Synthesis, Characterization and Antimicrobial Evaluation of Lanthanide(III) Complexes with Meloxicam

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Three new complexes of lanthanides (La, Pr, Nd) with meloxicam as ligand of the type $Ln_2(HMel)_2(CH_3COO)_4 \cdot H_2O$ were synthesized. The formula of complexes have been assigned from elemental chemical analysis, IR, UV-Vis-NIR, TG/DSC, magnetic and conductivity data. In all complexes meloxicam acts as bidentate ligand coordinating to the metal ions through the nitrogen atom of the thiazole ring and amidic oxygen atom. All complexes and meloxicam were screened for their antimicrobial activity against 7 microbial strains: Enterococcus faecium E5, Escherichia coli ATCC 25922, Candida albicans 1760, Pseudomonas aeruginosa ATCC 27857, Bacillus subtillis ATCC 6683, Staphylococcus aureus ATCC 6538, Klebsiella pneumoniae IC 13420. The ability of compounds to inhibit the microbial adherence ability to the inert substratum was also evaluated. The results demonstrated that some of the tested compounds exhibit very good antimicrobial and antibiofim activity.

Keywords: meloxicam, lanthanides, coordination compounds, antimicrobial activity

Synthesis of metal complexes containing active drugs as ligands is a research area of increasing interest for bioinorganic, pharmaceutical and medicinal chemistry, the main goal being to develop new antiinflammatory drugs, with a high rate of efficacy and less side effects. The study of these metal complexes is important because of the synergistic action of the beneficial effects of the ligand and the activity of the metal, different active functions combining in the same molecule.

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most common drugs taken worldwide for the treatment of analgesic, antipyretic and anti-inflammatory actions. It is generally accepted that their therapeutic effects are attributed to the inhibition of cyclooxygenase (COX) [1]. Meloxicam [4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide] is an oxicam derivative, used to treat rheumatoid arthritis, osteoarthritis and other joint pains. It is selective COX-2 inhibitor with fewer adverse side effects and has a superior gastrointestinal tolerability [2,3]. Several authors have reported inhibitory effects of meloxicam on colorectal cancer, non-small-cell lung cancer, osteosarcoma cells [4].

A number of transition metals complexes of meloxicam have been reported earlier [2, 5-10], and by screening the literature, no studies concerning the synthesis, characterization and biological activity of meloxicam with lanthanide ions are reported. Lanthanide ion is a subject of increasing interest in bioinorganic and coordination chemistry. A sustained research activity has been devoted to lanthanide complexes, because of their successful application as diagnostic tools in biomedical analysis as MRI contrast agents [11]. Lanthanide complexes have been found to exhibit antitumor and fungicidal properties [12]. In the last years, a big number of lanthanide complexes have been synthesized and their cytotoxicity evaluated. Based on the importance of lanthanide ions, we considered that a study concerning the interaction between meloxicam and lanthanide ions would be interesting.

As a part of our continuing work on the synthesis, characterization and application of metal complexes with nonsteroidal anti-inflammatory drugs [13], herein, we report the synthesis, characterization and *in vitro* antimicrobial activity against seven pathogenic bacteria of three new complexes of lanthanides (La(III), Pr(III), Nd(III)) with meloxicam. The coordination manner of the ligand to the metal centre was investigated by means of FTIR and UV-Vis-NIR spectroscopy, magnetic and conductance measurements, elemental chemical and thermal analysis.

Experimental part

Reagents

$La(CH_3COO)_3 \cdot H_2O$, $Pr(CH_3COO)_3 \cdot xH_2O$, $Nd(CH_3COO)_3 \cdot xH_2O$ (Strem Chemicals, France), meloxicam (Boehringer-Ingelheim, Germany), triethyl-amine (Sigma, Germany), absolute ethanol (Chimreactiv, Romania) were used without further purification. The deprotonation of H_2Mel was realized by the addition of equimolar quantities of warm ethanol solution of H_2Mel and triethylamine. The resulting anions $HMel^{-1}$ were not isolated and were used as solution.

Physico-chemical measurements

Elemental analysis was carried out with a Perkin Elmer CHNS/O Analyzer 2400 Series II. Molar conductance of 10^{-3} M solutions in DMF was measured at room temperature on a Mettler Toledo SevenGo Duo SG23 conductivity meter. The IR spectra were recorded using KBr pellet technique

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on a Jasco FTIR 4100 spectrophotometer in wavenumber region 4000–400 cm^{-1}. Absorption spectra were recorded at room temperature with a JASCO V-670 spectrophotometer. The molar magnetic susceptibilities were measured on powdered samples using the Faraday method. The thermal decomposition of the compounds was followed with a Netzsch TG 449C STA Jupiter. Samples were placed in alumina crucible and heated with 10 °C/min from room temperature to 900 °C, in air.

Synthesis of the complexes

The complexes were prepared according to the following procedure: a hot ethanolic solution of lanthanide(III) acetate (La(III), Pr(III), Nd(III)) (5 mL, 1 mmol) was added to a hot ethanolic solution of deprotonated meloxicam (20 mL, 3 mmol). The resulting clear yellow solution was refluxed under stirring for approximately 3 h. After ~ 10-15 min of mixing a microcrystalline powder started to precipitate. It became increasingly abundant during the refluxing time. The solid compounds were filtered, washed with hot ethanol, and then dried in dessicator under P_4O_{10} at room temperature.

The prepared complexes are stable at ambient temperature, insoluble in water and soluble to a limited extent in methanol and ethanol while freely soluble in DMF and DMSO. The complex compounds were dissolved in DMF and the molar conductivities of 10^{-3} M of their solutions at 18°C were measured. Table 1 shows the molar conductance values of the complexes. The obtained results indicate that these complexes are nonelectrolytes [14].

Antimicrobial activity

The antimicrobial activity was performed against 7 microbial strains: *Enterococcus faecium* E5 (waste water Bucharest), *Escherichia coli* ATCC 25922, *Candida albicans* 1760 (tracheal secretion), *Pseudomonas aeruginosa* ATCC 27857, *Bacillus subtillis* ATCC 6683, *Staphylococcus aureus* ATCC 6538, *Klebsiella pneumoniae* IC 13420, on Mueller–Hinton agar medium.

The compounds (ligand and complexes) were solubilised in DMSO and the starting stock solution was of 10 mg/mL. The qualitative screening of the susceptibility spectra of different microbial strains to the complexes was performed by adapted disk diffusion method. The quantitative assay of minimal inhibitory concentration (MIC) value was performed by the liquid medium serial microdilution method. Sterility control (wells containing only culture medium) and culture controls (wells containing culture medium seeded with the microbial inoculum) were used. The influence of the DMSO solvent was also quantified. The MIC values were considered as the lowest concentration of the tested compound that inhibited the visible growth of the microbial cultures.

The assessment of the complexes influence on the microbial ability to colonize the plastic inert substratum was performed by the micro-titre method, following previously described protocols [15].

Results and discussions

The complexes have been formulated on the basis of elemental chemical analysis, IR spectra, magnetic and conductivity measurements as it is shown in table 1.

IR spectra

The assignments of IR bands were made by comparing the spectra of the complexes with that of the free ligand.

In the spectrum of free meloxicam the narrow and intense band at 3290 cm^{-1} can be attributed to the stretching vibrations of N–H amide bond. The bands at 1619, 1550 and 1530 cm^{-1} are mainly associated with the O=C–N–H amide group [8]. In the spectra of the complexes the stretching vibration for the amide C=O group at 1619 cm^{-1} for free meloxicam is slightly shifted at 1616 cm^{-1} indicating the involvement of the C=O of the amide moiety in chelate formation. The band related to N–H stretching mode can not be observed in the region 3200–3300 cm^{-1} probably because the N–H group of $HMel^-$ is involved in intramolecular hydrogen bond to enolate oxygen [16]. After complexation, this region is dominated by two strongly broadened bands related to the stretching modes of the water molecules. Based on these data we can assume that the $HMel^-$ anion acts as bidentate chelator through the nitrogen atom of the thiazole moieties and through the amidic oxygen atom.

The presence of acetate anions in the spectra of the complexes is confirmed by the appearance of two new bands at 1565 and 1434 cm^{-1} respectively, which can be assigned to antisymmetric and symmetric stretching vibrations of acetate ligand. The difference in frequency, $(\nu_{as}(COO^-) - \nu_s(COO^-))$, is 131 cm^{-1} (164 cm^{-1} for free acetate ion), indicates the bridging coordination mode of the acetate group to metal ions [17].

Absorption spectra

The UV-Vis-NIR spectra of the compounds are presented in figure 1. The band in the region 250 - 480 nm is most probably due to a π–π^* transition of the organic ligand. This band overlaps the less intense and sharp transitions characteristic to the Ln(III) ions. The sharp absorption bands in the absorption spectra are characteristic to the transitions within the $4f^n$ configuration for Ln(III) ions.

Generally the absorption spectrum of Pr(III) (f^2 configuration) in the visible region has four bands due to the transitions from the ground state ($^3H_4$) to $^3P_2$, $^3P_1 + {}^1I_6$, $^3P_0$ and $^1D_2$ levels (435, 463, 480 and 588 nm) [18]. The absorption spectrum of $Pr_2(HMel)_2(CH_3COO)_4 \cdot H_2O$ shows only one band located at ~ 588 nm assigned to $^3H_4 \rightarrow {}^1D_2$ transition. In the near infrared region the absorption spectrum shows two bands at ~1435 and 1535 nm assigned to $^3H_4 \rightarrow {}^3F_4$ and $^3H_4 \rightarrow {}^3F_3$ transitions of Pr(III) ion.

Compound	Elemental chemical analysis % Found (Calcd.)				χ_g ($cm^3\ g^{-1}$)	Molar conductance ($\Omega^{-1}\ mol^{-1}\ cm^2$)
	C	H	N	S	294K	
$La_2(HMel)_2(CH_3COO)_4 \cdot H_2O$	35.46 (35.06)	3.34 (3.08)	6.85 (6.81)	10.38 (10.38)	Diamagnetic	28.6
$Pr_2(HMel)_2(CH_3COO)_4 \cdot H_2O$	35.10 (34.92)	3.35 (3.07)	6.76 (6.79)	10.27 (10.34)	6.63×10^{-6}	23.8
$Nd_2(HMel)_2(CH_3COO)_4 \cdot H_2O$	34.79 (34.75)	3.19 (3.06)	6.73 (6.75)	10.29 (10.29)	7.75×10^{-6}	24.2

Table 1
ELEMENTAL CHEMICAL ANALYSIS AND PHYSICAL PROPERTIES OF THE COMPOUNDS

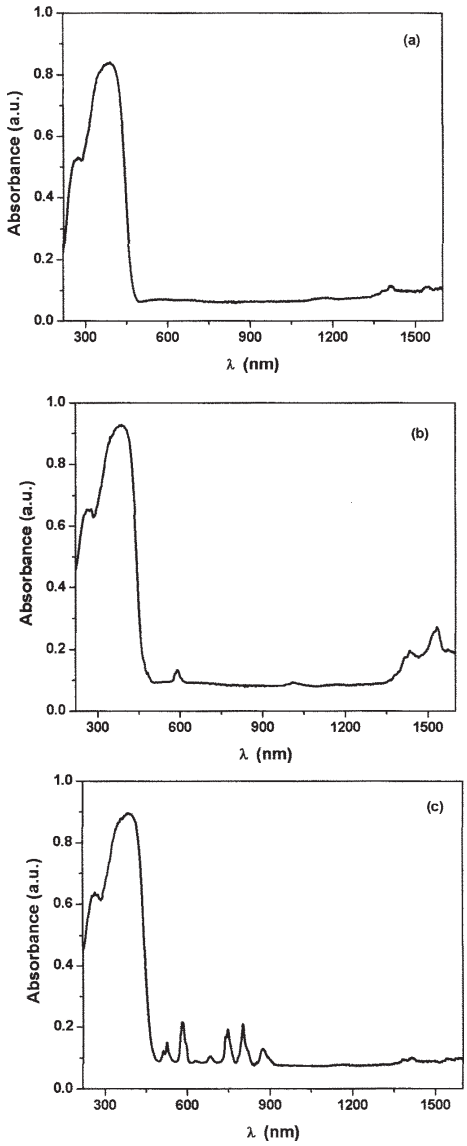

Fig. 1 UV-Vis-NIR spectra of: a) $La_2(HMel)_2(CH_3COO)_4 \cdot H_2O$; b) $Pr_2(HMel)_2(CH_3COO)_4 \cdot H_2O$; c) $Nd_2(HMel)_2(CH_3COO)_4 \cdot H_2O$

The absorption spectra of Nd(III) ion (f^3 configuration) contain many bands due to the transitions from ground state $^4I_{9/2}$ to the excited levels [18]. The sharp bands located at 510 (522), 580, 683, 742, 800 and 872 nm correspond to $^4I_{9/2} \rightarrow {}^4G_{9/2}$, $^4G_{7/2}$, $^4I_{9/2} \rightarrow {}^4G_{5/2}$, $^4I_{9/2} \rightarrow {}^4F_{9/2}$, $^4I_{9/2} \rightarrow {}^4F_{7/2}$, $^4I_{9/2} \rightarrow {}^4F_{5/2}$, $^2H_{9/2}$ and $^4I_{9/2} \rightarrow {}^4F_{3/2}$, $^3H_{9/2}$ transitions, respectively [18].

The value of the $\chi_M T$ products at room temperature are 2.65 $cm^3 mol^{-1} K$ ($Pr_2(HMel)_2(CH_3COO)_4 \cdot H_2O$) and 3.01 $cm^3 mol^{-1} K$ ($Nd_2(HMel)_2(CH_3COO)_4 \cdot H_2O$), which are lower than the calculated ones (3.2 $cm^3 mol^{-1} K$ for both compounds) corresponding to the sum of the contributions of the two uncoupled ions (1).

$$(\chi_M T)_{HT} = \frac{N\beta^2}{3k} g_J^2 \sum_i J_i(J_i+1) \qquad (1)$$

$$g_J = 1 + \frac{J(J+1)+S(S+1)-L(L+1)}{2J(J+1)} \text{ with } g_J = {}^4/_5 (Pr^{3+}); g_J = {}^8/_{11} (Nd^{3+})$$

Thermal analysis

Thermal analysis curves (TG/DSC) for all the studied compounds in air atmosphere are given in figure 2. Their decomposition profiles are similar and occurs in four well-defined steps. The first step, 120-160 °C, which is endothermic, corresponds to water molecules loss. After dehydration, the thermal degradation of organic part occurs progressively in three steps in the temperature range 200–900 °C. The first decomposition stage (mass loss ~ 20%), from 200 to 308 °C corresponds to the oxidation of the acetate moiety, process accompanied by an exothermic effect. The last two steps between 308–540 °C (continuous mass loss) and 540–900°C (sharp mass loss) are due to the thermal decomposition and oxidation of the intermediate leading to the corresponding lanthanide oxide and are accompanied by strong exothermic effects on DSC curve.

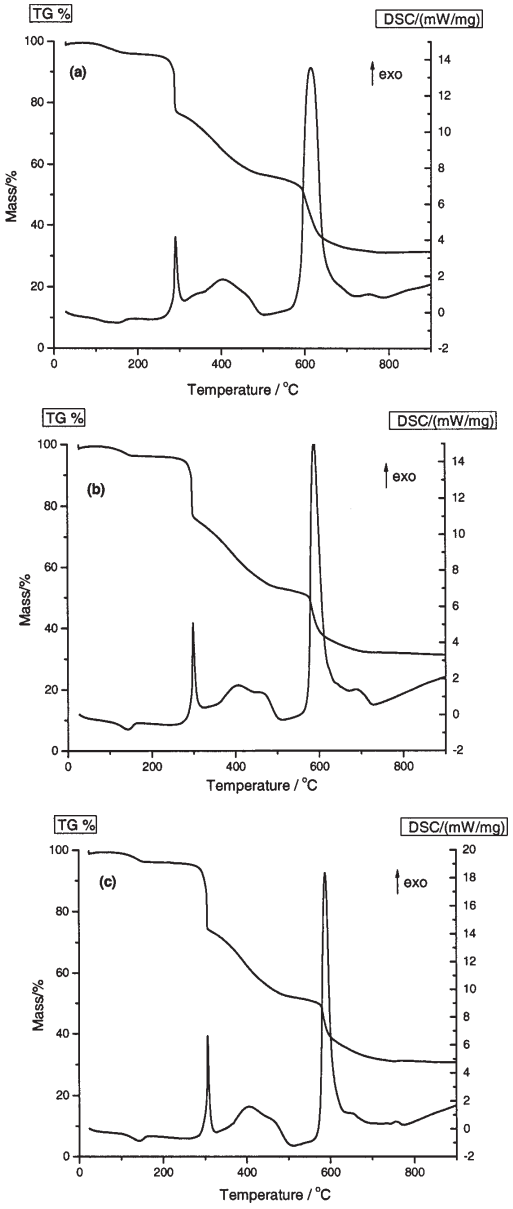

Fig. 2 TG / DSC curves of: a) $La_2(HMel)_2(CH_3COO)_4 \cdot H_2O$; b) $Pr_2(HMel)_2(CH_3COO)_4 \cdot H_2O$; c) $Nd_2(HMel)_2(CH_3COO)_4 \cdot H_2O$

On the basis of the above data the proposed coordination for the complexes is as it follows (fig. 3).

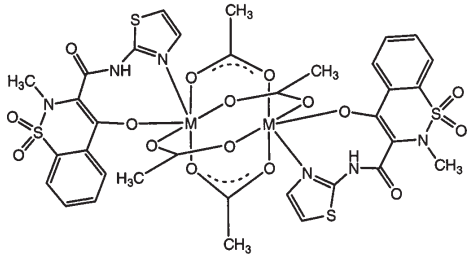

Fig. 3 The proposed molecular structures for complexes

Compound	*E. faecium* E5	*E. coli* ATCC 25922	*C. albicans* 1760	*P.aeruginosa* ATCC 27857	*B. subtillis* ATCC 6683	*S. aureus* ATCC 6538	*K. pneumoniae* IC 13420
H_2Mel	5	8	8	5	7	0	6
$La_2(HMel)_2(CH_3COO)_4 \cdot H_2O$	7	9	5	6	8	0	8
$Pr_2(HMel)_2(CH_3COO)_4 \cdot H_2O$	9	6	4	5	5	0	7
$Nd_2(HMel)_2(CH_3COO)_4 \cdot H_2O$	6	6	5	5	6	0	9
DMSO	0	4	6	4	4	0	0

Table 2
THE INHIBITORY EFFECT OF THE TESTED COMPOUNDS ON PATHOGENIC BACTERIA (DIAMETERS OF INHIBITORY ZONES, IN mm)

Compound	*E. faecium* E5	*E. coli* ATCC 25922	*C. albicans* 1760	*P.aeruginosa* ATCC 27857	*B. subtillis* ATCC 6683	*K. pneumoniae* IC 13420
H_2Mel	1	1	1	1	0.062	1
$La_2(HMel)_2(CH_3COO)_4 \cdot H_2O$	1	1	1	0.5	1	1
$Pr_2(HMel)_2(CH_3COO)_4 \cdot H_2O$	1	1	1	1	0.25	1
$Nd_2(HMel)_2(CH_3COO)_4 \cdot H_2O$	1	1	1	1	1	1
DMSO	>1	>1	1	1	1	1

Table 3
MINIMUM INHIBITORY CONCENTRATIONS (MIC) OF THE COMPOUNDS AGAINST TESTED MICROBIAL STRAINS (mg/mL)

Compound	*E. faecium* E5	*E. coli* ATCC 25922	*C. albicans* 1760	*P.aeruginosa* ATCC 27857	*B. subtillis* ATCC 6683	*K. pneumoniae* IC 13420
H_2Mel	>1	>1	>1	>1	0.125	>1
$La_2(HMel)_2(CH_3COO)_4 \cdot H_2O$	>1	>1	>1	>1	0.25	>1
$Pr_2(HMel)_2(CH_3COO)_4 \cdot H_2O$	>1	>1	>1	0.062	0.25	>1
$Nd_2(HMel)_2(CH_3COO)_4 \cdot H_2O$	>1	>1	>1	>1	0.25	>1
DMSO	>1	>1	1	0.5	>1	>1

Table 4
MBIC VALUES (mg/mL)

Antimicrobial activity assays

The qualitative screening of the antimicrobial activity indicated that all tested compounds show a broad spectrum of antimicrobial activity being active against all Gram positive and Gram negative bacteria and fungi strains tested, excepting *S. aureus* ATCC 6538 strain. The antimicrobial activity was evidenced by the appearance of growth inhibition zones. The results of qualitative analysis of antimicrobial activity, calculated by measuring the diameters of inhibitory zones (in mm) are shown in table 2.

Quantitative measurememnts of antimicrobial activity were made only for the compounds that showed antimicrobial activity at qualitative screening. Among the compounds tested $Pr_2(HMel)_2(CH_3COO)_4 \cdot H_2O$ showed moderate antimicrobial activity with MIC value of 0.250 mg/mL against *B. subtilis* ATCC 6683 strain, while for the other compounds MIC values were higher (indicating a weak antimicrobial activity) ranging from 1 to 0.5 mg/mL (table 3). Very good antimicrobial activity was recorded for meloxicam against *B. subtilis* ATCC 6683 strain (MIC – 0.062 mg/mL).

The results concerning the influence of the tested compounds on the development of microbial biofilms on inert substrate showed a very good antibiofilm activity of $Pr_2(HMel)_2(CH_3COO)_4 \cdot H_2O$ against *P. aeruginosa* ATCC 27853 strain (minimum biofilm inhibitory concentration - MBIC 0.062 mg/mL) (table 4). All the compounds showed a moderate inhibition activity on the development of microbial biofilms of *B. subtilis* ATCC 6683 (MBIC values between 0.250 and 0.125 mg/mL). For the other strains all the compounds showed high MBIC values (ranging from 1 to 0.5 mg/mL) indicating a weak antibiofilm activity (table 4).

Conclusions

La(III), Pr(III), Nd(III) complexes with meloxicam were synthesized and characterized by elemental analysis, IR, electronic spectra, molar conductance, TGA and magnetic measurements. IR data indicated that meloxicam acts as a monoanionic bidentate ligand, coordinating through the nitrogen atom of the thiazole moieties and amidic oxygen atom to the metal ions. A four-stage decomposition process is shown in the thermogravimetric analyses of all the complexes. The antimicrobial studies revealed that the praseodymium complex and meloxicam show a good activity against *B. subtilis* ATCC 6683 strain. Praseodymium complex also showed a very good antibiofilm activity against *P. aeruginosa* ATCC 27853 strain.

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