Synthesis, Spectroscopic and Biologically Assessments of Calcium(II), Zinc(II), Palladium(II) and Gold(III) Sulfacetamide Sodium Complexes: Gold(III) Nano Medical Complex as an Anticancer Agent

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Abstract
The preparation of metal-sulfa drug complexes has an important topic due to their biological applications as chemotherapeutic agents against bacterial infections in microorganism. This paper report the synthesis, characterization and antimicrobial screening of synthesized Ca(II), Zn(II), Pd(II) and Au(II) sulfacetamide sodium (SAM-Na) drug complexes. These complexes were assigned based on elemental analyses, molar conductance and some of essential spectral techniques like IR, 1H-NMR, and electronic spectroscopy. These analyses prove that the sulfacetamide sodium salt was reacted with Ca(II), Zn(II) and Pd(II) metal ions as a bidentate ligand through both oxygen atoms of acetamide and sulfonyl groups. On contrary, the SAM-Na acts as a monodentate ligand through its –NH2 amino in case of gold(III) complex. These complexes were placed in the following formulas: [Ca(SAM-Na)(Cl)2]·5H2O (1), [Zn(SAM-Na)(Cl)3]·3H2O (2), [Pd(SAM-Na)(Cl)2]·3H2O (3) and [Au(SAM-Na)(Cl)3]·4H2O (4). The molar conductance data reveals that all SAM-Na complexes is non-electrolyte. The morphological structures of SAM-Na complexes were checked using X-ray powder diffraction (XRD) and scanning electron microscope (SEM) analyzer.

Keywords: Metallo-antibiotics; Sulfacetamide sodium; Complexation; Spectroscopic; Anticancer; Nano-size

Introduction
Metal-drug interactions are one of an interesting topic in the field of bioinorganic chemistry, that metal-drug complexes were made improvement in the biological properties of free drug [1-6]. The various types of metal ions constituted the backbone for the metallo-antibiotic skeletons, which were helpful to enhance of biological and medical applications [7-10]. Antibiotics metal complexes as well as mixed antibiotics metal complexes were found more effective as chemotherapy agents than their parent antibiotics [8-10]. Metalloantibiotics interact with DNA, RNA, proteins, receptors and lipids, making them very unique and specific. In literature survey, the great attention has been drawn to studies of the antitumor activities of inorganic especially metal complexes [11,12]. The transfer of metal ion from the ligand to the viruses associated with cancer is a mechanism for releasing the anticancer drug in the locality of the tumor [12].

The sulfonamides antibiotic drugs have been effective chemotherapeutic agents to be used for the prevention and cure of bacterial infection in human body. The sulfa-drugs spend their antibacterial influence dependent on the inhibition properties for the dihydropterasine synthetase enzyme towards the substrate P-aminobenzoate [13,14]. In literature survey, many authors have been reported the antimicrobial activity of sulfa-drugs and their metal complexes [13-17]. The title complexes were synthesized by mixing 1:1 molar ratio of sodium salt of sulfacetamide (Figure 1) with Ca(II), Zn(II), Pd(II) and Au(III) chloride ions, which were further complexed. These complexes were then discussed by physical, elemental analysis and spectroscopic characterizations, thermal stabilities. Assessments of antimicrobial activity of these complexes were carried.

Experimental
Chemicals
Sulfacetamide sodium (SAM-Na) antibiotic drug was received from the Aldrich chemical company. All of chemicals used in this study were of analytically reagent grade, commercially available from BDH and used without previous purification like CaCl2, ZnCl2, PdCl2 and sodium tetrachloroaurate(III) dehydrate (NaAuCl4·2H2O).

Synthesis
The Ca(II), Zn(II), Pd(II) and Au(II) SAM-Na complexes were prepared similarly according to the following procedure: 1.0 mmol of SAM-Na ligand was dissolved in 25 mL methanol then mixed with 25 mL of methanolic solution of 1.0 mmol of each metal ions (CaCl2, ZnCl2, PdCl2 and NaAuCl4·2H2O). A mixture of 1:1 ratio (metal ions:

\[
\begin{align*}
H_2N & \quad S \quad N \quad O \\
\quad & \quad Na
\end{align*}
\]

Figure 1: Sulfacetamide sodium (SAM-Na) antibiotic drug.

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SAM-Na) was heated under reflux and continuous stirring at 60–70°C for about 3 h. The mixtures were left overnight until precipitation occurred. The precipitates obtained were filtered off and washed by methanol then left over anhydrous calcium chloride. The yield percent of the products collected were about 76–84%.

**Instruments**

Carbon, hydrogen and nitrogen analyses have been carried out in Vario EL Fab. CHNS. The amount of water and the metal content percentage were determined by gravimetric analysis method. Infrared spectra of the SAM-Na complexes were recorded on Bruker infrared spectrophotometer in the range of 400–4000 cm⁻¹. The molar conductances of 10⁻³ M solutions of the complexes in DMSO solvent were measured on a HACH conductivity meter model. All the measurements were taken at room temperature for freshly prepared solutions. The electronic spectrum of the complexes were measured in DMSO solvent with concentration of 1×10⁻³ M, in rang 200-800 nm by using Unicam UV/Vis spectrometer. The effective magnetic moment (µₑ) of complexes was measured at room temperature using Gouy’s method by a magnetic susceptibility balance from Johnson Metthey and Sherwood model. 'H-NMR spectra were recorded as DMSO solutions on a Bruker 600 MHz spectrometer using TMS as the internal standard. Thermogravimetric analysis (TGA) experiments were conducted using Shimadzu TGA-50H thermal analyzers. All experiments were performed using a single loose top loading platinum sample pan under nitrogen atmosphere at a flow rate of 30 mL/min and a 10°C/min heating rate for the temperature range 25–800°C. SEM images were obtained using a Jeol Jem-1200 EX II Electron microscope at an acceleration voltage of 25 kV. X-ray diffraction (XRD) patterns of the samples were recorded on X Pert Philips X-ray diffractometer. All the diffraction patterns were obtained by using CuKα radiation, with a graphite monochromator at 0.02 °/min scanning rate.

**Antimicrobial assessments**

Antimicrobial activity of the tested samples was determined against (fungi as Aspergillus flavus; Gram (-) bacteria as Staphylococcus aureus, Bacillus subtilis; Gram (+) bacteria as Escherichia coli, Pseudomonas aeruginosa and yeast as Candida albicans) using a modified Kirby-Bauer disc diffusion method [18]. Standard discs of Tetracycline (Antibacterial agent), Amphotericin B (Antifungal agent) served as positive controls for antimicrobial activity but filter disc impregnated with 10 µL of solvent (distilled water and DMSO) were used as a negative control.

**Anti-cancer activities**

Human colon carcinoma (HCT-116) cells and human hepatocellular carcinoma (HePG-2) cells were obtained from the American type culture collection ATCC, Rockvill, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 µg/mL gentamicyn. The cells were maintained at 37°C in a humidified atmosphere with 5% CO₂ and were subculture two to three times a week. The cells were grown as monolayers in growth RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 µg/mL gentamicyn. The monolayers of 10000 cells adhered at the bottom of the wells in a 96-well micro titer plate incubated for 24 h at 37°C in a humidified incubator with 5% CO₂. The monolayers were then washed with sterile phosphate buffered saline (0.01 M pH 7.2) and simultaneously the cells were treated with 100 µL from different dilutions of the test sample in fresh maintenance medium and incubated at 37°C. A control of untreated cells was made in the absence of the test sample. Six wells were used for each concentration of the test sample. Every 24 h the observation under the inverted microscope was made. The number of the surviving cells was determined by staining the cells with crystal violet [19,20] followed by cell lysing using 33% glacial acetic acid and read the absorbance at 490 nm using ELISA reader (Sun Rise, TECAN, Inc, USA) after well mixing. The absorbance values from untreated cells were considered as 100% proliferation. The number of viable cells was determined using ELISA reader as previously mentioned before and the percentage of viability was calculated as [1 – (ODt/ODc)] 100% where; ODt is the mean optical density of wells treated with the test sample and ODc is the mean optical density of untreated cells. The 50% inhibitory concentration (IC₅₀), the concentration required to cause toxic effect in 50% of inactivated cells, was estimated from graphic plots.

**Results and Discussion**

**Analytical and physical data**

The reactions between sulfacetamide sodium salt and different metal chlorides like CaCl₂, ZnCl₂, PdCl₂ and NaAuCl₃.2H₂O lead to a white, yellowish white, olive green and reddish brown solid complexes, respectively. The synthesized SAM-Na complexes exhibit a tetratetradentate environment. The different place of coordination between them were identified in the infrared spectra in the region corresponding to signals of the −CO, SO₂ and −NH₂ groups. The molar ratio and physical results of the SAM-Na drug complexes are summarized in Table 1. These complexes are stable with higher melting points in comparison with parent drug. The complexation mode of Ca(II), Zn(II), Pd(II) and Au(III) metal ions toward SAM-Na was assigned with the helpful of infrared and 'H-NMR spectra as well as thermal analyses and molar conductance. The found and calculated percentage of carbon, hydrogen, nitrogen and metal ions are agreements. The SAM-Na complexes have a non- electrolytic nature [21] due the exits of chloride ions inside the coordination sphere. The magnetic moment of SAM-Na complexes were determined at room temperature and have a diamagnetic character. The speculated formulas are as follows: [Ca(SAM-Na)(Cl)]_n.2H₂O (1), [Zn(SAM-Na)(Cl)]_2.3H₂O (2), [Pd(SAM-Na)(Cl)]_2.3H₂O (3) and [Au(SAM-Na)(Cl)]_2.4H₂O (4) (Figure 2).

**Infrared spectra**

The IR assignments of free SAM-Na antibiotic drug and their metal complexes are summarized in Table 2. The infrared spectra of the Ca(II), Zn(II), Pd(II) and Au(III) complexes are tabulated in Table 2 and shown in Figure 3. The infrared spectra of the Ca(II), Zn(II), Pd(II) and Au(III) SAM-Na complexes show the distinguish bands of the −CO, SO₂ and −NH₂ stretching vibration motions [22]. This band is shifted to higher frequency (20.55) (3.23) (5.99) (22.76) cm⁻¹ compared to the free SAM-Na ligand. The existed band at 3381 cm⁻¹ is assigned to the −NH₂ stretching vibration motions [22]. This band is shifted to higher frequency (20.55) (3.23) (5.99) (22.76) cm⁻¹ compared to the free SAM-Na ligand.

<table>
<thead>
<tr>
<th>Empirical formula</th>
<th>Color</th>
<th>m.p/ °C</th>
<th>Am (µS)</th>
<th>Elemental analysis, % Found % (Calcd.)</th>
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<td><strong>SAM-Na</strong></td>
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<tr>
<td>1 White</td>
<td>White</td>
<td>&gt;300</td>
<td>21</td>
<td>21.83</td>
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<td></td>
<td>White</td>
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<td>(21.97)</td>
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<td>2 Yellowish white</td>
<td>Yellowish white</td>
<td>&gt;300</td>
<td>24</td>
<td>22.42</td>
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<td>3 Olive Green</td>
<td>Olive Green</td>
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<tr>
<td>4 Reddish brown</td>
<td>Reddish brown</td>
<td>&gt;300</td>
<td>26</td>
<td>15.39</td>
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<td>(15.71)</td>
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**Table 1**: Elemental analyses and physical data of SAM-Na free drug ligand and their metal complexes.
wavenumbers 3384, 3445 and 3440 cm\(^{-1}\) for the Ca(II), Zn(II) and Pd(II) complexes, respectively, which symptomatic of the uncoordination of the –NH\(_2\) group to respected metal ions. In case of gold(III) complex the significant bands of –NH\(_2\) group is shifted to lower wavenumber (3376 cm\(^{-1}\)) due to sharing in the chelation environment. The stretching vibration bands of the ν(C=O) carbonyl of amido group exhibit at 1684 cm\(^{-1}\) in case of free SAM-Na free ligand, this band is disappeared for Ca(II), Zn(II) and Pd(II) complexes due to the involvement in the coordination towards these mentioned metal ions. On the other hand, the ν(C=O) carbonyl group in case of Au(III) complex is shifted to higher frequencies at 1703 cm\(^{-1}\) [22]. The increasing for the carbonyl vibration motion can be assigned upon the location of the C=O faraway the place of coordination. The stretching vibration bands of sulfonyl group ν(SO\(_2\)) are exhibit at 1355 ν\(_s\)(SO\(_2\)) and 1148 cm\(^{-1}\) ν\(_as\)(SO\(_2\)), these bands are shifted to lower wavenumbers at (1134 and 1327 cm\(^{-1}\)), (1094 and 1315 cm\(^{-1}\)) and (1133 and 1305 cm\(^{-1}\)) for the Ca(II), Zn(II) and Pd(II) complexes, respectively, but its shifted to higher wavenumbers (1153 and 1405 cm\(^{-1}\)) for the gold(III) complex. These results proved that the chelation of SAM-Na occurred through the oxygens of both carbonyl amido and sulfonyl groups in the manner of Ca(II), Zn(II) and Pd(II) complexes, but the chelation toward gold(III) metal ions take place through the nitrogen atom of –NH\(_2\) group. The presence of a medium-to-weak bands at 540-635 cm\(^{-1}\), can be assigned to ν(M-O) and ν(M-N) stretching vibration motions [22].

The UV-Vis absorption spectra of the free SAM-Na drug and gold(III) complex within the range of 200-1000 nm are shown in Figure 4. The absorption spectrum of SAM-Na drug has one distinguish absorption regions at 258 nm due to the intraligand excitation indicating the effect of the functional groups [23]. Based on complexation, there are some electronic changes due to the interaction of SAM-Na ligand with gold(III) metal ions. The UV-visible absorption spectrum of gold(III) complex has π→π* transitions at 280 and 310 nm due to aromaticity of double bond characters [24]. On the other side, the n→π* transitions were recorded at the wavelengths 375 nm which can be assigned to amino, amido, and sulfonyl groups [25]. The magnetic moments of Ca(II), Zn(II), Pd(II) and Au(III) complexes at room temperature have a diamagnetic character. The gold(III) complex also has a diamagnetic nature as expected for low spin d\(^8\) complexes, which assigned to square planar geometry [26].

\(^1\)H-NMR spectra

\(^1\)H-NMR chemical shifts of SAM-Na drug are assigned in Scheme 1. The \(^1\)H-NMR spectra of [Zn(SAM-Na)(Cl)\(_2\)]\(_3\)H\(_2\)O (2) and [Au(SAM-Na)(Cl)\(_3\)]4H\(_2\)O (4) complexes are shown in Figure 5 and their chemical shifts are listed and assigned in Table 3.
The $^1$H-NMR spectrum of SAM-Na ligand show peaks at $\delta=$ 6.61 and 7.61 ppm due to aromatic protons. The protons of $-\text{CH}_3$ of acetamide group $-\text{C}O-\text{CH}_2$ with singlet peak existed at $\delta= 2.04$ ppm. The two protons of $-\text{NH}_2$ group appears at $\delta= 6.27$ ppm with integration equivalent to two protons. The absence of singlet protons of the $-\text{NH}$ amido group at 11.59 ppm, indicating that SAM-Na is present as a sodium salt molecule.

The Zn(II) and Au(III) complexes have different pathway of coordination. In case of [Zn(SAM-Na)(Cl)$_2$.3H$_2$O (2)] complex, the protons of $-\text{NH}_2$ group are shifted to higher chemical shift at $\delta=$ 7.38 and 7.45 ppm, respectively, rather than free SAM-Na drug. This higher result may be due to uncoordination of $-\text{H}_2\text{N}$ toward zinc(II) metal ions. On the other hand, the chemical shift of $-\text{NH}_2$ protons (at 6.27 ppm in free SAM-Na drug) in case of [Au(SAM-Na)(Cl)$_3$.4H$_2$O (4)] complex is moved to lower chemical shift at 6.08 ppm, this due to the involvement of nitrogen of $-\text{NH}_2$ group in complexation. The chemical shifts of aromatic protons are shifted to lower or higher ppm values rather than free ligand (Table 5), this can be assigned upon the difference in place of complexation as well as electronic configuration of SAM-Na in state of complexation. The presence of new bands at $\delta= 3.34$ and 3.37 ppm, respectively due to water molecules inside the coordination sphere.

**Thermal analyses**

TGA curves of the SAM-Na complexes are presented in Figure 6 and their assignments are tabulated in Table 4. The first degradation step for all complexes occurs within temperature range of 30-200°C due to the loss of uncoordinated water molecules. The second-to-third decomposition steps take place within the temperature range of 200-800°C due to the loss of chlorine gas and decomposition of SAM-Na.
molecule. The calcium(II) oxide CaO, zinc(II) oxide, palladium(II) oxide and gold metal are the final products remains stable till 800°C as final residues.

X-ray powder diffraction and SEM studies

X-ray powder diffraction patterns of [Au(SAM-Na)(Cl)₃].₄H₂O (4) complex is shown in Figure 7. The XRD of gold(III) complex has a crystalline feature and the crystalline data was estimated upon the highest diffraction patterns using the Deby-Scherrer formula equation 1 [27]. Where λ is the wavelength of x-ray (1.5418 Å) for Cu Kα radiation, K is constant taken as 0.94, β full width at half maximum (FWHM) of prominent intensity peak (100% relative intensity peak), θ is a peak position. The grain sizes using Deby-Scherrer formula is 4 nm for the Au(III) complex. The strain (ε=0.003*10⁴) was calculated from the slope of β cos θ versus sin θ plot using the relation (equation 2). Since the dislocation density and strain are the manifestation of dislocation network in the complexes, the decrease in the strain and dislocation density indicates the formation of high quality complexes. The dislocation density (δ= 0.063*10¹².lin.m⁻²) was evaluated as equation 3 [28].

\[ D = \frac{K\lambda}{\beta \cos \theta} \]

\[ \beta = \frac{\lambda}{D \cos \theta - \epsilon \tan \theta} \]

\[ \delta = \frac{1}{D^2} \]

The SEM image of the [Au(SAM-Na)(Cl)₃].₄H₂O (4) complex is shown in Figure 8. This figure gave an impression about the images of NPs grown by complexation process. It is obviously from the SEM images that the synthesized products are NPs, which grown as uniform...
shape. This figure exhibits the high resolution images with regular cubic.

Antimicrobial assessments

The samples of free SAM-Na drug and their Ca(II), Zn(II), Pd(II) and Au(III) complexes were performed against Gram (+) bacteria (Staphylococcus aureus, Bacillus subtilis), Gram (-) bacteria (Escherichia coli, Pseudomonas aeruginosa) and Fungi (Candida albicans, Aspergillus flavus) with standards like tetracycline as antibacterial agent and amphotericin B as antifungal agents. The biological assessments are listed in Table 5. The gold(III) complex show higher biological activity than standards. The higher activity of gold(III) complex can be considered upon the increasing lipophilic character of gold(III) ion than the free ligand and other complexes. The increasing of lipophilicity enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms [29].

In vitro cytotoxicity assessment of the gold(III) complex was performed on human colon carcinoma (HCT-116) cell line and human hepatocellular carcinoma (HepG-2) cell line in the presence of doxorubicin standard drug. The results evaluated upon the determination of inhibitory concentration of 50% (IC50), the data was listed in Table 6. In comparison between data of gold(III) complex and doxorubicin standard, the gold(III) complex has IC50 equal 2.77 and 3.41 μg for HepG-2 and HCT-116 cell line, respectively. From these data we can deduced that gold(III) complex has an effective against HepG-2 cell line rather than HCT-116 cell line.

Table 5: The inhibition zone diameter (mm/mg sample) of SAM-Na and their complexes against some kind of bacteria and fungi. The inhibition zone diameter (mm/mg sample) of SAM-Na and their complexes against some kind of bacteria and fungi.

Table 6: The inhibitory activities against colon carcinoma and hepatocellular carcinoma cells for the [Au(SAM-Na)(Cl)3].4H2O (4) complex and doxorubicin drug.

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References
2. Refat MS (2013) Spectroscopic and thermal degradation behavior of Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) complexes with thiopental sodium anesthetics drug. J Mol Str 1037: 170-185.


