Complex Compounds of Sm(III) with Chlorhexidine

Synthesis, characterization, luminescent properties and antibacterial activity

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Three new samarium(III) complex compounds with chlorhexidine as ligand and mixed ligands chlorhexidine/o-phenanthroline have been prepared and characterized by elemental and thermogravimetrical analyses, infrared, electronic and luminescence spectra. The complexes corresponded to the formulas: $[Sm(CHX)(NO_3)_2]'NO_3$, $[Sm(CHX)(o\text{-}phen)_2]'(NO_3)_3$ and $[Sm_2(CHX)(o\text{-}phen)_2(NO_3)_4]\times(NO_3)_2$, where CHX was the chlorhexidine. Chlorhexidine acted as neutral tetradentate NNNN donor, coordinating through the four imine nitrogen atoms. The two mixed ligands complexes showed a strong luminescent emission in solid state, characteristic of samarium(III) ion. The metal complexes and the chlorhexidine diacetate were in vitro evaluated for their antimicrobial activity against two Gram negative bacteria. The results revealed that all compounds were very effective in reducing the bacterial growth rate, even at low concentration.

Keywords: Lanthanides, samarium, luminescence, biguanides, chlorhexidine.

Biguanide derivatives are a very important class of drugs, due to their antimalarial, antidiabetic, antimicrobial and antifungal properties [1-4]. The biguanides have a remarkable ability to form chelates with transition metal ions. This interaction is of the greatest importance for coordinative chemistry and many studies have been devoted to this field [5-9].

The most used antimicrobial agent of the biguanide class is chlorhexidine, 1,1'-hexamethylene-bis-[5-(*p*-chlorophenyl)-biguanide] (fig. 1), especially as an active ingredient in mouthwash for plaque and other oral bacteria control [10,11]. Chlorhexidine has been also used in non-dental applications, such as general skin and surface cleansing, instrument sterilization and pre-operative skin preparation [12].

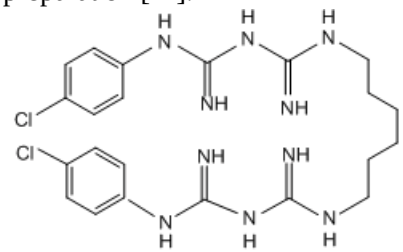

Fig.1. Chlorhexidine (CHX)

In recent years, many studies have focused on increasing the effectiveness of chlorhexidine when combined with metal ions. Thus, several studies have demonstrated synergistic inhibitory effect of some metal ions, such as Zn^{2+}, Ag^{+}, Cu^{2+} or Sn^{2+} with chlorhexidine on various bacteria and fungi [13-16].

In our previous works, we have reported the synthesis and the characterization of some copper(II), zinc(II) and silver(I) complex compounds of chlorhexidine in 1:1 and 2:1 metal:ligand ratio [17-20]. The antimicrobial screening *in vitro* has shown an increase of activity for most complexes comparatively with chlorhexidine.

The aims of the present study were the synthesis and the characterization of new complex compounds of samarium(III) with chlorhexidine diacetate. It has been known that lanthanide ions coordinate to some biological compounds such as flavonoids [21,22] or vital drugs (metformin) [23]. Literature survey revealed the absence of any reports on the interaction of chlorhexidine with lanthanide ions. Such complexes may be important for their antimicrobial action and also for their possible fluorescent properties. Taking into account these aspects, we reported in this paper the synthesis, characterization, antimicrobial studies and luminescent properties of three complex compounds of samarium(III) with chlorhexidine. In order to improve the luminescent properties, two metal complexes have mixed ligand such as chlorhexidine and *o*-phenanthroline.

Experimental part

Materials and physico-chemical analyses

All the chemical used were of reagent grade and were purchased from Sigma-Aldrich and Merck. The metal content was gravimetrically detected, as samarium(III) oxide, Sm_2O_3. Carbon, hydrogen and nitrogen were detected using a Euro EA Elemental analyzer. Thermogravimetric analysis was carried out in static air atmosphere, at a heating rate of 10°C/min, using a Perkin Elmer STA 6000 derivatograph. Infrared spectra (in KBr pellets) were recorded on a BIORAD FTIR 135 spectrophotometer, in the range 4000-400 cm^{-1}. UV-Vis diffuse reflectance spectra were registered on a UV-Vis Jasco 650 spectrophotometer, in the range 200-900 nm. Fluorescence measurements were made on a Jasco FP 6500 spectrofluorimeter, on solid sample.

Antibacterial analysis

The *in vitro* biological effects of the ligand and its Sm(III) complexes were monitored by the growth inhibition tests on two bacterial species, *Escherichia coli* (ATCC 25922) and *Citrobacter freundii* (ATCC 8090), purchased from ATCC. Bacterial g*rowth medium*, lauryl sulphate broth, was purchased from National Research and Development Cantacuzino (Bucharest, Romania). Every bacterial strain

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was initially grown on nutrient agar plates O/N at 37 °C, then a single colony was grown in the nutrient broth to a density of 1 OD at an absorbance of 600 nm (OD600nm). The bacterial growth inhibition test was performed in 96 wells plate in presence or in absence of chemical compounds at various concentrations (ranging from 0 to 50mM). Bacterial growing rate was monitored by spectrometry at an absorbance of 600nm (A_{600nm}) using Clariostar Microplate Reader (BMG Labtech GmbH).

The toxic effect of chemical compounds was quantified in function of bacterial growing rate compared to control samples, no compounds treatment. Each microbiological step had a positive and negative control to ensure quality outcomes and efficiency of working methods.

Sm(III) compounds were solubilized in ethanol, stock solutions of 0.25M.

Synthesis of metal complexes

[Sm(CHX)$(NO_3)_2$]' NO_3 (complex **1**): 0.6435 g (1 mmol) of chlorhexidine diacetate monohydrate were dissolved in 30 mL ethanol, with stirring. To this solution was added 0.4445 g (1 mmol) of $Sm(NO_3)_3 \times 6H_2O$ and the resulting solution was stirred at 35-40 °C for 2 h. The white precipitate obtained was filtered off, washed with ethanol and ether and dried in air. mp = 216°C.

[Sm(CHX)(*o*-phen)$_2$]' $(NO_3)_3$ (complex **2**) and $[Sm_2(CHX)(o\text{-phen})_2(NO_3)_4] \times (NO_3)_2$ (complex **3**) were prepared as follows: 0.6435 g (1 mmol) of chlorhexidine diacetate monohydrate were mixed with 0.3600 g (2 mmol) of *o*-phenanthroline in 50 mL ethanol. A solution of $Sm(NO_3)_3 \times 6H_2O$ in ethanol (0.4445 g, 1 mmol for the complex **2** and 0.8890 g, 2 mmol for the complex **3**) was added to the mixture of the ligands and the resulting solutions were stirred at 40-50 °C for 2 h, when solid white products were separated out. These were filtered off, washed with ethanol and ether and dried at air. Complex **2**: mp = 220°C; complex **3**: mp = 231C (decomp).

Results and discussions

The results of the elemental analysis and the proposed formulas based on analytical, thermogravimetrical and IR spectral data were presented in table 1.

All these complexes were partly soluble in acetone, ethanol and acetonitrile, easily soluble in dimethyl-formamide (DMF) and dimethyl sulfoxide (DMSO). They were quite stable at air, at room temperature and could be stored for several months.

Infrared spectra

The assignments of the important infrared bands of the chlorhexidine diacetate monohydrate and its samarium(III) complexes were listed in table 2.

Compound	Analysis Found / (Calculated) %			
	C	H	N	Sm
$[Sm(CHX)(NO_3)_2]\cdot NO_3$	31.04 (31.37)	3.60 (3.56)	21.40 (21.62)	17.61 (17.83)
$[Sm(CHX)(o\text{-phen})_2]\cdot(NO_3)_3$	45.52 (45.94)	3.90 (3.83)	19.41 (19.81)	12.05 (12.48)
$[Sm_2(CHX)(o\text{-phen})_2(NO_3)_4]\cdot(NO_3)_2$	35.44 (35.91)	3.15 (2.99)	18.02 (18.21)	19.51 (19.51)

Table 1
ANALYTICAL DATA OF THE METAL COMPLEXES

Assignments	Chlorhexidine	(1)	(2)	(3)
ν(OH) H_2O	~3400 m	-	-	-
ν(NH) Alkyl-NH-Aryl $(Alkyl)_2NH$	3338 s	3334 m	3335 m	3340 m
ν(=NH)	3181 s	3222 m	3224 m	3220 m
ν_{as} (NH_2^+) ν_{sym} (NH_2^+)	3140 m	-	-	-
ν(C=N)	1644 s	1635 vs	1634 s	1626 s
δ(NH_2^+)	1613 m	-	-	-
ν_{as}(COO)	1549 s	-	-	-
ν_{sym}(COO)	1417 s	-	-	-
δ(NH) + ν(C-N)	1574 s 1337 m	1580 m 1349 m	1579 m 1350 m	1590 m 1348 m

Table 2
CHARACTERISTIC BANDS IN THE IR SPECTRA OF CHLORHEXIDINE DIACETATE MONOHYDRATE AND ITS SAMARIUM(III) COMPLEXES ($\tilde{\nu}_{max}$, cm^{-1})

$\nu(C=C)_{arom.}$	1536 vs 1491 s	1531 vs 1493 m	1530 vs 1492 s 1457 m (*o*-phen) 1418 m (*o*-phen)	1540 s 1485 vs 1425 vs (*o*-phen)
$\nu(C_{aliph}-N)$	1249 m	1251 m	1253 m	1257 m
$\nu(NO_3)$	-	1383 vs (NO_3^- ionic) 1020 m (ν_2) 1348 m (ν_1) 1568 m (ν_5) (NO_3^- bidentate)	1384 vs (NO_3^- ionic)	1384 vs (NO_3^- ionic) 1030 w (ν_2) 1306 s (ν_1) 1490 s (ν_5) (NO_3^- bidentate)
ν(Sm-O)	-	630 w	-	639 w
ν(Sm-N)	-	615 w	620 w	615 w

Table 2
CONTINUATED

The infrared spectrum of chlorhexidine diacetate monohydrate showed many absorptions bands in the range of high wave numbers, due to the stretching vibrations N-H of the groups Alkyl-NH-Aryl and $(Alkyl)_2NH$ (at 3338 cm^{-1}) and to the stretching vibration of the group =NH (at 3181 cm^{-1}) [17,19,24]. Other important bands appearing in the same region were assigned to ν(OH) absorption (~3400 cm^{-1}) and to the symmetric and asymmetric N-H stretching modes of NH_2^+ group, at 3140 cm^{-1} [17,19,24]. The presence of this last band was in accordance with the protonation of the chlorhexidine in its diacetate salt, in solid state.

The strong absorption band, at 1644 cm^{-1}, could be assigned to the stretching vibration of the imine function, n(C=N) [19,25]. This band was expected to be strongly affected by coordination.

The bands occurring at 1574 and 1337 cm^{-1} could be attributed to d(NH) + ν(C-N), while the medium absorption band, at 1249 cm^{-1}, was due to $\nu(C_{aliph}-N)$ [26].

The infrared spectrum of the chlorhexidine diacetate also showed bands due to the stretching vibrations of the acetate group: 1549 cm^{-1} - ν_{as}(COO) and 1417 cm^{-1} - ν_{sym}(COO) [27].

The comparison between the IR absorption bands of the complex compounds and those of chlorhexidine diacetate provided information regarding the nature of groups involved in coordination to the metal ion.

The most obvious change in the IR spectra of the complexes was the shift toward lower wave numbers of the band assigned to ν(C=N), indicating the coordination of the imine nitrogen atoms to the metal ion [24,26]. This hypothesis was also supported by the shift of the bands due to the coupling vibrations δ(NH) + ν(C-N) and the strong upward shift of the stretching vibrations ν(=NH) [28,29].

The band due to the stretching vibrations of NH_2^+ group disappeared in the IR spectra of the complexes, in according to the deprotonation of the chlorhexidine and its involvement in complexation as neutral ligand [28]. Normally, the wave number of C=N stretching vibration should increase as a consequence of deprotonation. The negative shift of this band was an indicative of a strong metal-ligand bond.

The very strong band, appearing at 1383-1384 cm^{-1} in the spectra of all the complexes was due to the stretching vibrations of ionic nitrate [27]. The IR spectra of the complexes (**1**) and (**3**) showed also characteristic bands of coordinated nitrate, in the regions 1020-1030 cm^{-1} (ν_2), 1348-1306 cm^{-1} (ν_1) and 1568-1490 cm^{-1} (ν_5) [27]. The separation of two highest frequency bands, ν_5 - ν_1 was approximatively 220/184 cm^{-1}, in accordance with the participation of the nitrate as bidentate ligand [27].

The IR spectra of the complexes showed supplementary bands at low wave numbers, which could be assigned to ν(Sm-N) and ν(Sm-O) [27].

The free n(OH) band of crystalline water was observed in the IR spectrum of chlorhexidine diacetate monohydrate around 3400 cm^{-1}, but a not corresponding band was observed in the spectra of the metal complexes. The absence of the lattice or coordination water was also confirmed by the thermal analysis of the complexes.

On the base of the IR spectra we can conclude that chlorhexidine acts as neutral tetradentate NNNN donor ligand in all the three complexes, coordinating through the four imine nitrogen atoms. It was also confirmed the presence of ionic nitrate in all the complexes and coordinated nitrate in the complexes (**1**) and (**3**).

Thermal analysis

The results concerning the thermal decomposition of complex compounds were summarized in the table 3.

The complexes were stable up to 200°C, which was a proof of absence of any crystalline or coordinated water.

The thermal decomposition of the complex (**1**) undergoes in three steps. The first decomposition step, an exothermic one, in the temperature range of 200-270°C, with DTG peak at 250°C, corresponded to ionic nitrate decomposition [30]. The second stage was related to the decomposition of coordinated nitrate and takes place in the temperature range of 290-320°C, with a maximum rate at 300°C. The last strong exothermic decomposition step corresponded to the oxidative degradation of chlorhexidine ligand [19]. The mass losses observed for each step were backed up by the calculated values. The final residue of decomposition was Sm_2O_3 and the metal percentage

Table 3
THERMAL DECOMPOSITION DATA FOR Sm(III) COMPLEXES WITH CHLORHEXIDINE

Complex	Step	Thermal effect	Temperature range (°C)	$\%\Delta m_{exp}$	$\%\Delta m_{calc}$	Chemical process
1	I	Exothermic	200-270	7.12	7.37	NO_3 ionic loss
	II	Exothermic	290-320	15.23	14.74	$2NO_3$ coordinated loss
	III	Exothermic	320-490	58.10	57.21	oxidative degradation of CHX
2	I	Exothermic	220-290	15.26	15.48	$3NO_3$ ionic loss
	II	Exothermic	310-430	14.65	42.04	oxidative degradation of CHX
	III	Exothermic	430-590	29.44	29.97	Oxidative degradation of *o*-phen
3	I	Exothermic	210-300	15.86	16.12	$2NO_3$ ionic, $2NO_3$ coordinated loss
	II	Exothermic	300-375	41.40	40.91	$2NO_3$ coordinated, oxidative degradation of CHX
	III + IV	Exothermic	375-480, 480-600	10.07 + 12.58 = 23.42	22.65	Oxidative degradation of *o*-phen

detected from this was in according to the theoretically metal content.

The complex (**2**) started to decompose at 220°C, with the decomposition and the loss of ionic nitrate [30]. The next step of thermal decomposition was related to the oxidative degradation of chlorhexidine ligand and undergoes over a temperature range of 310-430°C, accompanied by a strong exothermic peak. The last step was a complex one and took place at a temperature range of 430-590 °C. According to the literature data, this step can be related to oxidative degradation of *o*-phenanthroline [31].

For the complex (**3**) the first exothermic stage of thermal decomposition started at 210 °C, with a DTG peak at 254 °C and could be associated with the decomposition and the loss of ionic nitrate and a half of coordinated nitrate. The second step, in the temperature range of 300-375 °C, corresponds to the loss of remaining coordination nitrate and oxidative degradation of CHX. The last decomposition step, consisting in two defined processes, could be associated with the oxidative degradation of *o*-phenanthroline [31].

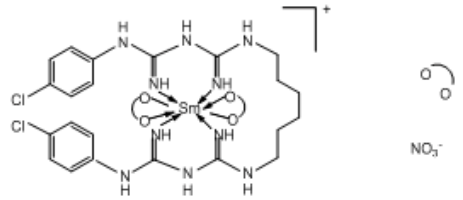
According to the determinations presented above, the following structures have been proposed for the complexes (fig. 2).

Electronic spectral data

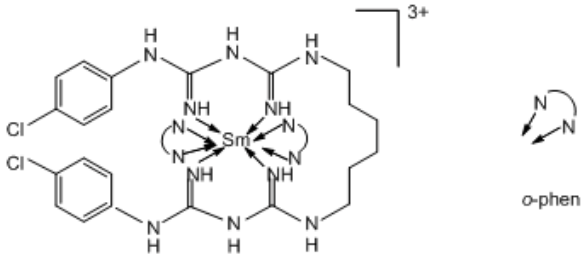
For all the compounds, the electronic spectra were recorded in solid state.

The chlorhexidine diacetate monohydrate exhibited strong absorption bands in the UV region, with peaks at 208 nm (48100 cm^{-1}), 255 nm (39215 cm^{-1}), 298 nm (33500 cm^{-1}) and 344 nm (29000 cm^{-1}), which could be assigned to n - σ*, π - π * and n - π * transitions, respectively [17-19].

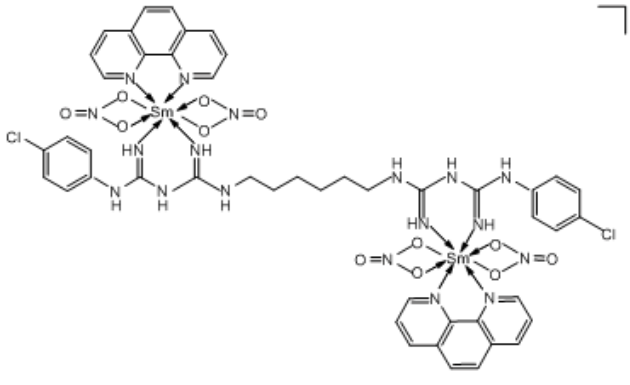
The UV-Vis spectra of Sm(III) complexes were remarkably different from that of the chlorhexidine ligand. The strong absorption bands in the ultraviolet region, characteristic of the ligand, were slightly shifted to higher or lower energy, as result of the complexation. The shoulders observed in the spectra of the complexes with mixed ligands around 240 nm were due to the absorption of *o*-phenanthroline [32]. In addition, the weak absorption bands, in the visible region, may be attributted to *f-f* transitions of Sm^{3+} (table 4) [33-35].



$[Sm(CHX)(NO_3)_2]\cdot NO_3$ (complex **1**)



$[Sm(CHX)(o\text{-phen})_2]\cdot(NO_3)_3$ (complex **2**)



$[Sm_2(CHX)(o\text{-phen})_2(NO_3)_4]\cdot(NO_3)_2$

Fig. 2. Proposed structures for the Sm(III) complex ions

Complex	Absorptions (cm^{-1})	Assignments
$[Sm(CHX)(NO_3)_2]\cdot NO_3$	16730	$^6H_{5/2} \rightarrow {}^4G_{5/2}$
$[Sm(CHX)(o\text{-phen})_2]\cdot(NO_3)_3$	13600 16850 20450	$^6H_{5/2} \rightarrow {}^4F_{11/2}$ $^6H_{5/2} \rightarrow {}^4G_{5/2}$ $^6H_{5/2} \rightarrow {}^4G_{7/2}$
$[Sm_2(CHX)(o\text{-phen})_2(NO_3)_4]\cdot(NO_3)_2$	13500 17100 20830	$^6H_{5/2} \rightarrow {}^4F_{11/2}$ $^6H_{5/2} \rightarrow {}^4G_{5/2}$ $^6H_{5/2} \rightarrow {}^4G_{7/2}$

Table 4
VISIBLE ABSORPTION BANDS FOR SAMARIUM(III) COMPLEXES

Luminescence studies

The emission luminescence spectra were recorded on solid sample; the excitation and the emission slit widths were 5 nm.

The chlorhexidine showed a relative strong and broad emission band, in the range 320-550 nm, with maximum at 390 nm (excitation at 300 nm) (fig. 3).

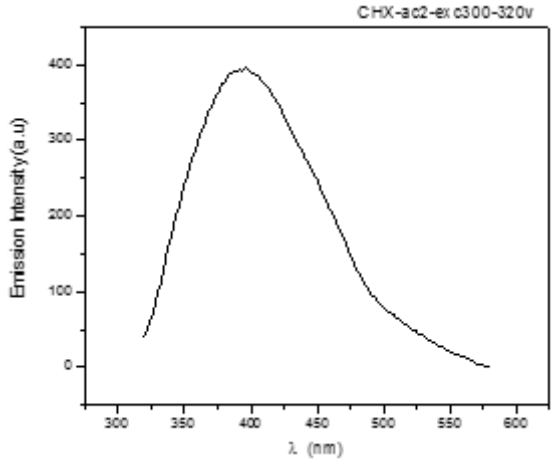


Fig. 3. Emission spectrum of chlorhexidine (λ_{exc} = 300 nm)

It was known that the emission of Sm^{3+} complexes consists of three peaks, in the range 550-650 nm, due to the transitions from the resonant $^4G_{5/2}$ level to ground levels $^6H_{5/2}$, $^6H_{7/2}$ and $^6H_{9/2}$, respectively [33-37].

The fluorescence spectrum of $[Sm(CHX)(NO_3)_2]' NO_3$ showed a broad emission band, with maximum at 430 nm, which was mainly due to the emission of chlorhexidine ligand. This fact pointed out that the ligand did not very effectively protect the metal from chemical environment, although any water molecule was detected in the first coordination sphere of the metal ion.

The emission of the complex $[Sm(CHX)(o\text{-phen})_2]' (NO_3)_3$ was larger than that of chlorhexidine and it expanded at wavelengths greater than 500 nm, where three clearly-defined weak peaks can be found (fig. 4). They can be attributed to the characteristic transition of Sm^{3+}: $^4G_{5/2} \rightarrow {}^6H_{5/2}$ (540 nm), $^4G_{5/2} \rightarrow {}^6H_{7/2}$ (600 nm) and $^4G_{5/2} \rightarrow {}^6H_{9/2}$ (650 nm).

The only compound which shows a typical emission spectrum for Sm^{3+} ion was $[Sm_2(CHX)(o\text{-phen})_2(NO_3)_4]\times(NO_3)_2$ (fig. 5).

The three strong and sharp emission bands observed at 565 nm, 600 nm and 648 nm arise from the transitions: $^4G_{5/2} \rightarrow {}^6H_{5/2}$, $^4G_{5/2} \rightarrow {}^6H_{7/2}$ and $^4G_{5/2} \rightarrow {}^6H_{9/2}$, respectively and any emission from the ligands can be observed in this case. This fact suggests an efficient sensitization process between the ligands and the lanthanide ion, with the ligands acting as an antenna.

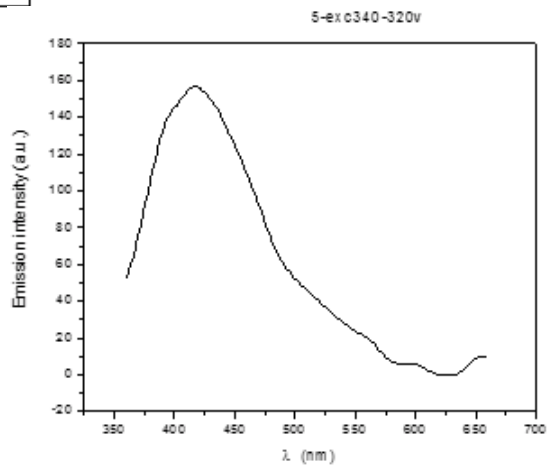


Fig. 4. Emission spectrum of $[Sm(CHX)(o\text{-phen})_2]' (NO_3)_3$ (λ_{exc} = 300 nm)

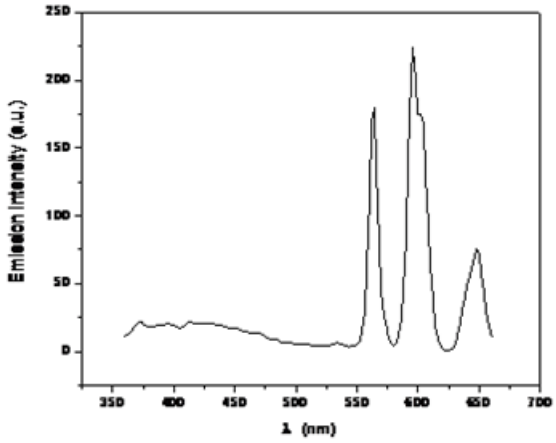


Fig. 5. Emission spectrum of $[Sm_2(CHX)(o\text{-phen})_2(NO_3)_4]\times(NO_3)_2$ (λ_{exc} = 300 nm)

Biological activity

In this study, we analysed the effect of chlorhexidine diacetate and its Sm(III) complexes on two bacterial strains which are naturally present in the environment, *C. freundii* and *E. coli*. The variation of the bacterial growth inhibition over time due to the presence of the complexes or of the chlorhexidine diacetate was shown in figures 6 and 7.

Bacterial growth was monitored by spectrometry at an absorbance at 600 nm up to 6 h and the percent of growth inhibition effect of these compounds was compared to the control (bacterial strains without incubation in presence of the compounds).

The results pointed out that these compounds at very low concentrations such as 1mM and 5mM were very effective in reducing the bacterial growth rate, for both *C. freundii* and *E. coli*, respectively. The 50% inhibition rate was attained very

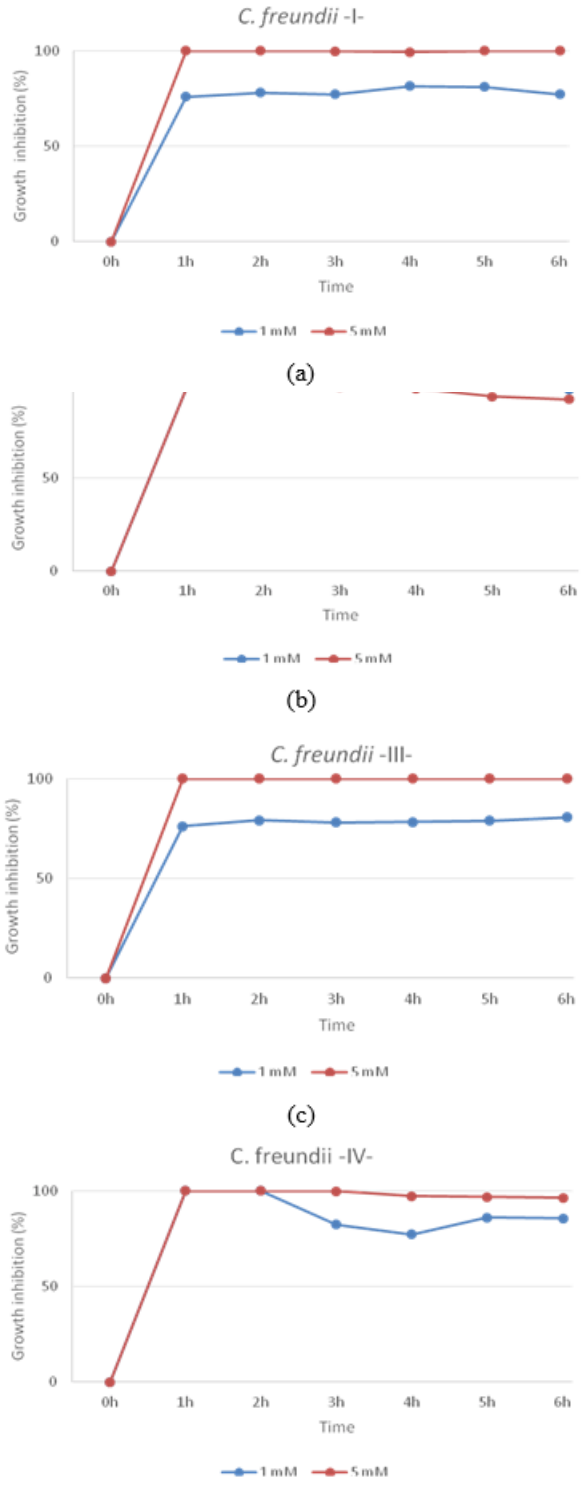


Fig. 6. Variation of growth inhibition (%) over time for *C. freundii* in presence of various concentrations of compounds (1mM and 5mM): (a) complex (**1**); b) complex (**2**); c) complex (**3**); d) chlorhexidine diacetate.

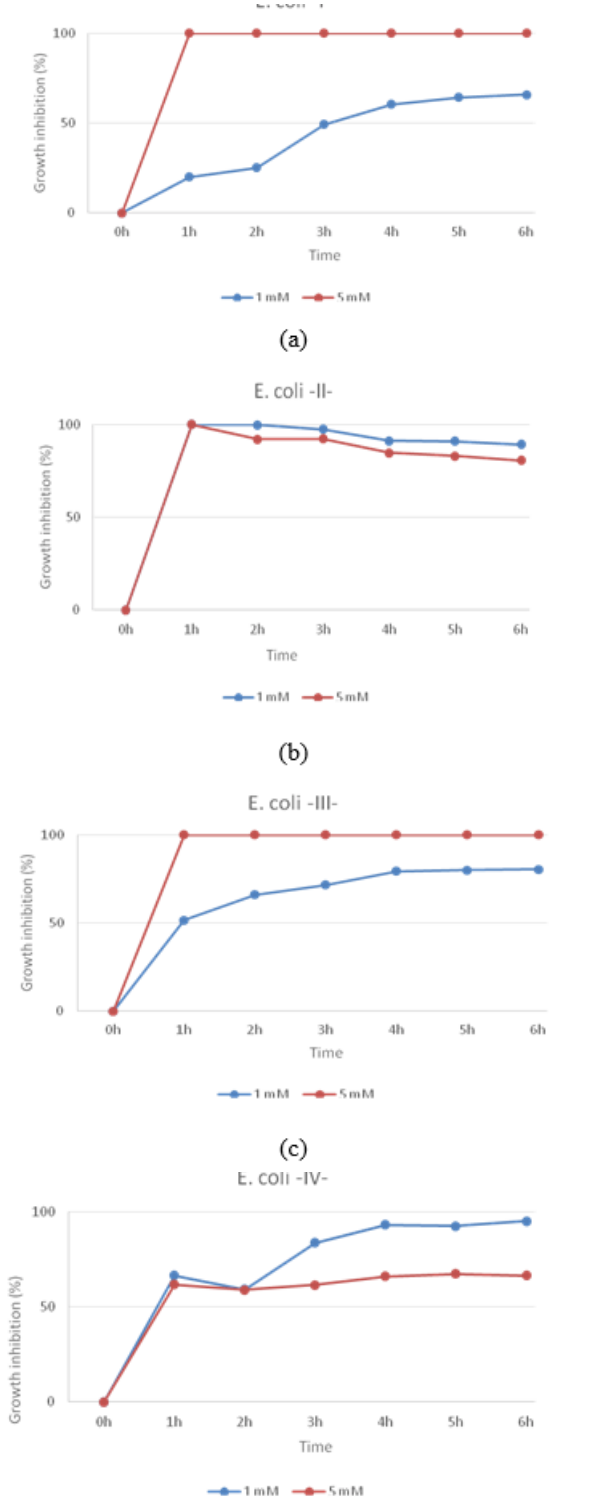


Fig. 7. Variation of growth inhibition (%) over time for *E. coli* in presence of various concentrations of compounds (1mM and 5mM): (a) complex (**1**); b) complex (**2**); c) complex (**3**); d) chlorhexidine diacetate.

fast, after 1h of bacterial growth, with one exception when *E. coli* was incubated in presence of 1mM complex **(1)** for 3h to reach 50% growth inhibition.

Interestingly, *E.coli* incubated in presence of 1 and 5 mM complex **(2)** showed, over time, a decrease of the inhibition growth, suggesting a possible defense mechanism such as the efflux pumps [38].

We also tested 50 mM for all the three complexes, but the results were comparable to 5 mM, since 5 mM was very effective to inhibit the growth rate.

C. freundii growth inhibition revealed almost the same profile for all the three complexes due to their toxic effects, the *E. coli* growth performance in the presence of complex (**2**) showed a great *strive to survive* capacity of bacterial population.

Although, chronic toxicity tests using invertebrates and vertebrates' organisms were used to assess the environmental pressures [39,40], the microbial toxicity evaluation biotests were more and more preferred due to their rapidity and efficiency.

Conclusions

We have prepared three new complex compounds derived from samarium(III) nitrate and chlorhexidine diacetate and *o*-phenanthroline as ligands, in 1:1 and 2:1 metal:chlorhexidine molar ratio. The complex corresponding to 2:1 samarium:chlorhexidine molar ratio, $[Sm_2(CHX)(o\text{-}phen)_2(NO_3)_4]\times(NO_3)_2$, exhibited strong luminescent emission in solid state, characteristic of samarium(III) ion.

These complexes showed a strong antibacterial activity against *C. freundii* and *E. coli*, being very effective in reducing the bacterial growth rate.

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