Advances in Environmental and Life Sciences 4(2) (2023) 15-21

Contents lists available at Egyptian Knowledge Bank

Advances in Environmental and Life Sciences

journal homepage: https://aels.journals.ekb.eg

Luminescence properties of the interaction between Ln-complex towards Ciprofloxacin antibiotics

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Abstract

Ciprofloxacin, a fluoroquinolone antibiotic, is commonly used to treat bacterial infections in humans owing to its broad antibacterial range. On the other hand, an overdose or excessive usage of ciprofloxacin may have a number of unfavorable consequences, not only on people but also on microbes. These side effects can be caused by ciprofloxacin. Using luminescence, an investigation was conducted to look at the interaction that occurs between ciprofloxacin and the Eu(III)-coumarin-3-carboxylic acid (Lc) complex. The changes in luminescence of the complex in solution were used as the basis for this inquiry. This investigation was based on the luminescence variations of the complex in solution in the absence and presence of ciprofloxacin. The effect of solvents on the interaction between the complex and antibiotic was investigated. The information obtained in this paper will be used in the development of new simple and efficient detection method of ciprofloxacin based on luminescent Eu(III)-complex as a chemosensor.

Keywords: Ciprofloxacin, Luminescence, Lanthanide complex, Europium

1. Introduction

Ciprofloxacin (Cipro), a third-generation synthetic fluoroquinolone antibiotic, is highly active against a broad spectrum of gram-negative and gram-positive bacteria. It is widely used for the treatment of infection caused by the bacteria that are resistant to other antibiotics including aminoglycosides and β-lactams [1]. At the same time, there is an increasing concern about bacterial resistance to antibiotics including Cipro, by means of mutations of altering the A subunit of DNA gyrase and other processes such as decreased drug permeation [2]. As the high risk of bacterial resistance could be a clinical issue, efficient analytical techniques are required to monitor residue levels of antibiotics such as Cipro in complex biological matrices. Accordingly, several analytical methods have been reported for determining Cipro, which include high performance liquid chromatography (HPLC), high performance thin layer chromatography [3–6]. Widely used HPLC methods for the determination of Cipro or similar fluoroquinolone derivatives in biological material involve tedious extraction and clean-up steps prior to chromatography. As such, these methods are also not suitable for high throughput analysis for a pharmacokinetic study. The clinical significance of the potency and activities of Cipro in vivo depends on its pharmacology, efficiency, and toxicity, which again depend on the bioavailability or achievable serum level upon administration to body. Hence, there is a strong need to develop a new strategy towards the detection and quantification of antibiotics, which could be readily applied to biological fluid samples. In this purpose, fluorescent detection could be an ideal means due to its high sensitivity and easy sample preparation procedures and

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doi 10.21608/AELS.2023.206278.1033
Received: 1 April 2023, Revised:15 April 2023
Accepted:16 April 2023, Published: 1 July 2023

non-invasive nature [7]. Cipro, being a representative quinolone carboxylic acid derivative, shows native fluorescence emission at 440 nm (ΦF = 0.28) upon excitation at 278 nm in water [4]. Based on the native fluorescence of Cipro, a number of detection methods are reported such as spectrophotometry [8], spectrofluorimetry [9, 10], and flow-injection with chemiluminescence detection [11, 12]. In recent years, use of lanthanide complexes (especially Eu^{3+} and Tb^{3+}), specifically sensitized lanthanide luminescence, has been explored for the development of sensitive and selective detection methods for biological analytes [13]. The main advantages of this technique include emission at the longer wavelength, long luminescence lifetimes, large Stokes shifts, and narrow emission bands, the properties of which can be used to alleviate the scattering interference and the background autofluorescence from the biomolecules. Cipro containing α-keto-acid functionality could chelate with lanthanide ions and act as an antenna to transfer its emission energy to the lanthanide ion. This sensitized emission with a large Stokes' shift and narrow emission band in the longer wavelength region is expected to enable us to detect Cipro in biological samples without any sample preparation or pretreatment procedures. Nevertheless, only a few Ln^{3+}-based fluorescent methods for Cipro detection are reported up to date, and those are often performed in conjunction with sample separating techniques [4, 12, 14, 15]. There are two reports on the terbium-sensitized luminescence detection of ciprofloxacin or quinolone drugs, in which such sample extraction or separation procedures are not performed [16, 17].

The information obtained in this paper will be used in the development of new simple and efficient detection method of ciprofloxacin based on luminescent Eu(III)-complex as a chemosensor.

2. Experimental

2.1. Chemicals

Materials and solvents utilized in our analysis are of analytical grade quality. The compounds were purchased from Sigma Chemical Company. They were as the following: $EuCl_3.6H_2O$, $TbCl_3.6H_2O$ from Sigma was dissolved in ethanol, Antibiotics understudy include Ciprofloxacin (A1), was from EGY Pharm, and Coumarin-3-carboxylic acid (Lc) was from Merck. All solvents used are of analytical grade quality including {Ethanol, Methanol, Acetonitrile and, Tetrahydrofuran (THF)} from Sigma-Aldrich. The Structure of the studied Ligand and Ciprofloxacin is shown the Figure (1).

2.2. Stock solutions

36.6 mg of $EuCl_3.6H_2O$ has been dissolved in 100 ml of ethanol to get 1.00×10^{-3} mol. dm^{-3} final concentration. 19.0 mg of solid ligand was dissolved in 100 ml of ethanol to obtain a final concentration of (1.00×10^{-3} mol. L^{-1}). The stock solution of ciprofloxacin hydrochloride monohydrate (A1) was prepared by dissolving 3.94 mg in 10 ml methanol to obtain a final concentration of (1.00.x 10^{-3} mol. L^{-1}). All stock solutions were stored at 4 °C. The working solutions of Eu (III), Coumarin-3-carboxylic acid, and ciprofloxacin were prepared daily via dilution of the stock solutions.

2.3. Instrumentation and measurements

Luminescence emission and excitation spectra have been acquired with JASCO-FP6300 spectrofluorometer with 1 cm quartz cell. The luminescence intensity of Eu(III) ion and its complex with ligand (Lc) have been measured at $\lambda_{ex/em}$ = 350/615 nm. Uv–vis absorption spectra have been acquired from Shimadzu-UV Probe Version 2.33 with 1 cm quartz cell. Luminescence and time-resolved measurements were obtained by a FLUOstar Optima microtiter plate reader (www.bmglabtech.com) with excitation and emission filters at $\lambda_{ex/\ em}$ = 340/615 nm. The incubation time was set to be 5 min and gain factor 1600 and the temperature was set to be at 25 ^{0}C. Specifically, the injection volume of working solutions was 150 μL for each well of microtiter plate. The average fluorescence intensity was calculated for five separated observations for each working solution.

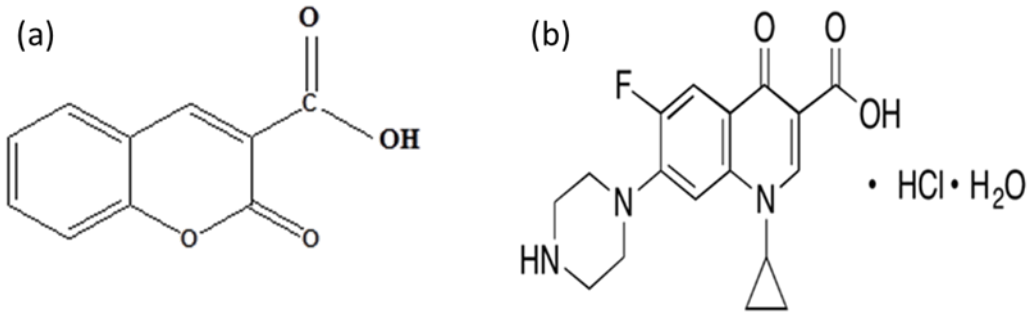

Figure 1: Structures of (a) Coumarin-3-carboxylic acid(Lc) and (b) Ciprofloxacin hydrochloride monohydrate antibiotic (A1)

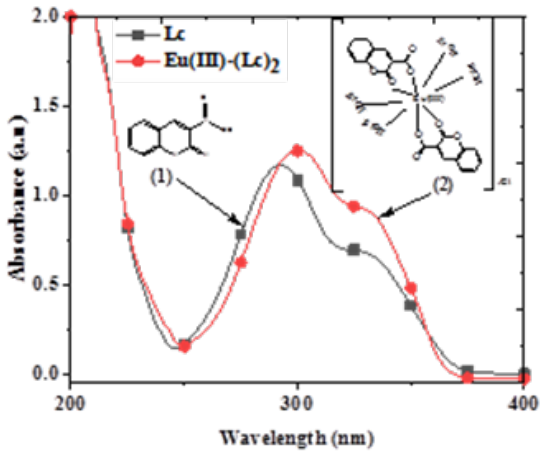

Figure 2: Absorption spectra of 2×10^{-5} M Lc (1), and 1×10^{-5} M Eu (III) + 2×10^{-5}Lc (2) in acetonitrile at room temperature

3. Results and Discussions

3.1. Steady-state of absorption and luminescence spectroscopy of Eu(III)-$(Lc)_2$ complex

The absorption spectrum of coumarin-3-carboxylic acid (Lc) in acetonitrile shows a maximum absorption band at 292.5 nm due to π–π^* transition, and shoulder around 330 nm due to n-π^* transition with extinction coefficient ($\varepsilon_{292.5nm}$ = 11.85 $\times10^4$ $mol^{-1}cm^{-1}L$, ε_{330nm} = 6.83 $\times10^4$ $mol^{-1}cm^{-1}L$). The addition of a solution of Eu (III) to the Lc solution enhances the absorbance with slightly red shift to 300 nm to the first band and enhances the absorbance value of the shoulder band to the higher wavelength (λ=350 nm) with extinction coefficient ($\varepsilon_{292.5nm}$ = 12.52 $\times10^4$ $mol^{-1}cm^{-1}L$, ε_{326nm} = 9.49 $\times10^4$ $mol^{-1}cm^{-1}L$) as shown in Figure (2), revealing the binding between the studied ligand and Eu(III) ion [18, 19]. The behavior of Eu(III) complex in ethanol at room temperature was monitored by UV–vis spectroscopy for 24 hours. Liberation of the ligand was not observed under these conditions. This suggests that the complex is stable under the conditions studied.

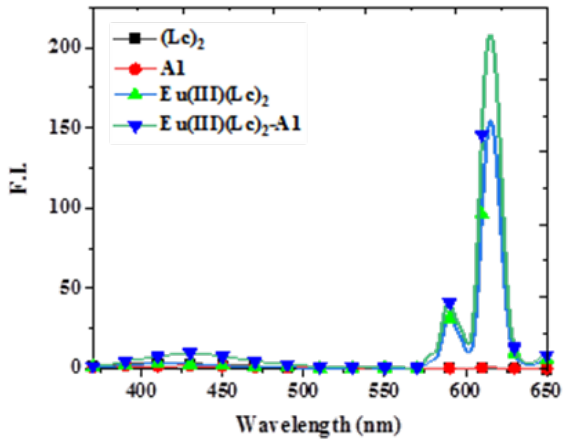

Figure 3: The emission spectra of Eu(III)-$(Lc)_2$ complex in the absence and presence of ciprofloxacin hydrochloride monohydrate antibiotic (A1) in acetonitrile at room temperature. Excitation wavelength was 350 nm and emission and excitation slit width were 5 nm

Figure (3) depicts the fluorescence spectra of the free ligand (Lc) and its complex with Eu(III) in acetonitrile. It was observed that, the free ligand has one emission peak at 425 nm, but two emission peaks were observed for its complex with Eu(III) at 588 and 615 nm which would be attributed to $^5D_0\rightarrow{}^7F_1$ and $^5D_0\rightarrow{}^7F_2$ transitions of the Eu(III) ion. The second emission peak at 615 nm is the predominant one for the studied complex. This is can be attributed to the efficiency of the intramolecular energy transfer from the triplet level

of the Lc ligand to the emitting level of the lanthanide ion, which depends on the energy gap between the two levels, probably the energy gap between the ligand triplet level and the emitting level of Eu(III) ion (antenna effect) which absorb energy and transfer it to Eu (III) ion, emitting the characteristic luminescence of Eu(III) ion.

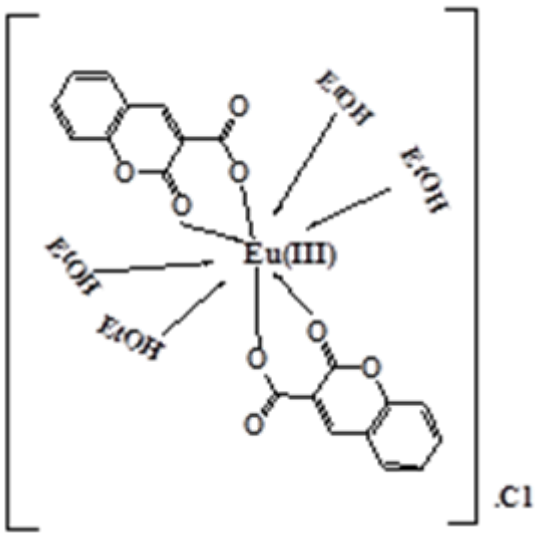

Figure 4: The proposed Chemical structure of Eu(III)-coumarin-3-carboxylic acid

According to our previous studies, the stoichiometry of the complex of Lc with Eu (III) ions was selected to be 1:2 for our present investigation. The proposed structure of Eu(III)-complex can be shown in Figure (4). The complex reaction can be written as:

Eu (III) + 2 (Lc)↔Eu(III) $(Lc)_2$

3.2. Ciprofloxacin Sensitized Lanthanide Luminescence

Among the lanthanides, europium ion Eu(III) is well known for its characteristic red luminescence at 615 nm and 588 nm, corresponding to the $^5D_0 \rightarrow {}^7F_2$ and $^5D_0 \rightarrow {}^7F_1$ transitions, respectively. Due to the limitation of the direct excitation of Eu^{3+}, a well-established phenomenon of using sensitized excitation of Eu(III) is possible by an appropriate organic ligand through energy transfer [20]. According to the Förster's resonance energy transfer theory, the rate of energy transfer depends upon the extent of overlap of the emission spectra of the donor with the excitation spectra of the acceptor and the distance between them [21]. Cipro has an intensely populated triplet state due to its relatively low energy gap between the S1 and T1 states (11.9 k cal·mol^{-1}) and shows high phosphorescence / fluorescence intensity ratio (P/F = 2.92) [4]. So, a coordination complex of lanthanide ion with Cipro, upon excitation of the Cipro moiety, would show a sensitized luminescence through the Förster's resonance energy transfer, where Cipro acts as energy donor and the lanthanide ion acts as energy acceptor. Keeping this in mind, we evaluated several Eu^{3+} complexes with different number of free co-ordination sites to find out the one that shows the largest sensitized luminescence change upon binding with Cipro. We chose Eu-Lc [Lc = Coumarin-3-carboxylic acid)], and $EuCl_3$ as the probe candidates, which have the available co-ordination site number of one, two, and nine respectively for an analyte, as the maximum co-ordination number for Eu^{3+} is nine (**Figure 4**). One equivalent of Cipro was added to each of metal complexes in acetonitrile, and the luminescence from Eu^{3+} at 615 nm was monitored under excitation at 350 nm, a maximum absorption wavelength of Cipro. A characteristic emission band of Eu^{3+} was enhanced in the presence of ciprofloxacin (**Figure 3**). It is thus concluded that Cipro coordinates effectively only to Eu-Lc, which has two vacant coordination sites, and Cipro acts as the antenna molecule for the europium excitation. It is likely that the europium metal is bound with Cipro by the two vacant co-ordination sites of the keto and carboxylate groups. Upon binding Cipro, the coordination sites of Eu^{3+} in Eu-Lc are saturated and thus there are no available coordination sites for water molecules. The result suggested that the Förster's resonance energy transfer from Cipro to europium is occurred. The efficient energy transfer could be rationalized by the higher extent of overlap between the emission spectra of the Cipro and the absorption spectra of the Eu(III)-complex, possibly owing to the relatively lower energy gap between the triplet state of Cipro and that of Eu^{3+}. The resultant ternary complex, Cipro-bound Eu-Lc shows the best performance, showing enhanced sensitized emission of Eu(III) ion at emission wavelength = 615 nm (**Figure 3**). The

sensing mechanism is shown in **figure 5**.

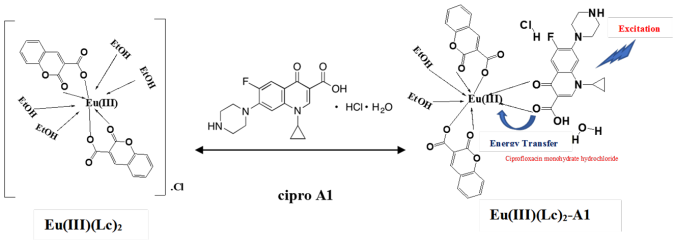

Figure 5: A sensing mechanism of Cipro with Eu(III)$(Lc)_2$ complex.

3.2.1. *Effect of solvents*

The influence of the different solvents on the fluorescence intensities of the Eu(III) complex in the presence of ciprofloxacin has been investigated as shown in Figure (6). Solvents have a remarkable effect on the fluorescence intensity of the considered Eu(III)-$(Lc)_2$ complex. The solvents used here are ethanol, methanol, water, acetonitrile and THF. The fluorescence intensities of Eu(III)–$(Lc)_2$ in different solvents at 615 nm are 357.98, 247.16, 70.62, 46.64 and 0.1669 for acetonitrile, ethanol, methanol, THF, and water, respectively. The best optimum solvent for our probe is chosen to be acetonitrile where the strongest energy transfer from the ligand to Eu(III) was observed in acetonitrile [16] as shown in Figure (6).

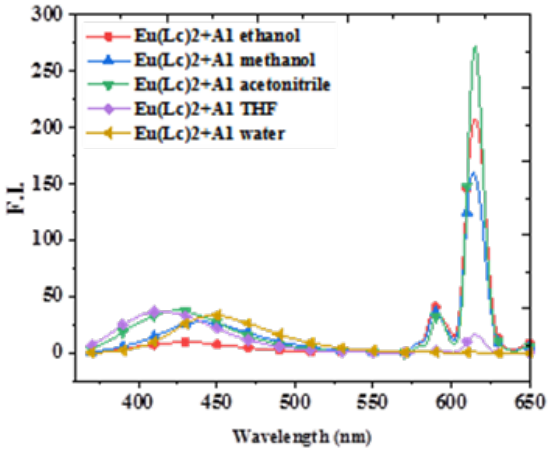

Figure 6: The fluorescence spectra of 1×10^{-5} M Eu(III)-$(Lc)_2$+ 1×10^{-5} Cipro (A1) in different solvents at room temperature with excitation wavelength at 350 nm

3.2.2. *Absorption spectroscopy of Eu(III) -$(Lc)_2$ complex with Ciprofloxacin antibiotic*

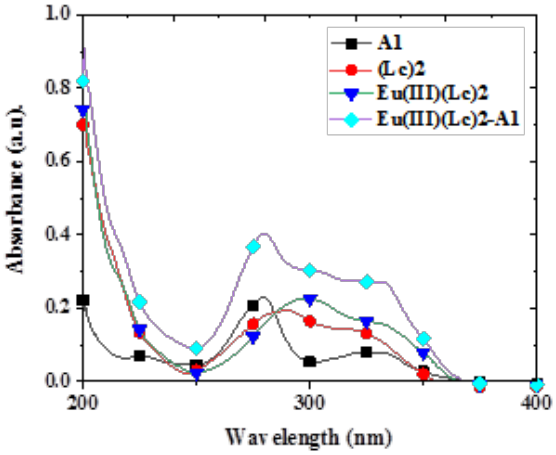

Figure 7: Absorption spectra of the Eu (III)$(Lc)_2$ complex in the absence and presence of $1x10^{-5}$ M Cipro (A1) in acetonitrile at room temperature.

Electronic absorption spectroscopy is one of the most useful techniques for studying the binding mode of metal complexes. The UV-vis absorption spectra of the Eu(III)-$(Lc)_2$ complex in the presence of antibiotic Ciprofloxacin monohydrate hydrochloride (A1) was studied in acetonitrile. The absorption band of Eu(III)-$(Lc)_2$ complex has been enhanced with a blue shift by the addition of antibiotic in an equal molar ratio, which indicates the binding between Eu(III)-$(Lc)_2$ complex and the examined antibiotic as shown in Figure (7). This reaction can be expressed according to the following expression:

Eu (III)-$(Lc)_2$ complex + A1 → Eu (III)-$(Lc)_2$-A1

3.2.3. *Effect of the Ciprofloxacin on the fluorescence intensity of Eu(III) -$(Lc)_2$ complex*

The above-mentioned findings prompted us to study the effect of Ciprofloxacin on the fluorescence intensity of Eu(III)–$(Lc)_2$ complex in acetonitrile. The fluorescence intensity of Eu(III)–$(Lc)_2$ complex at 615 nm was enhanced remarkably as the concentration Ciprofloxacin was increased (as shown in figure 8).

Figure (8) show the calibration plot of the antibiotic (Ciprofloxacin monohydrate hydrochloride)

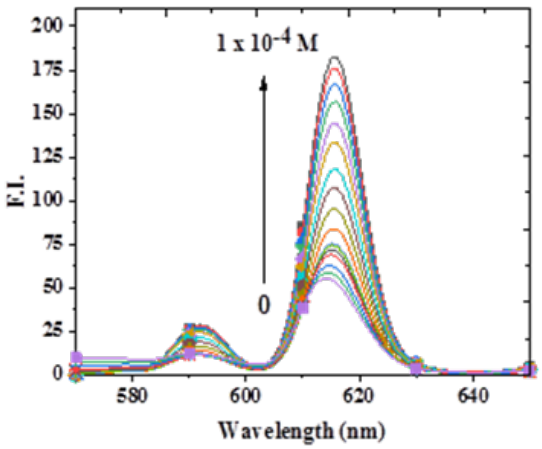

Figure 8: Effect of addition of Cipro (A1) on emission spectra of 1×10^{-5} M Eu(III)-$(Lc)_2$ complex in acetonitrile.

assay with Eu(III)-$(Lc)_2$ complex in acetonitrile. The linear range of the plot is described by the relation:

F=50 +65 [A] Equation (1)

where F is the fluorescence intensities of Eu(III)-$(Lc)_2$ complex. The linear range was 0.025-67.4 μM with correlation coefficients (R^2) equal 0.9501. The limits of detection (LODs) have been determined based on calibration curves using LOD = 3σ/slope. The LODs is found of 5.06 μM and limit of quantitation (LOQ) has been determined using LOQ = 10σ/slope is 12 μM.

The results of the present work indicated that the Eu (III)–$(Lc)_2$ complex formed containing two (Lc) moieties, can be modulated by changes in the coordinating ligand environment during the interaction of the ciprofloxacin. The luminescence emission of this complex was found to be modulated to different degrees when interacting with this antibiotic. This can be considered as a model for sensor for detecting ciprofloxacin.

Conclusion

This work describes the application of Eu(III)-$(Lc)_2$ complex as a probe for detection ciprofloxacin antibiotics using luminescence. A characteristic emission band of Eu^{3+} was enhanced in the presence of ciprofloxacin. The best optimum solvent for our probe is chosen to be acetonitrile. The linear range is 0.025-67.4 μM. The detection limit was 5.06 μM whereas the quantitation limit was 12 μM. This method has many advantages as it cheap, rapid direct for determination of the ciprofloxacin.

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