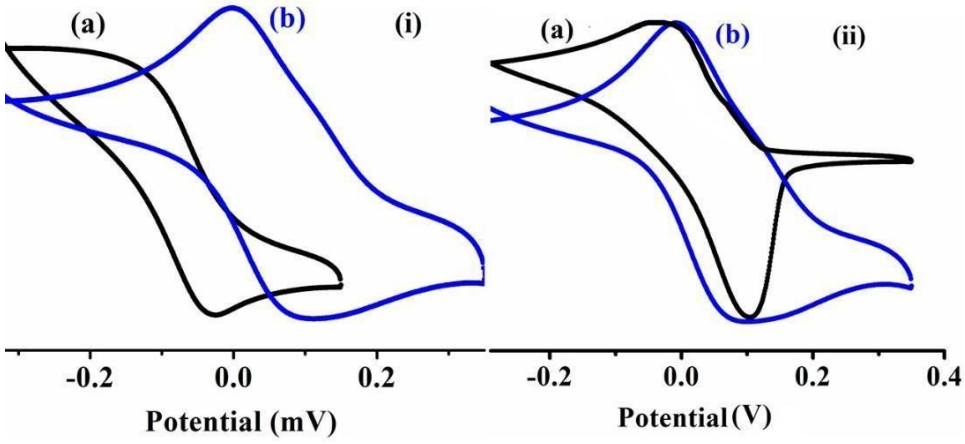

Figure 7: Cyclic voltammogram of complexes (i, a) **1** in buffer (i, b) in DMSO and (ii, a) 7 in bufferand (ii, b) in DMSO and Tris-HCl buffer solvents respectively

4. Conclusions

Nanoscale level metal complexes with bidentate [N_2MCl_2] and tetradentate (bi-ligated) chromophores as cisplatin analogues and intercalating models of, [M(phen)Cl_2] (1-3), [M(bpy)Cl_2] (4-6), [M(phen)$_2$] (7-9) and [M(bpy)$_2$] (10-12) (M = Cu(II), Ni(II) or Zn(II); phen = 1,10-phenanthroline and bpy = 2,2'-bipyridine) have been prepared by sonochemical method and characterized using elemental, various spectral and electrochemical techniques. Their nanorod or stick morphologies and crystalline nature were evaluated with scanning electron microscopy (SEM). Metal complexes, 1-6 exist with two labile chlorides similar to the well-known chemotherapeutic inorganic drug cisplatin. The preliminary studies have revealed that they may anticipate binding covalently with herring sperm DNA, While, complexes, 7-12 may undergo an intercalative mode ofbinding due to their structural differences relative to 1-6. Their detailed DNA interactions and subsequent anticancer activities towards human gastric cancer cell lines are under process in our laboratory.

Conflicts of interest

The authors declare no conflict of interest.

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