New metal based drugs: Spectral, electrochemical, DNA-binding, surface morphology and anticancer activity properties

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HIGHLIGHTS

The aim of this study, is to synthesize anticancer drugs as alternatives to the metal based drugs used in treatment of cancer.

An electrochemical FSdsDNA biosensor has been prepared by immobilizing FSdsDNA onto PGE surface.

The oxidation signal of guanine has been used as probe for the investigation of the interaction between compounds and FSdsDNA.

ABSTRACT

The NSAID piroxicam (PRX) drug was used for complex formation reactions with Cu(II), Zn(II) and Pt(II) metal salts have been synthesized. Then, these complexes have been characterized by spectroscopic and analytical techniques. Thermal behavior of the complexes were also investigated. The electrochemical properties of all complexes have been investigated by cyclic voltammetry (CV) using glassy carbon electrode. The biological activity of the complexes has been evaluated by examining their ability to bind to fish sperm double strand DNA (FSFsdsDNA) with UV spectroscopy. The binding constants of the compounds with FSdsDNA have also been calculated. The morphology of the FSdsDNA, PRX, metal ions and metal complexes has been investigated by scanning electron microscopy (SEM). To get the SEM images, the interaction of compounds with FSdsDNA has been studied by means of differential pulse voltammetry (DPV) at FSdsDNA modified pencil graphite electrode (PGE). The decrease in intensity of the guanine oxidation signals has been used as an indicator for the interaction mechanism. The effect of proliferation PRX and complexes were examined on the HeLA and C6 cells using real-time cell analyzer with four different concentrations.

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Introduction

Metals have an esteemed place in medicinal chemistry. Transition metals represent the d-block element which includes groups 3–12 on the periodic table. Their d shells are in process of filling. This property of transition metals resulted in the foundation of coordination complexes. Metal complex or coordination compound is a structure consisting of a central metal atom, bonded to a surrounding array of molecules or anions. Sophus Jorgensen in Denmark synthesized metal conjugates for the first time in the mid 1870s. In 1893 the major break-through in this field was occurred when Alfred Werner investigated a series of compounds, which contained cobalt, chloride and ammonia. He was awarded...
the Nobel Prize in 1913 for his work. The earliest reports on the therapeutic use of transition metal complexes in cancer and leukemia date from the sixteenth century. In 1960 the anti-tumor activity of an inorganic complex cis-diammine-dichloroplatinum(II) (cisplatin) was discovered. Cisplatin has developed into one of the most frequently used and most effective cytostatic drug for treatment of solid carcinomas. Other metal like gallium, germanium, tin, bismuth, titanium, ruthenium, rhodium, iridium, molybdenum, copper, gold were shown effective against tumors in man and animals [1].

Non-steroidal anti-inflammatory drugs are a well-known class of drugs that are antipyretic, analgesic and anti-inflammatory agents. They are used to reduce pain in different arthritis and other post-operative conditions. Besides this function, they show different types of other activities also [2]. They are utilized primarily as analgesics, anti-inflammatories and antipyretics and their side effects have been well studied. Their main known made of action is through inhibition of the cyclo-oxygenase-mediated production of prostaglandins [3]. They may also work via CO independent mechanisms [3,4] and they have been shown to modulate cell proliferation and cell death in cultured colon cancer cells lacking cyclo-oxygenase suggesting that non-steroidal and anti-inflammatory drug effects are not exclusively attributed to cyclo-oxygenase inhibition [4]. The chemopreventive and anti-tumorigenic activities of non-steroidal anti-inflammatory drugs have been recently reported [5,6] and it has been shown that the use of drugs reduces the number and size of carcinogen-induced colon tumors [7–10].

The importance of metal compounds in medicine is undisputed, as can be judged by the use of, for example antimony (anti-protozoal), bismuth (anti-ulcer), gold (anti-arthritic), iron (anti-malarial), silver (anti-microbial) and platinum (anti-cancer) compounds in the treatment of various diseases. In terms of anti-tumor activity a wide range of compounds of both transition metal and main group elements have been investigated for efficacy. The earliest report on the therapeutic use of metal containing compounds in cancer and leukemia date back to the sixteenth century [11].

PRX, chemically \((8\text{E})-8\text{-[hydroxy-(pyridin-2-ylamino)methylidene]-9-methyl-10,10-dioxo-10-6-thia-9-azabicyclo [4.4.0] deca-1,3,5-trien-7-one}\), the different chemical structures of PRX is shown in Fig. 1 [12] is a non-steroidal anti-inflammatory drug of the oxicam class used to relieve the symptoms of rheumatoid and osteoarthritis, primary dysmenorrhea, postoperative pain; and act as an analgesic, especially where there is an inflammatory component [13,14]. PRX is a patent anti-arthritic drug with a long biological half-life [15], almost no side effects and low acidity [16], which acts by inhibiting enzymes involved in biosynthesis of prostaglandins [17] and the cyclooxygenase activity [18]. PRX may...
exhibit differential anti-cancer effects on different cancer cell types [19,20]. It has been found to inhibit the growth premalignant and malignant human oral cell lines, without inducing apoptosis [21], it induced apoptosis in the HL-60 cells after 48-h incubation synergistically with eicosapentaenoic acid [22] and it is able to induce apoptosis under in vitro conditions in the fibro sarcoma (WEHI164) cell line [23].

Several M(II/III)-PRX complexes have been synthesized and characterized [24–28]. For example [M(H2L)2](A)2 y H2O (where H2L neutral position (Pir), A=Cl in case of Ni(II) or acetate anion in case of Cu(II) and Zn(II) ions and y = 0–2.5) and [M(H2L)2](A)y y H2O (A=SO42– in case of Fe(II) ion (z = 1) or Cl– in case of Fe(III) (z = 3) and Co(II) ions (z = 2) and y = 1–4) chelates are prepared and characterized by Mohamed and El-Gamel [29]. As well as some researchers have obtained single crystal of Cu(II)-PRX complexes. And they determined x-ray crystallography [30,27,31,26]. In this literature, in Cu(II)-PRX complexes, the metal ion have been hexacoordinated, having two drug moieties in the equatorial sites and the solvent molecules in the axial positions. Even if PRX is a potentially tetra dentate ligand, it is known to react as a mono dentate ligand through the pyridyl nitrogen and the amide nitrogen atoms to the metal ion. It is known to react as a mono dentate ligand in all complexes and has coordinated to the metal ion through the pyridine nitrogen and the amide nitrogen atoms. It is a deprotonated bidentate ligand in all complexes and has coordinated to the metal ion through the pyridine nitrogen and the amide oxygen. In this literature, the interactions of metal complexes of PRX with DNA have not been studied (except literature 1 [1]). In Roy’s studies, they have first shown that the PRX can form complex with Cu(II) in aqueous buffer near physiological pH. Besides, their results show that PRX-Cu(II) complex has been obtained to the metal ion through the pyridine nitrogen and the amide nitrogen atoms. It is a deprotonated bidentate ligand in all complexes and has coordinated to the metal ion through the pyridine nitrogen and the amide oxygen. The complexes have been tested for their ability to bind to FSdsDNA. The binding properties of the complexes with FSdsDNA have been investigated with UV and CV titration. In addition, the morphology of the FSdsDNA, PRX, metal ions, and metal complexes were investigated by scanning electron microscope (SEM). Therefore, the interaction of compounds double-stranded DNA (FSdsDNA) was studied by means of differential pulse voltammetry (DPV) at FSdsDNA modified pencil graphite electrode (PGE). The decrease in intensity of the guanine oxidation signals was used as an indicator for the interaction mechanism. The effect of proliferation PRX and complexes were examined on the HeLa and C6 cells using real-time cell analyzer with four different concentrations too.

Experimental

General

PRX was kindly provided by Pfizer Pharmaceutical Co. (Istanbul, Turkey). FSdsDNA was purchased from Sigma, NaCl, metal salts (CuCl2·2H2O, ZnCl2, and K2PtCl4), NaCl, 0.2 M Phosphate buffer at pH 2.0–12.0 and Tris-HCl were purchased from Merck. All the chemicals and solvents were reagent grade and were used as purchased. FSdsDNA stock solution was prepared by dilution of FSdsDNA to buffer solution (containing 150 mM NaCl and 15 mM tris-HCl at pH 7.0) after being centrifuged at 4 °C for three days [45], and kept at 4 °C for no longer than a week. The stock solution of FSdsDNA gave a ratio of UV absorbance at 260 and 280 nm (A260/A280) of 1.89, indicating that the DNA was sufficiently free of protein contamination [48]. The FSdsDNA concentration was determined by the UV absorbance at 260 nm after 1:20 dilution using e = 6600 M−1 cm−1 [49]. Elemental analyses (C, H and N) were performed using a LECO CHNS 932 elemental analyzer. Infrared spectra of the compounds were obtained using KBr discs (4000–400 cm−1) with a Perkin Elmer spectrum 400 FT-IR spectrophotometer. The electronic spectra were obtained in the 200–900 nm range by a Lenton 1100 MSD spectrophotometer. Magnetic measurements were carried out by the Gouy method using Hg[Co(SCN)4] as a standard. Effective magnetic moments were calculated from the expression \( \gamma M = 2.828 (\chi_{M} T)^{1/2} \) BM, where \( \chi M \) is the molar susceptibility corrected using Pascal’s constants for the paramagnetism of copper atom in the complexes. Mass spectra of the ligands were recorded on a LC/MS APCI API LENT 1100 MSD spectrophotometer. 1H NMR spectra were recorded on a Bruker 400 MHz instrument. TMS was used as internal standard and DMSO or acetone as solvents. The amount of metal in the complexes was determined using ICP–OES techniques. (Perkin Elmer Optima 2100. Operating parameters; Nebulizer flow: 0.8 L/min, auxiliary flow: 0.2 L/min, plasma flow: 1.7 L/min, sample flow rate: 1.5 mL/min, equilibrium time: 15 s, RF power: 1452 watts). The thermal analysis studies of the complex were performed on a Perkin Elmer STA 6000 simultaneous Thermal Analyzer under nitrogen atmosphere at a heating rate of 10 °C/min. Scanning electron microscopy associated with SEM Neo Scope JSM-5000 was used for morphological evaluation. Conductivity measurement were taken Thermo Scientific Orion 5 Star.
Electrochemical measurements

Glassy carbon working electrode

All voltammetric measurements at the glassy carbon working electrode were performed using a BAS 100 W (Bioanalytical System, USA) electrochemical analyzer. Glassy carbon working electrode (BAS: Φ: 3 mm diameter), an Ag/AgCl reference electrode (BAS: 3 M KCl) and platinum wire counter electrode and a standard one-compartment three electrode cell of 10 mL capacity were used in all experiments. Glassy carbon working electrode was polished manually with aqueous slurry of alumina powder (Φ: 0.01 mm) on a damp smooth polishing cloth (BAS velvet polishing pad), before each measurement. All measurements were realized at room temperature. 0.02 M Tris-HCl buffer solution at pH 7 have been used as electrolyte solutions. Mettler Toledo MP 220 pH meters was used for the pH measurements using a combined electrode (glass electrode reference electrode) with an accuracy of ±0.05 pH.

Pencil graphite electrode and morphology studies

Pencil graphite electrode (PGE) used for morphology studies. So, DPV was performed with an Autolab-PGSTAT 30 electrochemical analysis system with a General Purpose Electrochemical Software (GPES) 4.9 software package (Eco Chemie, Utrecht, The Netherlands). The three electrode system consisted of the PGE, as the working electrode, a reference electrode (Ag/AgCl) and a platinum wire as the auxiliary electrode. The renewable PGE process was described in literature [49]. A Rotring® pencil was used as a holder for Tomb® graphite leads. The pencil was hold vertically with 15 mm of the lead extruded outside (12 mm of which was immersed in the solution). Each measurement was performed using a new graphite lead. PGE was activated at +1.40 V for 1 min in 0.50 M tris-HCl buffer solution for electrode surface pre-treatment. The FSDsDNA was immobilized onto the pretreated PGE surface by applying a potential at +0.50 V during 240 s using 400 rpm stirring rate in 0.50 M tris-HCl buffer containing 4.00 μg/mL FSDsDNA. The electrode was then cleaned with blank acetate buffer solution for 5 s for the removal of the unbound FSDsDNA on the electrode surface. The FSDsDNA modified PGE was immersed into tris-buffer solution (30% ethanol) containing same concentrations of compound (PRX or M(II) complexes) during 180 s with 400 rpm stirring at open circuit system. The electrode was then rinsed with blank tris-HCl buffer solution for 5 s and SAM images were recorded. All SAM images have been taken on carbon strip.

DNA-binding studies

The interaction of the PRX and complexes with FSDsDNA has been studied with UV spectroscopy and DPV in order to investigate the possible binding modes to DNA and to calculate the binding constants to FSDsDNA (Kb). In UV titration experiments, the spectra of FSDsDNA in the presence of complex has been recorded for a constant FSDsDNA concentration in diverse [complex][FSDsDNA] mixing ratios (r). The intrinsic binding constant, Kb, of the complex with FSDsDNA has been determined through the UV spectra of the complex recorded for a constant complex FSDsDNAS concentration (4.2 × 10−8 M) in the absence and presence of FSDsDNA for diverse r values [49].

Anticancer activity studies of the compounds

Preparation of samples

Stock solutions of the samples were prepared in DMSO and diluted with Dulbecco’s modified eagle medium (DMEM). DMSO final concentration is below 1% in all tests.

Cell lines and cell culture

HeLa and C6 cancer cell lines were grown in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2% penicillin-streptomycin. The medium was changed twice a week.

Anticancer assay

Anticancer effects of the compounds were investigated on C6 cells (Rat Brain tumor cells) and HeLa cells (human uterus carcinoma) using impedance-based real time detection of cellular viability was conducted using the xCELLigence system Real-Time Cell Analyzer RTCA-MP (Roche Diagnostics, Penzberg, Germany). Recording of cell index values (CI) and normalization was performed using the RTCA Software 1.2 (Roche Diagnostics, Penzberg, Germany). A self check using RTCA Resistor Plate 96 was conducted prior to any experiment. Impedance measurements were carried out in designated 96 well E-plates (Roche, Penzberg, Germany). The impedance readout as recorded by the xCELLigence system is expressed as arbitrary cell index-values. Cultured cells were grown in 96-well plates (COASTAR, Corning, USA) at a density of 3104 cells/well. In each experimental set, cells were plated in triplicates and replicated twice. The cell lines were exposed to four concentrations (1, 10, 50 and 100 μl) of all compounds, for 48 h at 37 °C in a humidified atmosphere of 5% CO2. Oxaliplatin, carboplatin and cisplatin were used as standard compounds. Cells were then incubated for overnight before applying the xCELLigence assay reagent (Roche, Germany) according to manufacturer’s procedure. Results were reported as percentage of the inhibition of cell proliferation, where the optical density measured from vehicle-treated cells was considered to be 100% of proliferation. All assays were repeated at least three times using HeLa and C6 cells.

Synthesis of the complexes

Synthesis of copper(II), zinc(II) and platinum(II) complexes

All metal complexes were obtained according to a general procedure: A solution of a metal salt (1 mmol) dissolved in 5 ml of acetate buffer solution for 5 s for the removal of the unbound complexes. Cells were then incubated for overnight before applying the xCELLigence assay reagent (Roche, Germany) according to manufacturer’s procedure. Results were reported as percentage of the inhibition of cell proliferation, where the optical density measured from vehicle-treated cells was considered to be 100% of proliferation. All assays were repeated at least three times using HeLa and C6 cells.

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Synthesis of the complexes

All metal complexes were obtained according to a general procedure: A solution of a metal salt (1 mmol) dissolved in 5 ml of MeOH was added to a solution of PRX ligand (2 mmol) in 5 ml of distilled water and finally 15 ml of MeOH was added to mixture and the mixture was heated under reflux for 1 day. At the end of the reaction, determined by TLC, the precipitate was filtered off, washed with distilled water, ETOH and dried under vacuum. The proposed formulas of M(ll)-PRX complexes have been given in Figs. 2 and 3, respectively. Physical properties and other spectroscopic data are given experimental section.

[Cu(PRX)2(H2O)2][2H3O]; (C19H23CuN4O2S2).2H2O Yield: 75%, color: brown, m.p: 260 °C. Elemental analysis, found (calcd.%): C, 47.23 (48.55); H, 3.935 (3.875); N, 11.02 (10.78); S, 8.39 (8.24); Cu, 7.51 (7.88). Mass spectrum (LC/MS APCI): m/z 808.2, [C30H33CuN4O2S2](+2H)+ (2%), m/z 745.4, [C30H25CuN4O2S2](+3H)+ (5%), m/z 464.4, [C19H23CuN4O2S2](+4H)+ (100%), m/z 332, [C19H25CuN4O2S2](+5H)+ (55%). FT-IR: (KBr, cm−1): 3311, v(N-H), 1567 (v=C=Oamide), 1532 (υ(O=C)), 1132 (υ(S=O)), 767 υ(ν(N-M)), 502 υ(M-O), UV–vis: max nano: (5 cm−1) DMSO as solvent): 257, 6.35 × 104, 284, 5.65 × 104, 365 (6.5 × 104). Conductivity: 13.9 μS.

[Zn(PRX)2(H2O)2][4H2O]; (C19H23H5N4O2S2Zn) Yield: 82%, color: yellow, m.p: 274 °C. Elemental analysis, found (calcd.%): C, 47.15 (48.71); H, 4.239 (3.96); N, 11.00 (10.53); S, 8.39 (8.11); Zn, 7.16 (7.78). 1H NMR (C3D6O, δ ppm): 8.05–8.07 (s, N-H), 7.91–8.01 (m, Ar-H). Mass spectrum (LC/MS APCI): m/z 835, [C19H23H5N4O2S2Zn](+4H)+, m/z 464, [C21H25Zn2N4O2Zn–2H]+ (100%), m/z 465, [C21H25Zn2N4O2Zn–3H]+ (30%), m/z 466, [C21H25Zn2N4O2Zn–4H]+ (5%), m/z 391, [C19H23H5N4O2S2Zn](+2H)+, m/z 332, [C19H25H5N4O2Zn–4H]+ (12%) FT-IR: (KBr, cm−1): 3400, υ(N–H), 1608 υ(C–Oamide), 1575 υ(C=O), 1329 υ(S=O2), 1165 υ(S=O), 674 υ(M–N), 483 υ(M–O), UV–vis
The results of the elemental analysis and some physical characteristics of the obtained compounds were discussed. The complexes are air-stable with high-melting points, insoluble in water and most organic solvents except for acetone, DMSO, DMF and acetonitrile/water mixture are slightly soluble. The elemental analysis data of the complexes indicate that the 1:1 (metal:ligand) stoichiometry. The analytical data show the composition of the metal complexes to be [Cu(PRX)2(H2O)2]2.5H2O, [Zn(PRX)2(H2O)2]4H2O and [Pt(PRX)2]2.5H2O the ratios of the metal present by ICP–OES. The complexes have been decomposed in 1.5 N HNO3/H2O (1/1, v/v) and then dissolved 1.5 N HNO3. The amounts of metal have been given in experimental section. They support the structures given in Figs. 2 and 3. The molar conductivity measurement have been done all complexes are in DMSO (~1 × 10−2 M solutions). The complexes are very stable solids at room temperature without decomposition for a long time. The molar conductance values are in the 13.4–34.5 µS range, indicating the weak electrolytic nature of the complexes [50]. Diverse crystallization techniques have been employed in order to obtain a crystal suitable for the structure determination with x-ray crystallography. Nevertheless, the complexes have been collected as microcrystalline products.

The electronic spectrum of the ligand and its complexes were recorded in DMSO as a solvent. In the spectrum of the PRX, the band at the 355 nm may be assigned to the n–π’ transitions. The observed bands in the 290–280 nm range can be attributed to the n–π and n–δ transitions. Moreover, these bands 325 nm can be assigned to the dσ(L) → dσ(L) metal to ligand charge transfer transitions. The intra-ligand transitions n–π, π–π and δ–π’ in the metal(II) complexes are seen in the range 257–240 nm. The bands with higher energy in the UV region are of intra-ligand π–π type or charge-transfer transitions involving energy levels which are higher in energy than the ligand lowest unoccupied molecular orbital (LUMO).

The FT-IR spectra of PRX and its metal complexes are given in experimental section and Fig. 4. In order to clarify the mode of bonding and the effect of the metal ion on the ligand, the FT-IR spectra of the PRX and metal complexes were compared and assigned on the basis of careful comparison. As it is shown in Fig. 4, the peaks of PRX belonging to ν(N–H) and ν(O–H) enolate groups can be seen at 3337 and 3675 cm−1. The peaks seen as 3675 cm−1 belonging to ν(O–H)enolate groups were not seen in Cu(II), Zn(II) and Pt(II) complexes. This is consistent with the protonation of the enolate O–H group. The shift of the N–H amide group vibration from 3337 cm−1 to lower wavenumbers up to 3248 cm−1 is indicative of the weakening of the N–H bond due to an intramolecular H-bond between the deprotonated enolic oxygen atom and the hydrogen of the amide N–H group. The other series of weak bands between 2901 and 2987 cm−1 are related to ν(C–H) modes of vibrations. Some weak shoulders which are located between ~2000 and 1700 cm−1 can be attributed to implications of the aromatic rings. ν(C=O)amide occurs at ν(1628) cm−1 and ν(C=Npyr) at 1525 cm−1 in the IR spectrum of the PRX. The movement of ν(C=O)amide is declarative of the coordination mode and the number of coordination sites of piroxicam is upon complexation. When PRX acts as bidentate chelating ligand via Npyr and Oamide in Cu(II), Zn(II) and Pt(II) complexes [51], ν(C=O)amide is moved to certainly lower wavenumbers, in the range 1575–1506 cm−1. In any circumstance, coordination of the Npyr is usually implied by at 19–50 cm−1 move to a lower wave number in all the binary chelates. For Cu(II), Zn(II) and Pt(II) complexes, ν(C=O)amide occurs in the range 1607–1639 cm−1 and the ν(C=Npyr) absorption is moved to lower wavenumbers implying coordination of PRX as a result of Npyr and Oamide. The band, placed at 1148 cm−1 in the PRX spectrum is allocated to the antisymmetric stretching vibration of the SO2 group. The SO2 band is moved to lower (for Pt(II) complex ~32 cm−1) or higher frequencies in complexes (for Cu(II) ~7 cm−1 and for Zn(II) complex ~17 cm−1), in conformity with the assignment of Cini et al. [51] and Zayed et al. [52]. This move to higher frequencies must be associated to important hydrogen bonding effects as the SO2 group is not involved in metal binding. The

Results and discussion

Fig. 2. Proposed structure of Cu(II) and Zn(II) complexes.

Fig. 3. Proposed structure of Pt(II) complex.
FT-Far method were used aiming examining the substituent effect upon both the $\delta$- and $\pi$-contribution to the total metal–nitrogen and metal–oxygen bond strength. The $\nu$(M–N) bands for the pyridyl nitrogen seemed in the wavenumber range 676–625 cm$^{-1}$. New peaks of weak or medium intensity are discovered in the wavenumber range 522–483 cm$^{-1}$ which are imputed to $\nu$(M–O) vibrations of binuclear chelates.

The magnetic moments (as BM) of the copper complex were measured at room temperature. The Zn(II) and Pt(II) complexes are found in diamagnetic character and Zn(II) complex is

**Fig. 4.** The infrared spectra of the PRX and its metal complexes (a) MIR and (b) FAR.
The platinum(II) ion lies on the inversion center and is coordinated by two deprotonated PRX ligands to form a distorted square-planar geometry of [Pt(PRX)₂][2.5H₂O]. The PRX anions act as bidentate ligands, creating six-membered trans chelate rings. The structures of the mononuclear Cu(II) complex is supported by the magnetic moment data. The measured magnetic moment value of the Cu(II) complex of the PRX is 1.55 BM, which is close to that of the spin only value (1.73 BM) expected for a complex having one copper(II) ion with a single unpaired electron located in an essentially d^2z^2 orbital. This value suggests that the copper atom is in an octahedral geometry in its chelates [45].

The formulation of the complexes are deduced from analytical data, ^1H NMR and further supported by mass spectroscopy. The relatively low intensities of the molecular ion peaks, [M]^+, are indicative of the ease of fragmentation of the complexes, and this may reflect the number of heteroatoms present in each structure. For example, the main ion and another fragments achieved by cleavage in different positions in the [Cu(PRX)₂(H₂O)₂][2.5H₂O] molecule and the possible fragment ions are shown in Fig. 5 [30].

The spectrum of the [Cu(PRX)₂(H₂O)₂][2.5H₂O] complex shows peak at m/z 808.2. This peak can attributed to the molecular ion peaks [M+3]^+. Alike, the molecular ion peaks of [Zn(PRX)₂(H₂O)₂]4-H₂O and [Pt(PRX)₂][2.5H₂O] complexes showed at m/z 835 ([M]^+) and 806.5 ([M]^+), respectively. In the mass spectra of the copper(II) complex, the highest intensity peaks are at m/e 415 (100%) may be assigned to the [C₁₉H₂₂CuN₆O₈⁺][C₂₁H₂₈N₆O₈S₂⁺][2H⁺] ions which is formed by the loss of other parts of the molecular ions. Typical mass spectra of all complexes have been given in Fig. 6.

Nuclear magnetic resonance (NMR) is a physical phenomenon in which nuclei in a magnetic field absorb and reemit electromagnetic radiation. This energy is at a specific resonance frequency which depends on the strength of the magnetic field and the magnetic properties of the isotope of the atoms; in practical applications, the frequency is similar to VHF and UHF television broadcasts (60–1000 MHz). NMR allows the observation of specific quantum mechanical magnetic properties of the atomic nucleus. Many scientific techniques exploit NMR phenomena to study molecular physics, crystals, complexes and non-crystalline materials through NMR spectroscopy. By studying the peaks of nuclear magnetic resonance spectra, chemists can determine the structure of many compounds. It can be a very selective technique, distinguishing among many atoms within a molecule or collection of molecules of the same type but which differ only in terms of their local chemical environment. NMR spectroscopy is used to unambiguously identify known and novel compounds, and as such, is usually required by scientific journals for identity confirmation of synthesized new compounds. The H–NMR spectra of the PRX and its complexes were recorded using DMSO or acetone and obtained results are given in the Experimental section. In the spectrum of the PRX, the H[O14] shows at 11.2 ppm. In order of, other peaks: H[16N]; 7.87, H(34); 8.31–8.34, H (35); 8.26–8.23, H(25); 8.15–8.07, H(27); 7.99–7.96, H(36); 7.96–7.92. –CH₂ protons are seen at 3.40 ppm as singlet. The singlets in the 2.4–2.7 ppm range can be attributed to the methyl protons. The aromatic protons are shown at 7.02- and 7.34 ppm as singlets. In the spectra of the complexes, the –OH protons are not shown this situation confirms that the pyridine nitrogen and amide oxygen atoms of the –OH group coordinates to the metal ions. Typical NMR spectra of the PRX and its zinc complex have been given in Fig. 7.

In order to give more insight into the structure of the complexes, the thermal studies of the complexes have been carried out using thermogravimetry (TG–DTA) techniques. The thermogravimetric analyses for the metal complexes were carried out within the temperature range from ambient temperature up to 900 °C. The thermal curve and thermo analytical data of the all complexes are given in Fig. 8 and Table 1, respectively. The thermal

![Fig. 5. Mass fragmentation pattern of [Cu(PRX)₂(H₂O)₂][2.5H₂O] complex.](image-url)
behavior of all the complexes was almost the same. It was found from TG analysis that the mononuclear metal complexes start losing mass in the 30–216 °C and end in the 280–600 °C temperature range after losing big part of total mass, but the decomposition did not finish completely at this temperature range. The examination of TG curves showed that the all complexes decompose in four stages. These decomposition steps correspond to loss of the coordinated H₂O molecules and PRX ligands. Fourth step did not finish completely within the temperature range 900–975 °C. From the calculations, it follows that the final decomposition product can be CuO, ZnO and PtO products (see Table 2).

Cyclic voltammetry or CV is a type of potentiodynamic electrochemical measurement. In a CV experiment the working electrode potential is ramped linearly versus time like linear sweep voltammetry [53]. CV takes the experiment a step further than linear sweep voltammetry which ends when it reaches a set potential. When CV reaches a set potential, the working electrode’s potential ramp is inverted. This inversion can happen multiple times during a single experiment. The current at the working electrode is plotted versus the applied voltage to give the cyclic voltammogram trace. CV is generally used to study the electrochemical properties of an analyte in solution [58]. In CV, the electrode potential ramps linearly versus time as shown. This ramping is known as the experiment’s scan rate (V/s). The potential is applied between the reference electrode and the working electrode and the current is measured between the working electrode and the counter electrode. These data are then plotted as current (i) versus potential (E). As the waveform shows, the forward scan produces a current peak for any analytes that can be reduced (or oxidized depending on the initial scan direction) through the range of the potential scanned. The current will increase as the potential reaches the reduction potential of the analyte, but then falls off as the concen-

![Mass spectra of the complexes](image-url)
The redox couple is reversed when the applied potential is reversed, it will reach the potential that will deoxidize the product formed in the first reduction reaction, and produce a current of reverse polarity from the forward scan. This oxidation peak will usually have a similar shape to the reduction peak. As a result, information about the redox potential and electrochemical reaction rates of the compounds are obtained. The utility of CV is highly dependent on the analyte being studied. The analyte has to be redox active within the experimental potential window. It is also highly desirable for the analyte to display a reversible wave. A reversible wave is when an analyte is reduced or oxidized on a forward scan and is then reoxidized or reduced in a predictable way on the return scan. Even reversible couples contain polarization overpotential and thus display a hysteresis between absolute potential between the reduction ($E_{pc}$) and oxidation peak ($E_{pa}$). This overpotential emerges from a combination of analyte diffusion rates and the intrinsic activation barrier of transferring electrons from an electrode to analyte. A theoretical description of polarization overpotential is in part described by the Butler–Volmer equation and Cottrell equation [58]. Conveniently in an ideal system the relationships reduces to $|E_{pc} - E_{pa}| = 57 \text{ mV}/n$, for an $n$ electron
process. Reversible couples will display ratio of the peak currents passed at reduction ($i_{pc}$) and oxidation ($i_{po}$) that is near unity ($1 = i_{po}/i_{pc}$). This ratio can be perturbed for reversible couples in the presence of a following chemical reaction, stripping wave, or nucleation event. When such reversible peaks are observed thermodynamic information can be determined, however it often requires equal quantities of the analyte in both oxidation states. When a wave is non-reversible it is impossible to determine what their thermodynamic information is with CV. This $E_{1/2}$ can be determined, however it often requires equal quantities of the analyte in both oxidation states. When a wave is non-reversible CV cannot determine if the wave is at its thermodynamic potential or shifted to a more extreme potential by some form of overpotential. The couple could be irreversible because of a following chemical process, a common example for transition metals is a shift in the geometry of the coordination sphere. If this is the case, then higher scan rates may show a reversible wave. It is also possible that the wave is irreversible due to a physical process most commonly some form of precipitation as discussed below. Some speculation can be made in regards to irreversible waves however they are generally outside the scope of CV.

For instance if the electronic transfer at the surface is fast and the current is limited by the diffusion of species to the electrode surface, then the current peak will be proportional to the square root of the scan rate. This relationship is described by the Cottrell equation. The CV experiment then samples only a small portion of the solution, the material within the diffusion layer.

In earlier studies, Acuña et al. [54] described for piroxicam (PRX) and its complexes with Cu, Zn, and Pt. The CV study was conducted using conventional and chemically modified carbon paste electrodes. Samples of the compounds were studied over a wide pH range (1.0–12.0) with a glassy carbon electrode. Therefore, the electrochemical behaviors of compounds were studied under a wide pH range (1.0–12.0) with a glassy carbon disc electrode in buffered aqueous media.

In earlier studies, Acuña et al. [54] described for piroxicam determination based on adsorptive stripping voltammetric techniques, using conventional and chemically modified carbon paste.

---

**Table 1**
Thermo analytical data of PRX and its complexes.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Stage</th>
<th>TG results temp. peak (°C)</th>
<th>DTG results temp. C°</th>
<th>Weight loss Found (%) calculated (%)</th>
<th>Evolved moity</th>
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<tbody>
<tr>
<td>[Cu(PRX)$_2$(H$_2$O)$_2$]2.5H$_2$O</td>
<td>I</td>
<td>30–160</td>
<td>150</td>
<td>0.95</td>
<td>1/2H$_2$O</td>
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<tr>
<td></td>
<td>II</td>
<td>170–280</td>
<td>255</td>
<td>66.9</td>
<td>4H$<em>2$O•C$</em>{20}$H$_{20}$Cu$_2$N$<em>2$O$</em>{10}$</td>
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<tr>
<td></td>
<td>III</td>
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**Table 2**
Electrochemical data of all the compounds.

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<th>$E_{pc}$ (mV)</th>
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<th>$\Delta E_p$ (mV)</th>
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electrodes in micellar media. An application of a partial least squares calibration method for the simultaneous voltammetric determination of indomethacin, acemethacin, piroxicam and tenoxicam is suggested by Reguera et al. [55]. The electrochemical oxidation behavior of the piroxicam at the glassy carbon electrode in 10% ACN + 90% 0.2 M Britton–Robinson buffer is presented by Torriero et al. [56]. In this literature, CV, controlled potential electrolysis and spectroscopic techniques were used to obtain information about the reaction mechanism and product identification. Once and for all, the electrochemical behavior of piroxicam on a multi-walled carbon nanotubes electrode for the first time was investigated by Abbaspour and Mirzajani [57].

In our continued studies, PRX was electrochemically oxidized/reduced at the glassy carbon electrode in two steps depending on the pH value investigated. Therefore, several measurements with different electrochemical techniques such as cyclic, linear sweep, differential pulse and square wave voltammetry (Figs. 9–11) were performed using various supporting electrolytes and buffers in order to obtain such information. In CV studies, the scanning was started at −1.5 V in the positive direction, the anodic oxidation of PRX did not occur until about 0.0 V for GCE. After 0.0 V, PRX yielded two well-defined peaks were observed on the anodic and cathodic branch for GCE at 761 mV (at pH 2 BRB solution) and −1054 mV for 1 × 10⁻⁴ M PRX solution (at pH 2 BRB solution), respectively. The cyclic voltammetric behavior of PRX yielded
two well-defined peaks (anodic and cathodic) in acidic and close to strong acidic media such as some buffer solutions at 0 > pH < 6. (Fig. 9a–d). On the reverse scan no complementary reduction peak is observed for anodic peak, in all the pH values (pH 1–12). This attitude is typical for a fast irreversible chemical reaction couple to the charge transfer [56]. Over and above, in this instance, the chemical reaction gives electroinactive products. In the cathodic direction, a new cathodic peak is defined around 761 mV which corresponds to the reduction of piroxicam (Fig. 1a–d). These results agree with those of the previous report [60]. It was observed that at the second and higher cycles the PRX wave decreased and after third cycle the wave was nearly disappeared. This phenomenon may be partly attributed to the consumption of adsorbed PRX on the electrode surface.

The effect of pH on peak potential and peak current (anodic and cathodic direction) were studied by CV technique for PRX. The plot $E_p$ versus pH showed two different regions (Figs. 12 and 13). The first one appeared between pH 1 and pH 12.0. In this region, the peak potential shifted to less positive potential values according to the following equation:

\[
E_{pc} = -982 - 66.8 \times \text{pH} \times 0.9984 \quad \text{(between pH1.0 and 12.0)}
\]

\[
E_{pa} = 756 - 19.2 \times \text{pH} \times 0.9420 \quad \text{(between pH1.0 and 8.0)}
\]

As it can be seen from Fig. 12a one intersection point was observed at about pH 7.0. The intersection point can be explained by changes in protonation of the acid base functions in the molecule (Fig. 14) [59]. For cathodic peak the pH-independent zone above pH 8 means that there are no proton transfer steps before the electron transfer rate-determining step. So, the $E_{pc}$–pH equation of cathodic peak has been given between pH 1.0 and 8.0. In order to gain further information about the mechanism of electro-oxidation of piroxicam, an analysis of the dependence of $E_p$ versus pH was conducted. In water the proton transfer from or toward organic molecules is generally regarded fast, meaning that H\textsuperscript+ are in equilibrium in solution near the electrode. This type of situation should predominate in acidic or not extreme basic media, particularly when the site of protonation is an oxygen atom. A linear portion was detected at low pH with an incline of 0.068 V/pH. This incline is close to that presumed fora monoelectronic/monoprotonic electrode reaction, which is 0.0592 V/pH at 25 °C.

When examined of CV behavior of $[\text{Cu(PRX)}_2(\text{H}_2\text{O})_2]2.5\text{H}_2\text{O}$ and $[\text{Zn(PRX)}_2(\text{H}_2\text{O})_2]\text{H}_2\text{O}$ complexes in same medium, peak at 761 mV is shifted more positive potential values and their currents increased (+768 mV, $9.3 \times 10^{-6} \text{ A}$ for $[\text{Cu(PRX)}_2(\text{H}_2\text{O})_2]2.5\text{H}_2\text{O}$ and +769 mV, $3 \times 10^{-6} \text{ A}$ for $[\text{Zn(PRX)}_2(\text{H}_2\text{O})_2]\text{H}_2\text{O}$ at pH 2 in BRB solution). Unfortunately, we could not examine the electrochemical properties because of $[\text{Pt(PRX)}_2\text{H}_2\text{O}]$ complex precipitated after the addition of buffer solution as can be seen in Photo. A buffer solution contains an acid and its conjugate base or a base and its conjugate acid. Addition of the conjugate ion will result in a change of pH of the buffer solution. For example, if both sodium acetate and acetic acid are dissolved in the same solution they both dissociate and ionize to produce acetate ions. Sodium acetate is a strong electrolyte so it dissociates completely in solution. Acetic acid is a weak acid so it only ionizes slightly. According to Le Chatelier’s principle, the addition of acetate ions from sodium acetate will suppress the ionization of acetic acid and shift its equilibrium to the left. Thus the percent dissociation of the acetic acid will decrease and the pH of the solution will increase. The ionization of an acid or a base is limited by the presence of its conjugate base or acid. Similar situation were observed on the $[\text{Cu(PRX)}_2(\text{H}_2\text{O})_2]2.5\text{H}_2\text{O}$ complex after added pH 2.2 in BRB buffer.

According to Fig. 15, the redox mechanism of $1 \times 10^{-4} \text{ M} [\text{Cu(PRX)}_2(\text{H}_2\text{O})_2]2.5\text{H}_2\text{O}$ complex as below:

\[
\text{Cu(II)} \Leftrightarrow \text{Cu(III)} + \text{e}^- \quad (75 \text{ mV}, 14.55 \mu\text{A})
\]

\[
\text{Cu(II)} \Leftrightarrow \text{Cu(IV)} + \text{e}^- \quad (880 \text{ mV}, 0.43 \mu\text{A})
\]
Cu(IV) + e⁻ ↔ Cu(III) (-21 mV, 1.35 μA)

Cu(III) + e⁻ ↔ Cu(II) (-419 mV, 1.57 μA)

The peak that belong to the PRX molecule at 761 mV (3.81 μA, anodic direction) is shifted at 771 mV (9.31 μA) after complexation reaction. The peak at -1054 mV (5.85 μA) in cathodic direction no observed because of complexation procedure. The binding of PRX to Cu(II) occurs mainly via PRX Npyr atom and Oamide atom of the pyridine ring in solid state. The [Zn(PRX)₂(H₂O)₂]·4H₂O complex have similar mechanism.

The pH of the supporting electrolyte has a significant effect on the electro-reduction/oxidation of the [Zn(PRX)₂(H₂O)₂]·4H₂O at the GCE. Plots of pH versus Epa or Epc and Ip have been investigated using CV techniques too. The peak potential (Eₚ) at the redox process moved to less positive potential values by raising the pH. The plot of the peak potential versus pH showed one straight line between pH 2.0 and 7.0, which can be expressed by the following equations in BRB;

Eₚa (mV) = 795.12 – 34.92 pH r : 0.9961 (between pH 2.0 and 12.0)

Eₚc (mV) = -887.52 – 73.14 pH r : 0.9934 (between pH 2.0 and 8.0)

Typical comparative CV voltammogram of [Zn(PRX)₂(H₂O)₂]·4H₂O complex has been given in Fig. 16.

The effects of pH on peak current of all compounds in the range of pH 1.0–12.0 were also evaluated. The best and sharpest peak and reproducible results were obtained at pH 4.0 in BRB for PRX (anodic peak), at pH 1.0 in 0.1 M H₂SO₄ for PRX (cathodic peak) and [Cu(PRX)₂(H₂O)₂]·2.5H₂O complex (anodic peak) and at pH 3.0 in BRB for [Zn(PRX)₂(H₂O)₂]·4H₂O (anodic peak). Therefore these
medium were chosen in this study as the supporting electrolyte for the electroanalytical investigations. Scan rate studies were carried out to investigate whether the process at the GCE was under diffusion or adsorption control. The effect of the potential scan rates (10−1000 mV/s) and against an internal ferrocene−ferrocenium standard on the peak current and potential of all compounds were evaluated in pH 3 or 4 in BRB or 0.1 M H₂SO₄ solution. The typical current−potential (anodic or cathodic) curve of scan rate studies of PRX, [Cu(PRX)₂(H₂O)₂]2.5H₂O and [Zn(PRX)₂(H₂O)₂]4H₂O have been given in Figs. 17 and 18. The intrinsic binding constants, \( K_b \), were calculated from the intercepts of the straight lines of the plots of \( \log \theta \) vs. scan rate range. These linear relationship were obtained as follow (n = 10 in all studies):

\[
\log \theta = \log v \ (\text{mV s}^{-1}) - 0.193 \ (r : 0.970) \quad \text{for PRX (anodic direction)}
\]

\[
\log \theta = 0.43 \log v \ (\text{mV s}^{-1}) + 0.334 \ (r : 0.998) \quad \text{for PRX (cathodic direction)}
\]

\[
\log \theta = 0.43 \log v \ (\text{mV s}^{-1}) + 0.08 \ (r : 0.999) \quad \text{for [Cu(PRX)₂(H₂O)₂]2.5H₂O}
\]

\[
\log \theta = 0.47 \log v \ (\text{mV s}^{-1}) + 0.21 \ (r : 0.975) \quad \text{for [Zn(PRX)₂(H₂O)₂]4H₂O}
\]

The slopes (between 0.43 and 0.51) of the relationship are close to the theoretically expected (0.5) for an ideal reaction of solution species, so in this case the process had a diffusive component [59].

The mutual effect of the compounds with FsdsDNA has been studied with UV spectroscopy and CV in order to investigate the possible binding modes to FsDNA and to calculate the binding constants to FsdsDNA \( (K_b) \). In UV titration experiments, the spectra of FsdsDNA in the presence of each compounds have been recorded for a constant FsdsDNA concentration in diverse [compound]/[FsdsDNA] mixing ratios \( (r) \). The intrinsic binding constants, \( K_b \) of the compounds with FsdsDNA have been determined through the UV spectra of the complexes recorded for a constant compound 1.9 × 10⁻⁴ M DNA concentration in the absence and presence of FsdsDNA for diverse \( r \) values.

The transition metal complexes are known to bind to DNA via both covalent and/or noncovalent interactions [45,60]. In cova-

<table>
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<tr>
<th>Compounds</th>
<th>pH − direction</th>
<th>Scan rate (mV/s)</th>
<th>( E_{pc} ) (mV)</th>
<th>( E_{pa} ) (mV)</th>
<th>( E_{1/2} ) (mV)</th>
<th>( \Delta E_p ) (mV)</th>
<th>( E_{pa}/E_{pc} )</th>
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lent binding the labile ligand of the complexes is replaced by a nitrogen base of DNA such as guanine N7. Moreover, the noncovalent DNA interactions include intercalative, electrostatic and groove (surface) binding of metal complexes along outside of DNA helix, along major or minor groove. It has been reported that FSdsDNA can provide three distinctive binding sites for all metal complexes; namely, groove binding, electrostatic binding to phosphate group and intercalation [33]. This behavior is of great importance with regard to the relevant biological role of oxicams in the body. Fig. 19 illustrates the spectral changes occurred in 1 × 10^{-5} M methanolic solution of [Pt(PRX)2]2.5H2O upon addition of increasing amounts of FSdsDNA. Even though no appreciable change in the position of the intraligand band of metal complexes are observed by addition of FSdsDNA, the intensity of the band centered at 363 nm for [Pt(PRX)2]2.5H2O (from 363 to 360 for [Pt(PRX)2]2.5H2O, from 356 to 351 for [Zn(PRX)2(H2O)2]4H2O and from 360 to 357 for [Cu(PRX)2(H2O)2]2.5H2O complex is increased in the presence of DNA up to r = 9 and a blue shift of 3 or 5 nm is observed for higher amounts of DNA. The hypsochromic effect observed might be ascribed to external contact (electrostatic binding) or that all complexes could uncoil the helix structure of DNA and made more bases embedding in DNA exposed [33,48]. The intrinsic binding constant K_b of PRX, [Cu(PRX)2(H2O)2]2.5H2O, [Pt(PRX)2]2.5H2O and [Zn(PRX)2(H2O)2]4H2O complexes with FSdsDNA represents the binding constant per DNA base pair, can be obtained by monitoring the changes in absorbances between 363 and 351 nm with increasing concentrations of FSdsDNA from plots [DNA]/{\varepsilon}_0-\varepsilon_f versus [DNA] and is given by the ratio of slope to the y intercept, according to the following equation [35]:

$$\text{[DNA]} / (\varepsilon_0 - \varepsilon_f) = \text{[DNA]} / (\varepsilon_b - \varepsilon_f) + 1 / K_b (\varepsilon_b - \varepsilon_f)$$

where \(\varepsilon_0 = A_{\text{absd}}[\text{compound}], \varepsilon_f = A_{\text{absd}}[\text{compound}], \varepsilon_b = \text{extinction coefficient for the free complex and } \varepsilon_b = \text{extinction coefficient for } [\text{Cu(PRX)}_2(H_2O)_2]2.5H_2O, [\text{Pt(PRX)}_2]2.5H_2O and [\text{Zn(PRX)}_2(H_2O)_2]4H_2O \text{ in the fully bound form. The high value of } K_b \text{ obtained for } [\text{Pt(PRX)}_2]2.5H_2O, [\text{Cu(PRX)}_2(H_2O)_2]2.5H_2O, \text{ and } [\text{Zn(PRX)}_2(H_2O)_2]4H_2O \text{ suggest a strong binding of complexes to FSdsDNA. Indeed, it is much higher than } K_b \text{ calculated for PRX (1 × 10^6 M^-1), indicating that the coordination of PRX ligand to M(II) ion enhance significantly the ability to bind to FSdsDNA. This is an important point } K_b \text{ of } [\text{Cu(PRX)}_2(H_2O)_2]2.5H_2O, [\text{Zn(PRX)}_2(H_2O)_2]4H_2O \text{ is higher than the EB binding affinity for DNA (} K_b = 1.23 ± 0.07 \times 10^6 \text{) suggesting that electrostatic and intercalative interaction may affect EB displacement [61]. The } K_b \text{ values of other complexes have been given in Table 4.}

Fig. 20a–j presented the scanning electron microscopic (SEM) images of the free PGE (a), activated PGE (b), FSdsDNA immobilized onto PGE (c), PRX on FSdsDNA immobilized onto PGE (d), [Cu(PRX)2(H2O)2]2.5H2O on FSdsDNA immobilized onto PGE (e), [Zn(PRX)2(H2O)2]4H2O FSdsDNA immobilized onto PGE (f), [Pt(PRX)2]2.5H2O on FSdsDNA immobilized onto PGE (g), Cu(II) ions on FSdsDNA immobilized onto PGE (h), Zn(II) ions on FSdsDNA immobilized onto PGE(i) and Pt(II) ions on FSdsDNA immobilized onto PGE (j). The surface of the activated PGE (Fig. 20b) is smoother than the surface of the free PGE (Fig. 20a). When DNA is immobilized onto the surface of PGE (Fig. 20c), it has been seen that the surface of PGE changes and some plats occurs on the surface. The SEM image of the PRX on FSdsDNA immobilized onto PGE has been shown in Fig. 20d. The effective change on the surface and increase of the plats shows the interaction between PRX and DNA. SEM photographs of the synthesized copper and platinum complexes of PRX on FSdsDNA are illustrated in Fig. 20e and f, respectively. The [Cu(PRX)2(H2O)2]2.5H2O complex FSdsDNA immobilized on PGE has changed the surface much more than the [Zn(PRX)2(H2O)2]4H2O and [Pt(PRX)2]2.5H2O complexes FSdsDNA immobilized on PGE. In addition to all of these data, it has been recorded that SEM images of Zn(II) ions on FSdsDNA immobilized onto PGE (Fig. 20i) and Pt(II) ions on FSdsDNA immobilized onto PGE (Fig. 20j). On the contrary of copper complex, the Zn(II) and Pt(II) ions have caused big changes on the surface respectively to copper(II) ions.

This immobilization on the surface has also been recorded as voltammetric signal. The optimization of the immobilization step of FSdsDNA on the PGE is shown in Fig. 21 a as voltammetric signal. The guanine oxidation peak has been used as an indicator. Different concentrations of FSdsDNA have been immobilized and the maximum surface coverage the PGE has been obtained before interaction with PRX (or metal ions or metal complexes) by DPP technique. The guanine has been oxidized at about +1.0 V. For finding the optimum concentration of FSdsDNA, five different concentrations between 0.5 and 4.0 ppm have been studied. During this step, FSdsDNA has been immobilized at +0.5 V. Guanine peak current has been increased with increasing FSdsDNA concentration to 4 ppm and then leveled off. The effects of the experimental
parameters have been studied to find optimum analytical conditions for interaction between PRX and FSDNA (Fig. 21b). The binding of the PRX to the FSDsDNA modified PGE has been optimized depending on the interaction concentration by DPV in tris HCl buffer solution containing 30% ethanol. After the interaction with PRX, the guanine signal has been decreased linearly. In this way, different PRX concentrations have been studied between 0.0 and 8.0 ppm as seen Fig. 21b. The best results have been obtained at 4.00 ppm PRX concentration. Similar calibration studies have been done for [Zn(PRX)2(H2O)2]4H2O complex and the results have been given in Fig. 21c. According to voltammetric results, peak current of PRX-FSDsDNA immobilized onto PGE is greater than the [Zn(PRX)2(H2O)2]4H2O complex FSDsDNA immobilized onto PGE (Fig. 21d).

**Anticancer activity studies of the all compounds**

The antiproliferative activities of the PRX and its metal complexes were investigated against HeLa and C6 cell lines. According to the results; all of the compounds have shown cell selective activity against HeLa and C6 cell lines. However, the activities of the compounds have increased to depending increase of doses against all of the cell lines. The antiproliferative activities of the compounds against C6 cell line (a) and HeLa cell line (b) are given in Fig. 22a–c. The antiproliferative activities of the compounds and standard compounds showed the following order at 500 μM against HeLa cell line: [Pt(PRX)2]2.5H2O > oxaliplatin > carboplatin > cis-platine > [Zn(PRX)2(H2O)2]4H2O > [Cu(PRX)2(H2O)2]2.5H2O > PRX. The [Pt(PRX)2]2.5H2O complex has been found to show the highest
antiproliferative activity against HeLa cell line amongst the compounds prepared here.

4. Conclusion

The synthesis and characterization of three new PRX complexes with Cu(II), Pt(II) and Zn(II) have been synthesized with physicochemical and spectroscopic methods. The study of the complexes interaction with FSdsDNA has been performed with UV spectroscopy and DVP and it reveals that the complexes can bind to DNA. UV spectroscopic titrations have been used in order to calculate the binding strength of the complexes with FSdsDNA which is mirrored in the intrinsic binding constant $K_b$. The [Cu(PRX)$_2$(H$_2$O)$_2$]2.5H$_2$O complex exhibits much higher intrinsic binding constant than the other complexes. The results have been described in this study show that changing the metal environment can modulate the binding property of the complex with FSdsDNA.

Information obtained from the present is helpful to the development of nucleic acids molecular probes and therapeutic agents. In addition, it would be of considerable interest if the novel DNA adducts of [Cu(PRX)$_2$(H$_2$O)$_2$]2.5H$_2$O complex led to a broader spectrum of antiinflammatory activity. Cyclic voltammetric studies have shown that all complexes bind to FSdsDNA by both intercalation and electrostatic interaction. Most of bacterial infections now defy all known inflammations and the inflammation resistance is a growing problem in our environment. Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function. There is a great need for new inflammation agents and metal complexes as novel derivatives of oxicams can play an important role in this field. In most cases, it has been evidenced that the inflammation activity of the complexes is comparable to free PRX. According to our biological results, because of the chelate effect, [Pt(PRX)$_2$]2.5H$_2$O, [Zn(PRX)$_2$(H$_2$O)$_2$]4H$_2$O and [Cu(PRX)$_2$(H$_2$O)$_2$]2.5H$_2$O complexes exhibited higher anticancer activity than PRX against some cell line.

The investigation of drug–FSdsDNA interactions would provide new compounds to be tested for an effect on a biochemical target, for the design of DNA biosensors, which will further become DNA microchip systems [62]. In this paper, the interaction between antiinflammatory drug PRX and its Cu(II), Zn(II), Pt(II) complexes and FSdsDNA has been investigated by voltammetry and spectrophotometry for the first time. An electrochemical FSdsDNA biosensor has been prepared by immobilizing FSdsDNA onto PGE surface. The oxidation signal of guanine has been used as probe for the investigation of the interaction between compounds and FSdsDNA. As a result of the interaction of compounds in different concentrations with FSdsDNA, a decrease has been observed in the response based on the signal of guanine. Also, interaction of FSdsDNA in different concentrations with compounds, positive/ negative peak potentials shift may indicate that the compound binding to FSdsDNA by both intercalation and electrostatic interaction. It is clear that, the results of spectrophotometry and voltammetry used glassy carbon electrode are in accordance with the results of voltammetry used PGE. The utility of this electro-
Fig. 21. The effect of FSdsDNA concentration at oxidation signal at PGE for the optimization of immobilization of FSdsDNA (a), DP voltammograms for the interaction of 4 ppm FSdsDNA modified PGE with several concentrations of PRX (b), DP voltammograms for the interaction of 4 ppm FSdsDNA modified PGE with several concentrations of [Zn(PRX)$_2$(H$_2$O)$_2$]4H$_2$O complex (c), DP voltammograms for the interaction of 4 ppm FSdsDNA modified PGE with 4 ppm PRX and 4 ppm [Zn(PRX)$_2$(H$_2$O)$_2$]4H$_2$O complex (d).

Fig. 22. Antiproliferative activities of PRX and its metal complexes against C6 and HeLa cell lines compared with platinum-based anticancer drugs (a) C6 cell line and (b) HeLa cell line.
chemical biosensor for the interaction between F5dsDNA and compounds are cost effective and it provides rapid detection.

Acknowledgements

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Appendix A Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2014.06.144.

References