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An EXAFS spectroscopic study of Europium (III) complexation with dafone

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Abstract

An extended x-ray absorption fine structure (EXAFS) for L_{III} Eu crystal edge at 6977 eV energy was studied on an original complex based on europium. The ligand was coordinated entirely with respect to europium atom for [Eu(dafone)₂Cl₂.(H₂O)₃](Cl)(H₂O), where dafone is 4,5-diazafluoren-9-one. The EXAFS spectra showed close-neighbor correlations between europium and nitrogen atoms as well as to the adjoining carbon backbones on macrocyclic cages, which resulted in the bond lengths, the Debye-Waller factor, and coordination numbers. The Eu (III) complex displays marked antibacterial efficacy on a set of Gram-negative bacteria and Gram-positive bacteria showed that the complex displays marked antibacterial behavior. The minimum complex inhibitory concentrations showed that the Eu complex displays significantly higher antibacterial impact on conventional Staphylococcus aureus and Escherichia coli bacterial strains compared to those of silver sulfadiazine and europium nitrate. The Eu (III) bacterial inhibitions are closely linked to relevant DNA binding affinities.

1. Introduction

In the 1960s, it was discovered that cis-dichlorodiamineplatinum (II), i.e. cisplatin, elongated the bacteria, leading to the inhibition of cell division [1]. Currently, platinum-based inorganic compounds such as cisplatin, carboplatin and oxaliplatin are applied in an estimated 50%–70% of cancer treatment routines. However, there is a maximum tolerable dose of these drugs as they attach to the healthy cells [2]. Cisplatin, carboplatin, and oxaliplatin have been successful in clinical treatments entailing the placement of metal-based drugs at the forefront of the battle against cancer. Several mononuclear platinum (II) complexes were synthesized with their anti-cancer behavior being meticulously assessed using suitable biological models [3]. On the contrary to expectations, the majority of platinum (II) complexes studied have displayed biological behaviour similar to cisplatin but with no substantial therapeutic benefits [4]. In addition, the cancers treated with platinum drugs are scarce and entail resistance phenomena and other side effects [5]. Nonetheless, rare earth elements and other metal based products and complexes have been the subject of research for medical advances [6–10]. Rare earth elements are relevant in other technological [11, 12] and scientific [13, 14] advances which are not being discussed in this work, but the results obtained could be applied as well.

Detailed knowledge of the metal-organic and inorganic molecules and complexes is essential in their applications in science, technology, and medicine. As examples, x-ray crystallography [15, 16] and electron momentum spectroscopy (e,2e) [17, 18] are the two methods which derive information such as bond-length, bond-angle, and even the electronic structure of small or large molecules. These methods require specific target preparations to perform the test and intensive physical models to derive the final results. In an x-ray crystallographic experiment, the sample should be prepared in a crystal form of minimal size, while an (e,2e) test should be performed under a vacuum where the sample is prepared as a very thin film or in a gaseous state. The Eu-complex prepared in this work could not be prepared in either a crystal form or in a gaseous state. However, the powerful EXAFS method was used [19–21] to study the Eu complex. The theoretical model required to analyze the EXAFS spectrum is relatively simple and will be discussed in the following text. It should be noted
that FT-IR and UV spectra of the Eu complex demonstrate that the complex is formed. NMR spectrum for a lanthanide is complicate. Therefore, no results would be gained from NMR due to the presence of orbital f states.

Lanthanide ions have also been considered because of several characteristics, such as optical activities that discern them from various luminescent substances [22–24]. Europium, a lanthanide, has a half full f orbital with seven unpaired electrons in an \( ^{8}S_{7/2} \) ground-state configuration. There are many 4f states that are Laporte-forbidden with a lifetime of 1 ms. The first available Laporte-allowed transition, \( 4f_{6}5d_{1} \rightarrow 4f_{5} \), appears between 390–580 nm [25], [26]. Therefore, the magnetic, luminescence, and binding properties of Eu can be tuned by applying the coordination chemistry. Nonetheless, europium complex has been successfully implemented to probe DNA [27–30] and cancer treatment [31]. The synthesized Eu complex has major antibacterial effects on different bacteria, which is largely related to the chelate effect of the central metal. The attached ligand reduces the polarity of the central atom due to the overlap of the ligand orbitals. Therefore, the chelate effect of the whole metal complex is enhanced when the \( \pi \) electrons are not localized, increasing the lipophilic effect of the complex. The increased lipophilic effect allows the complex to enter the micro-organ [32].

In the present work, an Eu-complex with the 4,5-diazafluoren-9-one, (dafone) ligand, \([\text{Eu} (\text{dafone})_2 \text{Cl}_2 (\text{H}_2\text{O})_2] (\text{Cl} (\text{H}_2\text{O})) \) (scheme 1) was prepared according to a previously established method described by Henderson et al [33] starting with 4,5-diazafluoren-9-one (dafone). The EXAFS spectra of the samples were recorded at ALBA synchrotron, BL22–CLAESS beam line, Spain. The final spectrum derived for a typical sample was an average of eighteen spectra. The EXAFS data are analyzed under different software programs such as ATHENA, ARTEMIS, AVOGADRO [34] and FEFF. The energy shift of the photoelectron energy, amplitude reduction factor, variation in half-path length, degeneracy of similar atom to atom paths, and photoelectron mean free path was determined to lead to the final structure of the complex.

The results from the EXAFS analysis of the Eu-sample are essential to any work on the medical, optical or magnetic properties of inorganic molecules or complexes based on europium. We started a study on the antibacterial activities of the Eu complex shown in scheme 1. The minimal bactericidal concentration (MBC) and minimal inhibitory concentration (MIC) were implemented through the Mueller Hinton broth mixture techniques to quantitatively derive the antibacterial behavior of Eu complex [3, 35].

2. Experimental details

2.1. Materials and instrumentation

The Eu complex was prepared according to a well-known method described by Henderson et al [33] starting with 4,5-diazafluoren-9-one (dafone). The yield was 40%. The ‘Eu solution’ was added to a methanol solution.
containing 2mM of the dafone ligand in a dropwise manner. The resulting reaction concoction was refluxed for 2 h at 60 °C. After cooling down to room temperature, the resulting solution was filtered, and the yellow-colored precipitate was gathered and washed for three times using ethanol. Then the samples were cooled down for 8 h at 60 °C. A Perkin-Elmer 2400 primary analyzer was used to evaluate the N, H, and C atoms in the complex.

It should be noted that the sample was prepared to make use of a methanol solution combined with the metal chloride and the ligand, while their respective relative stoichiometric were carefully controlled. The reaction was completed, and the sedimentation was completed. Finally, the resulted sediment was washed with methanol and dried for further analysis.

The UV-Vis absorption spectra were documented via an Analytik Jena SPECCORD S100 UV-Vis spectrophotometer. Under room temperature, fluorescence measurement was conducted using a PERKIN ELMER, LS-3 spectrofluorophotometer that included 1-cm path length quartz cuvettes. The pH dependency of the complex on the fluorescence intensity was studied in the range of 2 to 11. The highest emission was at a pH level of 7. Therefore, the pH level of 7 was selected as the optimal pH for any later interaction studies with DNA.

The Eu samples were mixed with Boron Nitrate (BN) at 30/70 ratio at ALBA while the samples were rubbed, and homogeneous sample pills were prepared. Finally, 1 mm thick pills were made from the mixture under the pressure of 10 Pa.

2.2. EXAFS producer
At ALBA Synchrotron in Spain, the BL22-CLAESS beamline was used to conduct the EXAFS measurements. The average synchrotron electron beam current among the measurements was 200 mA at 3 GeV. The beamline source was wiggle based and was able to exhibit theoretical maximum energy of 70 keV.

Order-sorting, double silicon (111) crystal monochromators that could cover 2.4–63.2 keV energy range were employed. The set up consisted of interchangeable I0, I1, and I2 measurement chambers, including the photon flux ionization chambers before the specimen, after the specimen and after the reference. Spectra were gathered in transmission mode. The optical layout for the CLAESS beamline details is accessible elsewhere [36]. Six scans were collected at three different locations on the specimen to decrease the impact of radiation exposure. The sample was subjected to radiation for about 1 min for every scan at each location. The measurement of two spectra at every location led to the determination of the radiation-induced chemical impacts for the 1 min. The transmission XAFS signal at LIII europium edge was used, which is located at the approximate energy of 6.977 keV.

2.3. Antimicrobial assay
The antibacterial behavior of our Eu complex was evaluated via the broth dilution technique versus E. coli (ATCC 25922), K. pneumoniae (ATCC 10031), S. typhi (ATCC 1609), Methicillin-resistant Staphylococcus aureus (MRSA), Acinetobacter and Vancomycin-resistant Enterococci (VRE). The bacterium growth intermediary was determined as the Mueller Hinton broth, including 2% glucose. The diameter of the inhibition zone was derived from acquiring the Eu complex antibacterial spectrum. A 107 colony-forming units (CFU/ml) standard inoculum on Muller-Hinton agar plate was conducted via a swab. The swab was done before using filter paper discs infused via antibacterial agents (6 mg ml−1) on an agar plate. Incubation was held under 37 °C temperature and left overnight to achieve the constriction zone diameter.

The MBC and MIC were applied via the broth mixture techniques to derive the Eu complex antibacterial behavior quantitatively. The bacterial suspension (107 CFU ml−1) was placed in test tubes of 5 ml Mueller-Hinton broth, including 10-fold complex mixtures within the 0.003–36 mg l−1 range. Incubation was conducted aerobically under 37 °C for 24 h. The non-shaking tubes were then used to determine visible turbidity. MIC denotes the lowest complex concentration without bacterial progression. The tests were conducted three times to validate results as MIC (μg ml−1) pertaining to the strain. Upon determination of the MIC value, a 0.1 ml inoculum solution from the tubes of visible turbidity was subjected to subculture on the nutrient agar plate surface prior to incubation under 37 °C for 24 h. The resulting colonies count on the subculture was subjected to comparison with CFU ml−1 count on the initial inoculum. MBC denotes the complex’s minimum concentration permitted less than 0.1% of the initial inoculum in order to survive.

2.4. Antitumor activity in vitro assays
The Eu complex on A-549 cell line antitumor activities was assessed using the MTT assay in vitro, using a RPMI 1640 intermediary complemented with 10% FBS and 50 μg ml−1 penicillin–streptomycin. The A-549 cell lines (5 × 103 cells well−1) were incubated in 96 well-defined plates at a temperature of 37°C in a 5% CO2 humidified incubator for a period of 24 h, individually, with the existence and presence of various compound concentrations. Then, MTT dilution (12 mM, 10 μL) was placed in the wells, and incubation commenced for a
period of 4 h at a temperature of 37 °C. For the next stage, the media-dispensed and phosphate-buffered saline was used to wash the wells. DMSO (50 μl) was added and incubation was continued for 10 min. An ELISA reader (Bio-Tek, Elx 808, Germany) at 545 nm was used to derive the IC50 at 50% concentration of incubation based on:

\[
\text{%Cell Cytotoxicity} = \left[1 - \frac{\text{Abs(drug)}}{\text{Abs(control)}}\right] \times 100.
\]

The value of the IC50 denotes the drug leading reduction concentration within cellular viability pertaining to the Eu complex.

The A-549 cell line with cell culture (100 μl) medium possessing 0.12 μg Eu-complex (tuntamount to 100 μM concentration), correspondingly were incubated for 24 h in a 5% CO2 incubator. The resulting supernatant was dispensed, while the concoction, i.e. compound cells, was treated by CHCl3 and HNO3. Hence, Eu-complex contents were ascertained via ICP. The tests were performed in four repetitions and the quantity of complex wells tested/dose was two for every test.

3. Results and discussion

3.1. Spectral and elemental characterization

Figure 1 clearly represents the comparison between the FT-IR spectra of the ligand and its Eu complex. With the coordination of ligands with the metal center, the IR spectrum of the Eu complex was changed. In the complex, the stretching vibration for these groups shifted slightly to a lower wave number. The FT-IR spectrum of Eu complex included the characteristic bands of the \((O-H)\), \((C≡O)\), \((C≡N)\), \((C=C)\), and \((C\cdots H)\) at about 2616–3605, 1717, 1558, 1391–1492, and 782, 810 cm\(^{-1}\) (figure 1(b)). In addition, the spectra of Eu complex showed new absorptions in the regions of 1621, 485, and 452 cm\(^{-1}\) attributed to the H–OH, Eu–N, and Eu–Cl stretching modes, respectively.

The elemental analysis for C\(_{17}\)H\(_{11}\)N\(_3\) revealed 79.50% for C, 16.59% for N, and 4.17% for H, where the calculated values were 79.36%, 16.33% and 4.31%, respectively. The analytical calculation for the elements comprising C\(_{20}\)H\(_{16}\)N\(_2\)O\(_3\)EuCl\(_3\) showed 40.38% for C, 2.69% for H, and 4.71% for N, where the experimental results are 40.22%, 2.58%, and 4.63%, respectively. The elemental analysis as well as the FT-IR spectra presented in figure 1, proved the formation of Eu-complex.
3.1.1. EXAFS basic principles

XAFS is a spectroscopic method used to study complexes and clusters. High energy x-rays of intensity \( I_0 \) with energy \( h\nu \) are absorbed by an inner shell of the central atom energy \( E_0 \), where a wave of ejected electron leaves the central atom. In this experiment, the absorbing level LIII of the Eu atom is aimed to be about 6.977 keV. The absorption coefficient \( \mu(E) \) is measured as:

\[
\mu(E) = \ln(I_0/I)
\]

where \( I \) and \( E \) are the scattered x-ray intensity and the 'final' ejected electrons energy, respectively. A sample of collected data is shown in figure 2. Subtracting the background from the normalized absorption coefficient is:

\[
\chi(E) = \left[ \mu(E) - \mu_0(E) \right] / \mu_{\text{edge}}
\]

where \( \mu_0(E) \) is the background absorption spectra and \( \mu_{\text{edge}} \) is a normalization factor to be determined. It is evident from figure 2 that some oscillations are present after the absorbing edge of the central europium atom due to the scattering of the spherical wave of ejected electrons from the surrounding atoms of the complex, which is called the EXAFS spectrum. The transformation from an energy dependence spectrum to wave number space is needed in order to acquire \( \chi(k) \). The EXAFS spectrum of the observed electron is described by the quantity \( \chi(k) \) which is approximately written as [37]:

\[
\chi(k) = \sum_j (S^2_j N_j / (kr_j^2)) f_j(k) \exp(-2\sigma_j^2 k^2) \exp(-2r_j/l_j) \sin(2kr_j + \varphi_j(k) + 2\delta_j).
\]

where \( k = \sqrt{2m(E - E_0)/\hbar^2} \) and \( l_j \) are the ejected electron momentum and the photoelectron mean free path, respectively. The available structure within the quantity \( \chi(k) \) is due to the extensive scattering of the ejected electron wave from the atoms surrounding the central atom within a complex. Thus, the quantities involved in equation (3) are relevant to the physical and geometric properties of the complex, as explained elsewhere [36, 37]. To be self-contained, equation (3) is described briefly while one of the present data is provided by figure 3. Three phases in the sine function of equation (3) are associated with the ejected electron wave dispersed from the atoms of the jth coordination cell in the vicinity of the absorber. The ejected electron experiences a phase shift, \( 2kr_j \), by traveling towards the scatterer \( j \) located at \( r_j \) and moving back to the absorber. The electron experiences the phase shifts \( \varphi_j(k) \) and \( 2\delta_j \) due to the scattering from the scatterer and the absorber, respectively. The assumption is that \( N_j \) atoms exist for a shell about the absorber with a backscattering amplitude \( f_j(k) \). Parameters such as the ejected electron damping factor \( \exp(-2r_j/l_j) \), the amplitude reduction factor \( S^2_j \), and the relative thermal vibrational attenuation \( \exp(-2\sigma_j^2 k^2) \) are also included in equation (3). It is noteworthy that \( \sigma_j \) is the relative mean square displacement of the equilibrium path length as the atoms are vibrating with respect to each other.

3.1.2. EXAFS results

Experimental results of an EXAFS spectrum of a complex were studied comprehensively by using a suitable theoretical model [38]. As seen in equation (3) and figure (3), there is an oscillation subsequent to the resonance peak at about 6.977 keV exhibited by the EXAFS signal for the emitted electrons from the Eu atom. The molecule’s symmetry is considered for the determination of degeneracy for every path \( N_{\text{degens}} \). In the specific case of the singular scattering event, \( N_{\text{degens}} \) denotes the number of atoms within the relevant shell concerning the Eu
atoms. Figure (3) shows the original background-subtracted normalized spectrum in the k-space. In figure 4(a), this spectrum is plotted in r-space, which is the Fourier transform of χ(k). Figure 4(b) shows the inverse-Fourier transform of NFi(k) in the q-space. This is called the q-space to distinguish it from the k-space spectrum.

The spectrum in the wavenumber space enables us to find the k-weighted spectrum in momentum space, $k^n\chi(k) (n = 1 \text{ to } 3)$, as well as its Fourier transform in position space $\chi(r)$ for a detailed analysis. The EXAFS spectrum analysis typically initiates by seeking the potential paths and pertinent impact on the overall spectrum through a program named FEFF. In this regard, the heteroleptic europium complex structure [Eu(dafone)$_2$(OH)$_2$(Cl)$_2$(Cl)(H$_2$O)] (dafone = 4,5-diazafuoren-9-one) is examined. The FEFF version 6 was adopted to derive the mean photoelectron free path, the effective scattering phase shift, and the remaining parameters of equation (3) by simulating the scattering amplitude. To arrive at the best results under the FEFF software, one has to find the closest Eu complex with a known structure. We chose the complex [Eu(phen)$_2$(OH)$_2$(Cl)$_2$(Cl)(H$_2$O)] (phen = 1,10-phenanthroline), where its bond-length $r'_n$ is available from the literature [15, 39]. The parameter $\Delta r_n$ is defined as the difference between $r$ and $r'_n$. The remaining parameters, such as $\Delta E_0$ (energy shift of the photoelectron energy), $S_0^2$, and $N_{\text{degen}}$ (degeneracy of the path) were also derived. It is noteworthy that, $N_{\text{degen}}$ denotes the number of atoms within a shell concerning the Eu atoms for a single-scattering event [19]. Many trial runs of the FEFF program were performed to get the closest EXAFS spectrum between the Eu(phen)-complex and our experimental results of Eu(dafone)-complex. We arrived at the best statistical results.

Determining the pre- and post-edge spectra using the ATHENA software, while removing the background, is a straightforward task as shown in figure 3. To extract the essential parameters of the complex from an L_{III}-edge absorption spectrum, a normalized $\chi(k)$ was derived using ATHENA software where the steps are:

1. The selection of $E_0$ threshold by maximizing of the first derivative.
2. The pre-edge region was fitted to correct the background.
3. The post-edge curve was fitted by constant spline to average the oscillations. The resulting fit was subtracted for the extraction of the oscillating spectrum.

The parameters displaying various atoms producing shells around europium are obtained, i.e., relative shifts in ionization potential ($\Delta E_n$), EXAFS Debye-Waller factors ($\sigma^2$), interatomic distances ($r$) and coordination numbers (N). Grouping the paths using symmetry is helpful. This grouping can be done by multiplying the single contributions made to EXAFS signal by the degeneracy of every path, N. Figures 5–7 present the original and Fourier transformed background k-weighted EXAFS oscillations, $k^3\chi(k)$, for the current Eu-complex, respectively. Within the r-space range of 1.23 to 3.68 Å, the Fourier transform (FT) spectra are separated for the acquired Eu L_{III} data. Figure 5 depicts the well-separated peaks of the Fourier transformed EXAFS oscillation enabling the determination of parameters contributing to equation (3). The amplitude reduction factor, $S_0^2$, is kept at 1 among fittings. The FEFF6 path is utilized to ascertain the potential diffraction paths, whereas the best fit to the peaks is determined by applying the ARTEMIS software. A favorable fit for the peaks' inverse transforms are found using the Eu scattering parameters to the first peak (approximately 1.45 Å) and the second peak (approximately 2.55 Å), as depicted in figures 5–7. The difference in the bond length of our sample,
Eu(dafone)-complex, and simulated spectrum from Eu(phen)-complex, shown in the column $\Delta r$ of table 1, eventually led to the correct sample’s bond length. According to the result shown in table 1, bonds length of Eu with O is 2.36 Å, Eu – N is 2.52 Å, and Eu – Cl is 2.76 Å. The $\Delta r$ values are in the 2%-3% range of the derived bond length. Based on these results, the structure shown in scheme 1 is acceptable, and the Eu(dafone)-complex bond length is very close to the Eu(phen)-complex chosen for simulation. It is needed to understand the angular relation between two dafones in our complex which could be different than the Eu(phen)-complex.

Knowing that the angular relations between the two ligands bonded with europium defined the type of DNA binding, we simulated the angular relations between the two ligands. When the two ligands attached with
europium are parallel, the groove binding with DNA occurs. Otherwise, intercalation binding occurs and vice versa. Thus, we tried to find two examples of the UMPC complex having the same structure, while they differ in the angle of the ligand plates connected to the europium. We then imported the CIF file off into the VESTA software to arrive at the crystalline structure in the Cartesian coordinate system. In this study, the Auto Optimize tool was applied repeatedly to optimize the molecular geometry under the default force field in Avogadro software [34]. Two models, non-parallel and parallel ligand planes, were assumed to study the EXAFS data, which was not conclusive; i.e., other experimental techniques are required to establish more detailed structure of the Eu complex prepared.

Table 1. Structural parameters of complex [Eu(dafone)_2Cl_2(H_2O)_2](Cl)(H_2O) fitted to EXAFS oscillations at \( \Delta E_0 = 5.038 \pm 0.050 \) eV.

| Bond     | N   | \( \sigma^2, \text{Å}^2 \) | \( \delta r, \text{Å} \) | \( r, \text{Å} \) |
|----------|-----|--------------------------|-------------------|------------|
| Eu – O   | 1.94| 0.00491                  | -0.04744 ± 0.02318| 2.36386    |
| Eu – N   | 4.48| 0.00210                  | -0.08259 ± 0.01332| 2.52381    |
| Eu – Cl  | 1.89| 0.00500                  | 0.05584 ± 0.01386 | 2.76094    |

* The values and errors reported here are the ones derived from the software. However, physically the correct result for Eu-N bond, \( \delta r \), is \(-0.08 \pm 0.01\) as an example.

Figure 6. Best fit of the Fourier back-filtered of Eu L_{III} edge EXAFS spectra (red line) as compared with the experimental data (blue line). The top curves are the absolute values of \( k^3 \chi(k) \), while the lower ones are the real part of the same spectrum.

Figure 7. The best fit of the Eu L_{III} edge EXAFS spectra, Re \( \chi(q) \), in q-space (red line) as compared with the experimental data (blue line).
3.2. Antibacterial effects

Antibacterial behaviors pertaining to Eu-complex were investigated based on inhibition region diameter measurement, MBC, and MIC methods. The inhibition region diameter range on the Eu complex versus bacteria was within the 8.0 to 40.0 mm, as shown in Table 2. There was significant antibacterial behavior of the Eu complex, namely versus S. typhi and VRE. Table 2 shows the adequate efficacy of the Eu complex versus such bacteria. The MIC and MBC values of Eu complex versus bacteria were 16.0–14.0 μgm l⁻¹ and 0.14–2.22 mg ml⁻¹, respectively. There was significant antibacterial behavior for the Eu complex with no consideration for gram class, namely S. typhi and VRE, which is arduous to eliminate [35]. However, there were no antibacterial activities by the ligand. The antibacterial behavior of the Eu complex may be described according to the chelation concept [3]. Metal ion polarity may be condensed through chelation, because the positive charge is partially shared with donor groups and also due to the delocalization of electrons within the chelate ring. The condensation of metal ions may be intensified through the chelate lipophilic properties and interactions among the cell wall and metal ions. The charge distribution and geometries of a metal complex molecule are consistent with the interaction of the Eu complex with the bacterial cell wall, which enhances the penetration through the cell wall. Such unique structural adjustment can cause the collapse of the cell barrier and disrupt typical cell procedures. The interactions among the cellular complexes and metal ion are enhanced because of the diversity of the metal complex functioning groups. The chelated complex may cause the deactivation of numerous cellular enzymes, which modifies microorganism metabolic activities [40].

3.3. In vitro cytotoxicity studies

The MTT technique was utilized to evaluate the Eu-complex antitumor behavior to assay the behavior of A-549 cancer cell lines in vitro, presented in figure 8. The value of IC50 found for Eu complex is 8.43 mg ml⁻¹. The analysis results pertaining to variance indicated that the variations among IC50 means of Eu complex in regard to A-549 cell lines were statistically meaningful and not randomly created.

The complex displayed in vitro cytotoxicity versus selected cell lines. The enhanced concentrations of the complex have significantly reduced cancer cell proliferation as shown in figure 8, exhibiting the dose reliant growth hindering impact on these cells.
4. Conclusions

In this study, distorted octahedral structures of a europium (III) complex [Eu(dafone)$_2$(OH)$_2$(Cl)$_2$(Cl)(H$_2$O)] (dafone = 4,5-diazafulleren-9-one) were examined by utilizing EXAFS spectroscopy. The octahedral coordination at the vicinity of the metal ions containing two O, two Cl, and four N atoms was confirmed by EXAFS analysis. The atomic distance between the central Eu atom and the nearest neighbor atoms were determined. The coordination number for europium atom was calculated, which resulted in it having a similar structure with the assumed complex. Knowing the detailed structure of the europium complex, one can determine its potential in being attached to the bacterial DNA and cancerous cell in order to fight cancer. In this work, attempts were made to find the angular relation between the two planar ligands attached to the europium. An EXAFS result was simulated for two different angular separations of the ligands where their structures were known, which was not conclusive. The anisotropic EXAFS method in the Raman mode can be employed to find the angular bond separation [41] of the ligands.

The antibacterial characteristics of the Eu complex were validated against four bacterial strains. The resultant data emphasizes the more significant affinity of the Eu complex regarding DNA and the greater antibacterial behavior of this complex. Depolarization, hydrolysis, and many other effects will result in the bacterial destruction. A full understanding of a complex and its effect as an antibacterial and anticancer agent, while leaving the healthy cells unaffected, requires a detailed understanding of the molecular structure of the complex. A detailed analysis of the complex to determine its DNA and RNA binding cleavage is needed for future work.

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