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Short communication

A new homoleptic coordination compound of ruthenium and norfloxacin and its interaction with human serum albumin

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ABSTRACT

This work presents a new coordination compound of ruthenium and the norfloxacin antibiotics, as well as its interaction with human serum albumin (HSA). The ESI HRMS spectrum of [Ru(nor)$_3$] (nor = norfloxacinate, $C_{16}H_{17}FN_3O_3^-$) displayed the protonated molecular ion at *m/z* 1057.3, while the HRMS/MS spectra displayed peaks at *m/z* 958.4 and 639.3 assigned to the gaseous phase norfloxacin adducts $(Hnor)_3 \cdot H^+$ and $(Hnor)_2 \cdot H^+$. Coordination to Ru(III) remarkably enhances the water solubility of norfloxacin. The complex absorption spectrum in aqueous medium is dominated by the norfloxacin transitions in the UV region but also displays a broad shoulder around 370 nm, which possibly overlaps with ligand-field transitions. Stern–Volmer analysis of human serum albumin (HSA) fluorescence quenching by [Ru(nor)$_3$] showed that both dynamic and static mechanisms contribute to the quenching. Lifetime measurements demonstrated that the predominance of one pathway over the other depends on temperature variations. Finally, the analysis of thermodynamic parameters leads to the conclusions that the interaction of the novel complex with HSA is spontaneous and that this interaction occurs through the formation of hydrogen bonds and van der Waals forces ($\Delta H = -259.2$ kJ/mol and $\Delta S = -779.9$ kJ/mol·K).

The fluoroquinolones belong to one of the greatest class of antimicrobial agents used nowadays. They have applications both in human and in veterinary medicine, since they act against Gram-positive and Gram-negative organisms [1–7]. Last generation fluoroquinolones can act also against anaerobic bacteria [8,9]. The fluoroquinolones differ from quinolones by the presence of a fluorine atom at position 6 and a piperazinyl group. The introduction of fluorine leads to a broader spectrum of action, having an antibacterial activity 1000-fold higher than that observed for nalidixic acid, its predecessor [10,11].

These drugs are able to chelate metal ions through their carboxylate and pyridone groups [12], originating stable complexes [13–19]. Fluoroquinolones metallic complexes combined with ruthenium have good clinical application, since they present suitable pharmacological activity and interactions with relevant biomolecules such as DNA and low toxicity. These positive biological features of ruthenium complexes have been explained partially by ruthenium ability to mimic iron in biological environment and also due to its rich coordination chemistry, in terms of accessible oxidation states, rates of hydrolysis and photochemistry [20–27].

Albumin is the most abundant protein in the plasma. Therefore, any administered drug will interact with this macromolecule to some extent, which would fundamentally determine its bioavailability and toxicology [28–30].

Recently this research group has reported on the parent compound [Ru(cipro)$_3$] (cipro = ciprofloxacinate) and its perspectives to be used as a metalo-drug [22]. In this context, the aim of this study is to expand the available data on homoleptic ruthenium coordination compounds with fluoroquinolones by reporting the synthesis and characterization of the new complex [Ru(nor)$_3$] (nor = norfloxacinate, $C_{16}H_{17}FN_3O_3^-$), Fig. 1. Besides that, [Ru(nor)$_3$] interaction with human serum albumin (HSA) is also described. To this end, we have performed the investigation of HSA fluorescence quenching, analyzed by the Stern–Volmer model and life-time measurements. We also present the dependence of the quenching with temperature and, consequently, the preferential interaction mode between complex and protein.

In order to synthesize the molecule of interest, all reactants were commercially available (Sigma-Aldrich) and were used without further purification. The synthesis was performed according to Chattah *et al.* [31]. In a 25 mL flask 0.1094 g of $RuCl_3$ (0.48 mmol) and 0.4474 g of norfloxacin (1.4 mmol) were dissolved in 15 ml of water. The mixture was kept under magnetic stirring and heated to reflux for 3 h. The solution was cooled to room temperature and filtered. Then, 200 ml of acetone was added to the filtrate in order to precipitate a

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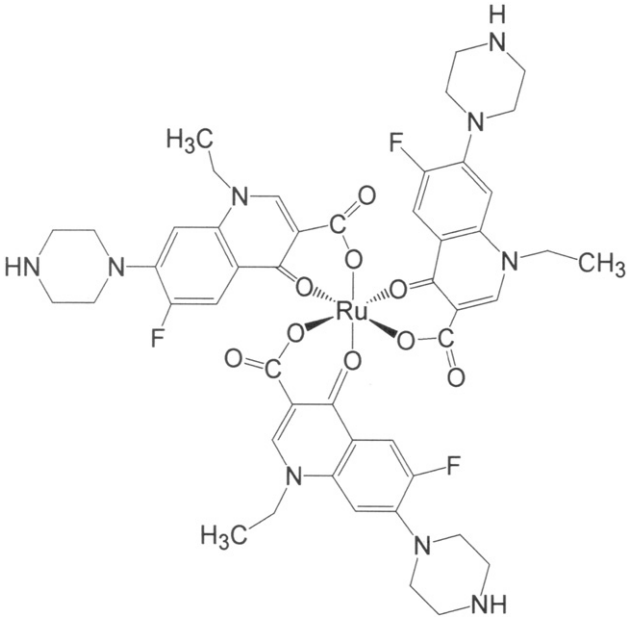

Fig. 1. Proposed structure for $[Ru(nor)_3]$.

brown solid. The crude product was isolated by filtration, dissolved in a minimum volume of water and then purified by exclusion chromatography in a Sephadex G-10 column. This procedure yielded m = 0.0517 g (η = 10.2%). $[Ru(C_{16}H_{17}FN_3O_3)_3]$: calcd. H 4.9, C 54.5, N 11.9; found H 5.0, C 53.1, N 12.1.

High resolution mass spectra (ESI–HRMS and MS/MS) were collected in positive mode with a Bruker Daltonics spectrometer (Billerica, MA, USA), model ultrOTOFQ–ESI–TOF, with an infusion pump flow of 300 μL/h and a 1 H_2O:1 CH_3CN mixture as mobile phase. Gentle fragmentation of the molecular ion was performed with 15 eV. Electronic spectra were recorded using quartz cuvettes of 1 cm optical path with a Hitach UV–visible spectrophotometer model U-3501. Infrared spectra were recorded using KBr pellets containing the sample of interest in the 4000–400 cm^{-1} region in a Shimadzu FTIR spectrophotometer, model Prestige-21.

Stock solutions of HSA (Sigma-Aldrich, 1.0×10^{-4} mol L^{-1}) and $[Ru(nor)_3]$ (1.0×10^{-6} mol L^{-1}) were prepared before each experiment in phosphate buffer (PBS), pH = 7.4. Fluorescence emission spectra were recorded on a Shimadzu fluorescence spectrophotometer model RF-5301PC using a quartz cell with 1.0 cm optical path. 3.0 ml of HSA (1.0×10^{-6} mol L^{-1}) was added to the quartz cell where successive aliquots of complex solution (1.0×10^{-6} mol L^{-1}) were added. Measurements were made for three incubation temperatures (298, 303 and 308 K). The incubation time, after each addition of $[Ru(nor)_3]$, was 5 min. The wavelength of excitation was set to 280 nm and the fluorescence emission was recorded in the range between 300 and 550 nm. The widths of the excitation and emission slits were 5 nm. Control experiments were performed, in which PBS 5×10^{-6} mol L^{-1} solutions of $[Ru(nor)_3]$ were excited at 275 nm and 345 nm, (excitation and emission slits = 10 nm) and its emission was recorded in the range between 300 and 600 nm. It was necessary to correct the intensity of fluorescence to eliminate the inner filter effect of HSA and complex solution, according to the equation [32]:

$$F_{corrected} = F_{observed} e^{\frac{(A_{emission}+A_{excitation})}{2}} \quad (1)$$

where $F_{corrected}$ and $F_{observed}$ are the corrected and observed fluorescence intensities, respectively. $A_{excitation}$ and $A_{emission}$ are the absorbance values of the drugs at the excitation and emission wavelengths, respectively. Lifetime measurements were recorded on Tsunami - Spectra Physics, the selected wavelength of excitation was 281 nm at two incubation temperatures (298 and 310 K). Into a solution of HSA (1.0×10^{-6} mol L^{-1}) it was added successive aliquots of $[Ru(nor)_3]$ solution (0; 0.75; 1.5; 2.25; 3.0; 6.0×10^{-5} mol L^{-1}), both prepared in PBS. The detected signal as pulse excitation is called IRF (instrument response function). The resulting curves were fit by an exponential adjust of Origin 8.0® program.

Typically, metal complexes combined with fluoroquinolones had their structure characterized by means of X-ray spectroscopy [4,6,7,13,14]. In our case, in the lack of a suitable crystal, we performed the structural characterization of $[Ru(nor)_3]$ using ESI–HRMS spectrometry and IR spectroscopy. Fig. 2 presents the match between the observed isotopic pattern for the protonated molecular-ion $[Ru(nor)_3]\cdot H^+$ and the theoretical prediction.

In the ESI–HRMS spectrum of complex $[Ru(nor)_3]$, there is a cluster of isotopologue ions centered at *m/z* 1057.3, assigned to the protonated complex $[Ru(nor)_3]\cdot H^+$. The isotopic multiplicity is characteristic of the presence of the ruthenium atom, which is a multiple isotope element (^{104}Ru (18.7%), ^{102}Ru (31.6%), ^{101}Ru (17.0%), ^{100}Ru (12.6%), ^{99}Ru (12.7%), ^{98}Ru (1.88%) and ^{96}Ru (5.52%)) [33]. The $\Delta m/z$ value is one, confirming the +1 charge for this fragment. Interestingly, in the MS/MS spectrum of $[Ru(nor)_3]$, the most intense peaks observed are centered at *m/z* 958.4 and 639.3 and their isotopic pattern matches with that of carbon [33]. This behavior has already been observed by us earlier, for the parent compound $[Ru(cipro)_3]$ [22]. These peaks were assigned as the monovalent species formed by the protonation of a gaseous phase adduct of three ($Hnor_3\cdot H^+$) and two ($Hnor_2\cdot H^+$) molecules of norfloxacin, respectively. The expansion of the peaks centered at *m/z* 958.4 and 639.3 are available as Supplemental Information.

Quinolones and fluoroquinolones have complex spectra in the infrared region, due to the presence of different functional groups in their structure. The most studied bands are the ones corresponding to the carbonyl stretches of the pyridone $\upsilon(C=O)$ around 1630 cm^{-1} and of the carboxylic acid $\upsilon(COOH)$, around 1725 cm^{-1} [34]. This last band tends to disappear in metal complexes with quinolones because, in order to coordinate to the metal ion, the quinolone carboxylic group is supposed to be deprotonated. This idea is supported by X-ray crystallography studies of some quinolone–metal complexes which show that the majority of these complexes have the quinolone coordinated to the metal ion through the carbonyl oxygen of the pyridone and other carbonyl oxygen atom of the carboxylate group [4,35,36]. However, in

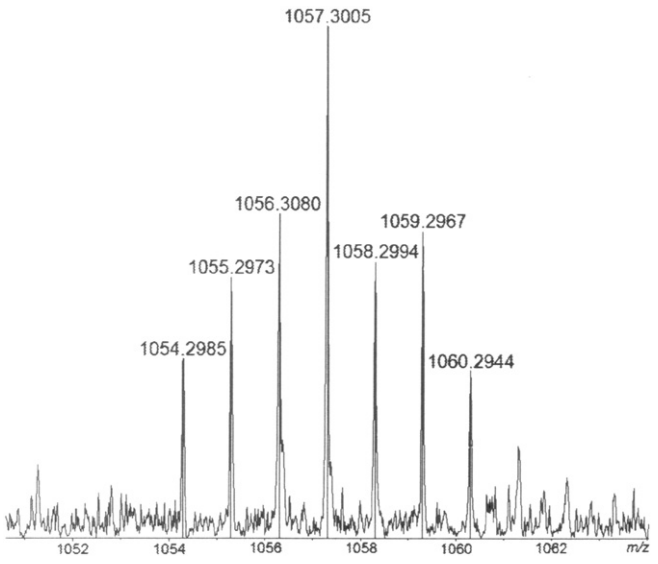

Fig. 2. Molecular ion observed for $[Ru(nor)_3]\cdot H^+$ in the ESI–HRMS spectrum, recorded from a 1 H_2O:1 CH_nCN mixture, overlaid with the theoretical prediction for its isotopic pattern.