FTIR, $^1$H NMR, TGA, TEM AND BIOLOGICAL STUDIES ON THE NEW SYNTHESIZED NANO ZIRCONIUM(IV) CAPTOPRIL HYPERTENSION DRUG COMPLEX

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Abstract

Captopril (H2cap) is currently used to treat arterial hypertension and some types of congestive heart failure and to prevent kidney failure due to high blood pressure and diabetes. In an alkaline medium, a new zirconium(IV) complex was synthesized through the chemical reaction between H2cap drug ligand and Zr(SO4)2 $\cdot$ H2O hydrated salt in a ratio of 1:1 (metal:ligand). The structure interpretation of Zr(IV) complex has been confirmed using microanalytical analysis, molar conductance, infrared (FTIR) and proton nuclear magnetic resonance ($^1$H NMR) spectra, thermogravimetric (TGA) and differential thermogravimetric (DrTGA) analysis. Spectroscopic analysis of the synthesized Zr(IV) complex in solid state form showed that the coordination occurs through the oxygen of carboxylate COO and amido groups C=O, as well as sulphur atom of –SH group. The H2cap ligand acts as a tridentate chelate after the deprotonation of both hydrogen atoms from the ligand in an alkaline medium, with the presence of three water molecules inside the coordination sphere of the complex. These results are supported by elemental analysis as well as by molar conductance and TGA/DrTGA measurements. The experimental results revealed that the Zr(IV) complex has an approximate octahedral geometry around zirconium metal ion with formula [Zr(cap)(H2O)3] $\cdot$ 7 H2O. Nanoscale of the synthesized complex was studied by transmission electron microscopy (TEM). The antimicrobial efficiency of Zr(IV) complex was carried out against Escherichia coli, Bacillus subtilis and antifungal (Aspergillus niger and Aspergillus flavus).

Rezumat

Captoprile (H2cap) este folosit pentru a trata hipertensiunea arterială și unele tipuri de insuficiență congestivă a inimii, dar și pentru a preveni insuficiența renală provocată de hipertensiunea arterială și diabet. În mediu alcalin, a fost sintetizat un nou complex de zirconiu(IV), prin reacția chimică dintre ligandul H2cap și sarea hidratată Zr(SO4)2 $\cdot$ H2O în raport 1:1 (metal:ligand). Structura complexului Zr(IV) a fost confirmată folosind analiza microanalitică, conductanța molară, spectrele în infraroșu (FTIR) și de rezonanță magnetică nucleară de protoni ($^1$H RMN), analiza termogravimetrică (TGA) și termogravimetría diferențială (DGTA). Analiza spectroscopică a complexului Zr(IV) sintetizat în stare solidă a demonstrat coordonarea prin oxigenul carboxilat COO și grupurile amido C=O, precum și prin atomul de sulf al grupurii –SH. Ligandul H2cap acționează ca un chelat tridentat, după deprotonarea ambilor atomi de hidrogen din ligand, într-un mediu alcalin cu prezența a trei molecule de apă în interiorul sferei de coordonare a complexului. Aceste rezultate sunt susținute de analiza elementară, precum și de măsurătorii ale conductanței molare și TGA/DGTA. Rezultatele experimentale au arătat că acest complex prezintă o geometrie octaedrică aproximativă în jurul ioniului metalic de zirconiu cu formula [Zr(cap)(H2O)3] $\cdot$ 7 H2O. Scala nanometrică a complexului sintetizat a fost studiată prin microscopie electronică de transmisie (TEM). Eficiența antimicrobiană a complexului Zr(IV) a fost demonstrată asupra Escherichia coli, Bacillus subtilis și a speciilor Aspergillus niger și Aspergillus flavus.

Keywords: captopril, coordination, FTIR, zirconium, TEM, antimicrobial

Introduction

Captopril (H2cap) is a well-known antihypertensive drug that acts as a potent inhibitor of angiotensin I, It is used in the management of high blood pressure. In heart failure, after myocardial infarction and in diabetic nephropathy [1]. It seems to be a good chelate towards metal ions, because it includes a number of donating groups, e.g. COOH, C=O, SH and proline nitrogen [2-4]. Mineral ions are important in the functioning of the vital functions of living organisms as they occur under the form of complex groups or chelates, as well as for methods of analysing and controlling medicinal substances by forming complexes [3-8]. In various pH media, zinc(II) captopril complexes have been synthesized and characterized by many authors [9-11]. The polymeric structure of zinc(II) complexes were isolated at pH less than 6, while within pH 6 - 8 range, the Na[Zn(cap)$_2$] complexes were precipitated [9]. The coordination between zinc(II) metal ions and two captopril molecules were occurred through sulphur and carbonyl oxygen. The hydrated
cadmium(II) and nickel(II) captopril complexes with general formula Na[Zr(cap)(H2O)2] • H2O were synthesized by Atzei et al. [12]. In these complexes the captopril molecules are chelated to the central metal ions through the thiolate sulphur and carboxylic oxygen. The pseudo octahedral cobalt(II) complex with general formula as [Co(cap)(OH)2] has been synthesized and characterized by different spectroscopic methods [13]. In this case, captopril chelate molecule coordinated to the cobalt metal ion via thiolate sulphur and amido oxygen. The organometallic Sn(IV) captopril complexes R2Sn(cap) with four substituted groups (R = methyl, ethyl, n-butyl and t-butyl) were synthesized and investigated using crystal X-ray crystal structure, FTIR, Raman, 119Sn NMR and mass spectroscopy [13]. The Sn(IV) metal ions are coordinated through the –SH group and the C=O of one captopril ligand in the equatorial axial set and axial coordination of carboxylate group of another ligand with trigonal bipyramidal geometry around Sn(IV) ion. Herein, this study aimed to synthesised and spectroscopic characterized of new nanostructured form of Zr(IV) complex with captopril.

Materials and Methods

Materials and Apparatus

The pure chemical materials e.g. captopril and Zr(SO4)2 • H2O have been received from Sigma-Aldrich Chemical Company, USA. These chemicals were used in the synthesis without further purification. The microanalytical, physical and spectroscopic apparatus used in this study are presented in Table I.

| Type of analysis       | Models                                                                 |
|------------------------|------------------------------------------------------------------------|
| Elemental analyses     | (Perkin Elmer CHN 2400, Waltham, Massachusetts, United States)         |
| Conductance            | (Jenway 4010 conductivity meter, Cole-Parmer, Beacon Road, Stone, Staffordshire, ST15 OSA, UK) |
| FTIR spectra           | (Bruker FTIR Spectrophotometer, Billerica, Massachusetts, United States) |
| 1H NMR spectra         | (Varian Mercury VX-300 NMR spectrometer, 300 MHz, Palo Alto, California) |
| Thermogravimetric      | (TG/DTG–50H, Shimadzu thermo-gravimetric analyser, 1, Nishinoskkyo-Kuwabara-cho, Nakagyo-ku, Kyoto 604-8511, Japan) |
| TEM                    | (JEOL 100s microscopy, Akishima, Tokyo 196-8558, Japan)                 |

Synthesis of Zr(IV) captopril complex

Equimolar amounts (1 mmole) of Zr(SO4)2 • H2O in distilled water (15 mL) and (1 mmole) of captopril pure drug in 30 mL methanol solvent were mixed. Alkaline solution of (1 M) NH4OH was added until the pH = 9 was achieved. The mixture was refluxed for two hours. The volume of the mixture was evaporated to half. The solid precipitate was formed, filtered off, washed with methanol then dried in glass desiccators under anhydrous CaCl2. The yield of pale-yellow solid product was ~ 74%. This complex has a higher melting point > 250°C. Anal Calcd. for [Zr(cap)(H2O)2] • 7 H2O: C, 22.21; H, 6.83; N, 2.88; S, 6.59; Zr, 18.75. Found: C, 22.07; H, 6.69; N, 2.74; S, 6.51; Zr, 18.62. Mol. Wt. 486.65 g/mol and molecular formula C2H33NO5O6SZr.

Biological assay

By using the disc diffusion method [14], antibacterial and antifungal tests were performed against two bacterial (Escherichia coli (G−), Bacillus subtilis (G+) and two fungi (Aspergillus niger and Aspergillus flavus) species. Briefly, 100 μL of the tested bacteria/fungi were grown in 10 mL of fresh media until they reached a count of approximately 10^8 cells/mL for bacteria or 10^5 cells/mL for fungi. 100 μL of the microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained. Isolated colonies of each micro-organism were selected from primary agar plates and tested for susceptibility by disc diffusion method [14]. The agar used was Müller-Hinton agar which was tested for composition and pH. The zones of inhibition have been determined for susceptible values according to CLSI (Clinical and Laboratory Standard institute). Blank paper disks (Schleicher & Schuell, Spain) with a diameter of 8.0 mm were impregnated with 10 μL of the tested concentration from the stock solutions [14].

Results and Discussion

Conductance measurements

The composition of the [Zr(cap)(H2O)3] • 7 H2O complex was assessed by using microanalytical and thermogravimetric methods, revealing the presence of endothermic DrTGA peak at 63°C and 347°C due to the presence of seven crystalline water molecules and three coordinated water molecules. The elemental analysis data referred to the Zr:2cap of 1:2 stoichiometry. The third endothermic differential thermal analysis peak at 493°C is assigned to the completely destruction process for the 2 captopril molecules. The prepared complex was found to be solid, insoluble in water, but it was soluble in selected organic solvents like dimethylsulphoxide (DMSO) and dimethylformamide (DMF). The lower value observed (λm = 12 cm2/ohm x mol) of molar conductivity in DMSO indicates the non-electrolyte behaviour of the Zr(IV) complex [15]. According to the elemental analysis, molar conductance, thermal analysis and spectroscopic data of (FTIR and 1H NMR) spectra, it can be concluded that captopril ligand coordinated to zirconium metal ion as a...
tridentate chelate while the three of water molecules are complete the coordination sphere (Figure 1).

Figure 1.
Proposed structure of [Zr(cap)(H₂O)₆] • 7 H₂O complex

Infrared spectra
The infrared spectrum of the [Zr(cap)(H₂O)₆] • 7 H₂O complex was performed within the range of 4000 - 400 cm⁻¹. The assignments of the distinguished frequency of FTIR spectrum of zirconium(IV) complex was compared with the free H₂cap drug ligand as well as the sodium salt of captopril NaHcap (Table II). It is worth noting that the H₂cap may coordinate metal ions via –COOH carboxylic, >C=O carbonyl and –SH thiol. In case of the FTIR spectrum of H₂cap ligand, the broad band exhibited at 2566 cm⁻¹ is assigned to the stretching vibration of ν(SH). This band disappeared after complexation as displayed in the spectrum of Zr(IV) complex, indicating the interaction of sulphur atom of this deprotonated group –SH with the Zr(IV) metal ions [16]. The strong intensity band at 1750 cm⁻¹ of free H₂cap ligand is attributed to the stretching vibration band of ν(C=O) regarding the –COOH group. This band is absent in the Zr(IV) complex and NaHcap sodium salt due to the deprotonated carboxylic group which chelates central metal ions through oxygen atom of carboxylate group. The absence of the carboxylic group frequencies (1750 cm⁻¹) in the free H₂cap and presence of two new stretching vibration bands at 1449 cm⁻¹ and 1281 cm⁻¹ in the complex, can be assigned to the νasym and νsym models of carboxylate group respectively, (Δν = νasym COO - νsym COO = 168 cm⁻¹), which is in agreement with the mono-dentate coordination of the carboxylate group [17]. In the case of NaHcap compound, these two bands are present at 1622 cm⁻¹ and 1400 cm⁻¹ (Δν = νasym COO - νsym COO = 222 cm⁻¹), showing the formation of an anionic carboxylate. Comparing between the Δν data of both Zr(IV) complex and NaHcap, it was found that the (Δν = 168 cm⁻¹) value of [Zr(cap)(H₂O)₆] • 7 H₂O complex was broader than the one observed for ionic NaHcap sodium salt compound (Δν = 222 cm⁻¹). This results confirmed the undentate coordination behaviour of the carboxylate group towards zirconium metal ions [16-18]. The stretching vibration band of ν(C=O) amino group in the free spectrum of free H₂cap ligand is located at 1610 cm⁻¹. This band is shifted to lower wavenumber 1589 cm⁻¹. This may be to the involvement of the oxygen carbonyl-amide group in the complexation with the zirconium(IV) ions [16]. The strong broad band at 3423 and shoulder intensity band at 3227 cm⁻¹ are assigned to νasym and νsym models of water molecules coordinated to the metal [16]. The new observed bands at 533 cm⁻¹ and 429 cm⁻¹ are assigned to the stretching vibration motions of ν(M-O) and ν(M-N) bonds [16]. According to these interpretations, it can be summarized that the captopril ligand acts as a tridentate ligand against zirconium ion through the oxygen of the carboxylic group, oxygen of the carbonyl amido group, and the sulphur of thiol group and three coordinated water molecules to complete the octahedral geometry around central metal ions.

Table II

| Assignments | Frequencies (cm⁻¹) | H₂cap | NaHcap | [Zr(cap)(H₂O)₆] • 7 H₂O |
|-------------|--------------------|--------|--------|------------------------|
| ν(OH)       | -                  | -      | 3423 br, 3227 sh |
| ν(SH)       | 2566 s             | 2561 w | -      |
| ν(C=O); COOH| 1750 s             | -      | -      |
| νasym(COO)  | -                  | 1622 s | 1449 s |
| νsym(COO)   | -                  | 1400 m | 1281 w |
| Δ (νasym - νsym) | -      | 222    | 168    |
| ν(C=O); amide| 1610 sh, 1592 s   | 1598   | 1589 vs |
| δ(SH)       | 901                | 917    | 850 w   |
| ν(M-O)      | -                  | 528    | 533, 429 |

¹H NMR spectra

The ¹H NMR spectrum of H₂cap free ligand in DMSO-d₆ has characteristic peaks δ (ppm) at 1.50, 1.19, 1.92 - 2.33, 2.67, 2.92, (3.51 & 3.41), 4.33 and 11.00 ppm due to the protons of mercapto –SH, –CH₃ methyl, CH₂-CH₂ of pyrrolidine ring, methylene CH₂-SH, CH-C=O, CH₂-N pyrrolidine ring, CH-COOH and COOH respectively [19]. In case of the ¹H NMR spectrum of [Zr(cap)(H₂O)₆] • 7 H₂O complex, the protons of mercapto, carboxylic and CH-C=O groups disappeared due to their involvement in the coordination with zirconium(IV) ion. The other protons have slightly shifted due to the electronic configuration change of captopril ligand. These peaks located at δ 1.15, 1.88, 135
2.96, 3.60 and 4.30 ppm which assigned to –CH₃ methyl, CH₂-CH₂ of pyrrolidine ring, methylene CH₂-SH, CH₂-N pyrrolidine ring, and CH-COOH respectively. These results confirm the sites of coordination around zirconium metal ions.

Thermo gravimetric analysis

At the temperature range of 25 - 800°C, the mass loss of [Zr(cap)(H₂O)₃] • 7 H₂O complex is 60%, corresponding to the decomposition of captopril molecule, and release of 10 H₂O coordinated and uncoordinated molecules. It was found that the solid Zr(IV) complex is thermally decomposed with three endothermic DrTGA peaks at 63, 347 and 493°C, respectively. This complex was actually decomposed in three steps and the residual was 40%, which was assigned to ZrO₂ polluted with few carbon atoms. The new DrTGA peak at 610°C is present without weight loss, due to solid-solid interactions.

TEM analysis

TEM image of the [Zr(cap)(H₂O)₃] • 7 H₂O complex showed spherical black spots with 85 - 93 nm (Figure 2).

Figure 2.
TEM images of [Zr(cap)(H₂O)₃] • 7 H₂O complex

Investigations regarding the use of transition metal complexes as medicines for human diseases have shown important advances, with transition metal complexes being the most widely used as chemo-therapeutic agents. Research in nanotechnology allows the development of nanomaterials below 100 nm, the specific physical and chemical properties of nanomaterials being common in all fields [6-8]. Studies showed that nanocomposites have distinct physical, chemical, and biological applications. In the present paper, a description is given on the preparation of zirconium(IV) captopril nano complex and evaluation of its antibacterial activity.

Biological analysis

The antimicrobial activity (anti-bacterial and antifungal species) of the zirconium(IV) complex was screened against bacteria (Escherichia coli, Bacillus subtilis) and fungi (Aspergillus niger and Aspergillus flavus). Dimethyl sulfoxide (DMSO) as a control sample is a polar organic molecule of amphipathic nature which is ideal for solubilizing poorly soluble polar and nonpolar molecules. DMSO is widely used as a solvent in toxicology and pharmacology, for cells cryopreservation, and as a penetration enhancer in topical treatments. DMSO is generally accepted as non-toxic less than 10% (v/v), and in practice the effects of DMSO are assumed to be negligible. The diameter of inhibition zone (cm) of zirconium(IV) complex were 2, 1.5, 1.5 and 1.8, respectively, for Escherichia coli, Bacillus subtilis, Aspergillus niger and Aspergillus flavus.

According to the CLSI guidelines, our results showed that the [Zr(cap)(H₂O)₃] • 7 H₂O complex has a good antimicrobial efficiency against all bacteria and fungi species tested. These results can be assigned to the ability of the metal complexes to penetrate the cell wall, thus interfering with the cellular cycle [20].

Conclusions

Zirconium(IV) complex with the proposed formula ([Zr(cap)(H₂O)₃] • 7 H₂O) has been synthesized and characterized. The synthesized metal complex was screened for antimicrobial activity against Escherichia coli (G+), Bacillus subtilis (G+) and antifungal (Aspergillus niger and Aspergillus flavus) by disc diffusion method.

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Conflict of interest

The authors declare no conflict of interest.

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