

Anti-inflammatory Drugs Interacting with Zn(II) Metal Ion

Synthesis, characterization and thermal behaviour of the complex with ketoprofen

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A complex of Zn(II) metal ion with the anti-inflammatory drug, 2-(3-benzoylphenyl)-propionic acid (Ketoprofen) was isolated and investigated. The complex was characterized by elemental analysis, Fourier transformed infrared spectroscopy (FT-IR), X-ray diffraction powder (XRPD) and thermal analysis. The thermal behavior was studied by TG/DTG and DTA methods under non-isothermal conditions in a dynamic air atmosphere. The results provided informations of the composition, structure, thermal behavior, dehydration and thermal decomposition. The spectroscopic study suggest that the carboxylate group of ketoprofen is coordinate to metal as bidentate bond.

Keywords: anti-inflammatory drug, ketoprofen, FT-IR spectroscopy, X-ray analysis, thermal analysis

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently used drugs in the world, primarily for symptoms associated with osteoarthritis and other chronic musculoskeletal conditions [1,2].

The anti-inflammatory activity of NSAIDs and most of its other pharmacological effects are related to the inhibition of the conversion of arachidonic acid to prostaglandins, which are mediators of the inflammatory process [3,4].

Ketoprofen (KT), 2-(3-benzoylphenyl)-propionic acid, a member of the NSAIDs, which structural formula is shown in figure 1, is an inhibitor of prostaglandin synthase. It is effective in the long-term management of rheumatoid arthritis, ankylosing spondylitis, osteoarthritis and acute out, as well as mild to moderate pain and dysmenorrhea [5-7], and has been used as model drug for such investigations.

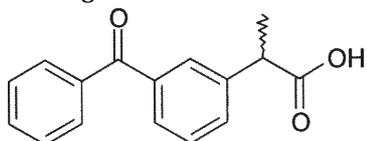


Fig. 1. The chemical structure of ketoprofen

The anti-inflammatory activity of NSAIDs and most of their other pharmacological activity are related to the inhibition of the conversion of arachidonic acid to prostaglandins, which are mediators of the inflammatory process. NSAIDs are potent inhibitors of cyclo-oxygenase *in vitro* and *in vivo*, thereby decreasing the synthesis of prostaglandins, prostacyclin and thromboxane products. Recently, two different cyclo-oxygenase isoforms have been characterized Cox-1 and Cox-2. Inhibition of ox-2 enzyme system results in anti-inflammatory action, while inhibition of the Cox-1 enzyme system reox-2 enzyme system results in anti-inflammatory action, while inhibition of the Cox-1 enzyme system results in anti-inflammatory action as well as gastric irritation [8]. New studies from the last years revealed that in addition to arthritis and pain, cancer and neurodegenerative diseases like Alzheimer's

disease could be potentially treated with Cox-2 inhibitors [9].

It is well established that metal ions play a wide range of important roles in biological systems. The presence of drugs that can compete with other biological molecules for the metal ions, changes the distribution of these ions in blood plasma and other fluids. On the other hand, presence of these metal ions can affect the bio-availability of these drugs [10-12].

Synthesis and study of metal complexes with active drugs as ligands is a research area of increasing interest for inorganic, pharmaceutical and medicinal chemistry and has concentrated much attention as an approach to new drug development. The goal is to prepare new compounds with better or different pharmaceutical profile than that of the free ligand. Knowledge of the species formed by combining a metal ion with a drug provides useful information to approach the mechanisms of action of the drug for a disease under treatment and ultimately this can also diminish collateral effects and enhance the efficacy of the parent drug.

It has long been suggested that the mode of action of many anti-inflammatory drugs may involve the chelation with some bioactive metals such as Zn(II), Cu(II), Cd(II) and it facilitates the transfer of the metal to and from a site of inflammation or pain.

Zinc, which is a relatively abundant element in biological organisms, plays an essential role in the large number of enzymatic reactions. Having the anti-bacterial and antiviral activities, zinc and its compounds may be used as a therapeutic agents and anti-sicking agent playing a role in the prevention of pain crisis in sickle-cell disease and in treatment of various sickness [13-15].

For the characterization of the new compounds with possible pharmacological properties, beside the classical methods such as UV-Vis and FT-IR spectroscopy, respectively X-ray diffraction, the thermal methods are used in an increased proportion.

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Thermal analysis is one of the most frequently used instrumental techniques in the pharmaceutical research, for the thermal characterizations of different materials from solids to semi-solids, which are of pharmaceutical relevance. The term thermal characterization refers to the thermal stability and decomposition of the substances of pharmaceutical interest. The evaluation of the thermal stability of a drug is realized especially by analysing its decomposition in isothermal and non-isothermal conditions. Usually, this takes place by irreversible mass loss.

The drugs' decomposition reactions have a theoretical, as well as a practical signification [16-18]. Research in thermal decomposition of drugs is of great interest in developing new products since it is often necessary to predict degradation rates at marketing temperatures from collected data on accelerated processes studied at elevated temperatures.

The thermoanalytical techniques, especially thermogravimetry/derivative thermogravimetry (TG/DTG) and differential scanning calorimetry (DSC) or differential thermal analysis (DTA), are used in solving some pharmaceutical problems, e.g. the determination of purity level, the qualitative and quantitative analysis of drug formulations, the polymorphism, the thermal stability with the determination of the corresponding kinetic parameters, etc. [19-21].

In our previous papers we provided the importance and the utility of the thermoanalytical techniques in the estimation of thermal stability of some pharmaceuticals, by thermal behaviour and kinetic analysis, respectively their compatibility [22-36].

The motivation for the preparation of zinc complex with ketoprofen is determined by the existence of other complexes with non-steroidal anti-inflammatory drugs, already reported in the literature [37-41].

In this paper we report the synthesis and characterization by elemental analysis, FT-IR spectroscopy, X-ray diffraction patterns and thermal analysis, of Zn(II) complex with anti-inflammatory drug, 2-(3-benzoylphenyl)-propionic acid.

Experimental part

Materials methods and equipment

All chemical used were analytical reagent products.

The KT drug was obtained from S.I.M.S., Italy, lot: 138315.

Zn(CH₃COO)₂·2H₂O and KOH were obtained from Merck, Germany.

Aqueous solution of ketoprofen potassium salt (0,1 mol L⁻¹) was prepared by neutralization aqueous ketoprofen suspension with 0.1 mol L⁻¹ potassium hydroxide solution and the pH was adjusted to 8.0.

Zinc (II) was used as its acetate and ca. 0.1 mol L⁻¹ aqueous solution of this ion were prepared by direct weighing and dissolution of the salt.

Solid state compound was prepared by adding slowly with continuous stirring, solution of zinc acetate solutions (1.0 mmoles = 0.2195 g) to the respective ketoprofen potassium salt (2.0 mmoles = 0.5846 g) until precipitation of metal ion. The white needle precipitate washed with distilled water in order to elimination of acetate ions, filtered through and dried on Whatman no. 42 filter paper, and kept in a desiccator over anhydrous calcium chloride.

Elemental analyse of C and H was carried out on a Vario El elemental analyzer.

The Zn (II) content was determined by complexometric titration with EDTA, in buffer solution (NH₃ - NH₄Cl) an pH = 10, using Eriochrome black T as indicator.

Infrared spectra (400 - 4000 cm⁻¹) for ketoprofen and its complex with Zn(II) were recorded on a Perkin-Elmer FT-IR 1600 spectrometer. The samples for the FT-IR spectra measurements were prepared as KBr discs.

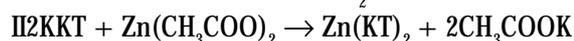
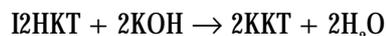
X-ray diffraction patterns (XRPD) were obtained with a Bruker D8 Advance X-ray diffractometer using MoK α radiation (Zr filter on the diffracted beam, 50 kW and 40 mA) in a Bragg-Brentano θ :2 θ configuration, with soller and fixed slits and a NaI scintillation detector. The measurements of 2 θ ranged between 0 and 30°. Data analysis and acquisition were performed using DIFRACT^{plus} software from Bruker AXS.

Simultaneous TG, DTG and DTA curves were obtained with thermal analysis system, model Netzsch-STA 449 TG/DTA. The purge gas was on air flow of 20 mL·min⁻¹. A heating rate of 10 °C·min⁻¹ was adopted, with samples weighing 20 mg. Platinum crucible was used and the heating range 25 - 1200 °C.

Results and discussions

Synthesis

The complex with Zn(II) metal ion have been prepared by simple reaction which involves deprotonation of the ligand by KOH in aqueous solution, followed by complexation with a metal salt.



The X-ray powder patterns showed that the white compound was obtained with low crystallinity degree.

To establish the combination ratio we have studied the systems Zn(II)-KKT in ratios 1:1; 1:2 and respectively 1:3. From these systems, we were able to isolate and characterize the following type of mononuclear complex: Zn(KT)₂. Figure 2 presents the chemical structure of the complex obtained.

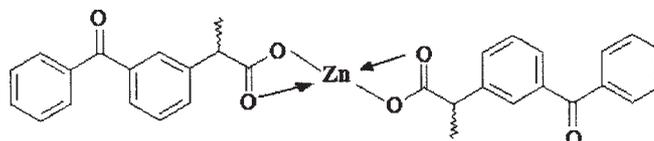


Fig. 2 The chemical structure of Zn-Ketoprofen complex

The formulae proposed for this compound was established on the bases of elemental chemical analysis correlated with physico-chemical investigations, (FT-IR spectroscopy and X-ray diffraction) and thermal analysis, especially for the determination of the co-ordination and crystallization water, as well the molecular formulae.

The results of the elemental analysis for the complex with the formulae Zn(KT)₂ ≡ C₃₂H₂₆O₆Zn (M = 572) are the following: Anal. (%) Calcd. C 67.13 ; H 4.55 ; Zn 11.43. Found: C 66.62 ; H 4.41 ; Zn 11.56.

Infrared spectroscopy

The FT-IR spectroscopy is the most suitable technique of the non-destructive spectroscopic methods and has become an attractive method in the analysis of pharmaceutical solids, since the materials are not subject to thermal or mechanical energy during sample preparation, therefore preventing solid-state transformation. The appearance, respectively disappearance of new absorption bands, broadening of bands, and alteration in intensity are the main characteristics to evidence the differences between substances (samples) [21,23,42,43].

Table 1
IR SPECTROSCOPIC DATA FOR POTASSIUM KETOPROFEN AND FOR ITS COMPLEX DATA WITH ZINC (II)

Compound	$\nu_{C=O} / \text{cm}^{-1}$	$\nu_{\text{asym}(\text{COO}^-)} / \text{cm}^{-1}$	$\nu_{\text{sym}(\text{COO}^-)} / \text{cm}^{-1}$	$\Delta\nu / \text{cm}^{-1}$
KKT	1698 ; 1656	1576	1420 ; 1370	181
Zn(KT) ₂	1678 ; 1655	1548	1448 ; 1411	118

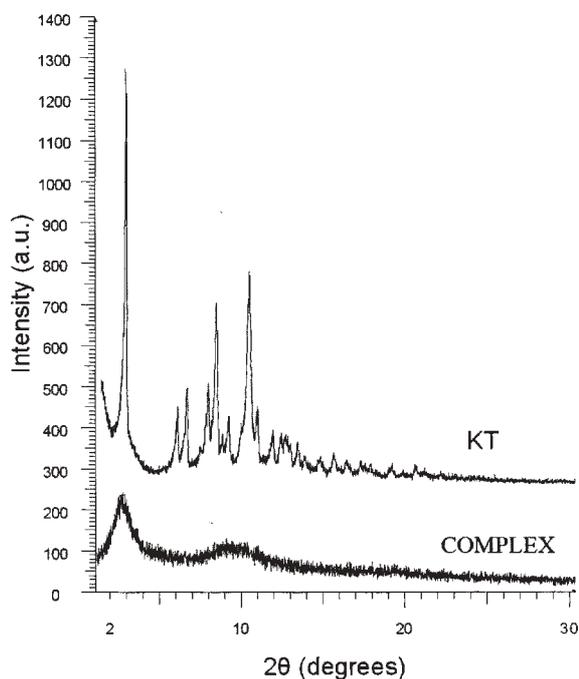


Fig. 3. X-ray diffractogram of ketoprofen, respectively of its complex with Zn(II)

The IR spectrum of complex exhibits absorption bands of ketoprofen ligand.

The principal bands are reported in table 1.

The bands found for ketoprofen (potassium salt) are centered at 1698, 1656 cm^{-1} (ketonic carbonyl stretches), 1576 cm^{-1} (asymmetrical carboxylate vibration) and 1420, 1370 cm^{-1} (symmetrical carboxylate vibration).

The ketonic carbonyl stretches vibrations frequency (1678; 1655 cm^{-1}) is unchanged in the complex in comparison with potassium salt. These data suggest the ketonic carbonyl does not participate in coordination with the metal center.

The major characteristic of the FT-IR spectrum of complex is the frequency of the $\nu_{\text{asym}(\text{COO}^-)}$ and $\nu_{\text{sym}(\text{COO}^-)}$ stretching vibrations. The frequency of these bands depends upon the co-ordinate mode of the carboxylate ligand. For the $-\text{COO}^-$ group, unidentate or bidentate modes of coordination have been observed. The unidentate mode of binding shows two very strong broad and ν_{sym} stretching bands in the region 1560 – 1620 cm^{-1} and 1370 – 1425 cm^{-1} respectively with an average $\Delta\nu$ value 190 cm^{-1} [10,11]. For the bidentate chelate co-ordination mode the $\nu_{\text{asym}(\text{COO}^-)}$ band occurs at a lower frequency, at 1520 – 1570 cm^{-1} , while the $\nu_{\text{sym}(\text{COO}^-)}$ stretching frequency increases to 1410 – 1450 cm^{-1} giving an average $\Delta\nu$ value of 115 cm^{-1} .

No distinguished differences between bidentate double bound and bidentate triple bound co-ordination mode could be extracted from $\nu_{\text{asym}(\text{COO}^-)}$ and $\nu_{\text{sym}(\text{COO}^-)}$ stretching frequencies or to $\Delta\nu$ values.

The calculated value of ($\Delta\nu$) (asymmetrical-symmetrical carboxylate vibrations) for the synthesized complex, shows smaller values (1548, respectively 1448, 1411 cm^{-1}) in comparison of those values obtained for the potassium salt (table 1). The average $\Delta\nu$ value is 118 cm^{-1} . These results suggests that the co-ordination of the

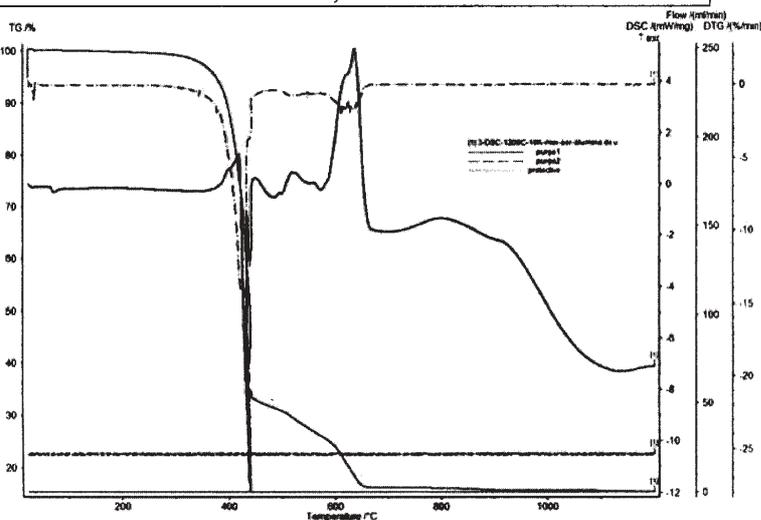


Fig. 4. TG, DTG and DTA curves of Zn(KT)₂

metal occurs in the carboxylate site of ketoprofen as bidentate bond [44].

X-ray diffraction patterns

To investigate the configuration of the complex obtained, besides the FT-IR spectroscopy which is a qualitative analysis technique, the X-ray powder diffraction (XRPD) has been used for qualitative and quantitative identification of crystallinity. The number of the speciality papers which uses XRPD is growing [45-47].

The appearance of new lines and disappearance of some of the lines present in the ligand, respectively the shifting of some of the diffraction lines of higher moderate and lower intensities in the complex, which are originally present in the X-ray diffraction patterns of the ligand indicates the presence of a new compound.

The X-ray diffraction patterns of ketoprofen and of its complex with Zn(II) are shown in figure 3.

From figure 3 it can be remarked a great difference between the diffractograms of the ligand, respectively complex, by the disappearance, respectively the appearance of some meaningful lines.

Thermal analysis

The thermal stability of Zn(II) complex was studied in air, the simultaneous TG, DTG and DTA curves are registered and are shown in figure 4.

The data obtained from thermoanalytical curves are supported by chemical and X-ray diffraction pattern investigations.

The thermal decomposition of the complex Zn(KT)₂ unfolds practically in three stages, without being able to afford to delimit the last two stages.

It is very difficult to specify the nature of the intermediate compounds because of the complexity of decomposition process with simultaneous and/or competitive reactions.

The complex obtained is stable up to 320°C.

The first step of thermoanalysis occurs in the range 321 – 452 °C. The process is characterised by an endothermic peak on the DTA curve ($\text{DTA}_{\text{peak}} = 438^\circ\text{C} = \text{DTG}_{\text{peak}}$), respectively by large mass loss (66.67%) which correspond to partially decomposition of organic ligand.

Decomposition of organic ligand continue in the second step (between 452 – 591 °C), with a loss of mass of 10.95% and the formation of ZnCO₃. The TG curve, but especially DTG and DTA show the complexity of thermal decomposition through the related DTg peaks (438 and 522 C), respectively DTA peaks (438 – endo; 495 – endo; 522 – exo; 570 – endo).

The last mass lost (8.09%) between 501 – 671°C (the third step of decomposition) with a strong exothermic effect (DTA_{peak} = 648°C; DTG_{peak} = 638°C) is due to the thermal decomposition of the intermediate (ZnCO₃), leading to the zinc oxide.

Calculations based on the mass loss up to 900°C are in agreement with the formation of ZnO as final residue (exp. 14.29% ; calc. 14.23%).

Conclusions

Ketoprofen is a very interesting ligand from point of view of its applications. It could form several complexes with metal (II and III) ions. In this work, the synthesis and properties of these types of compounds was investigated. The complex with the empirical formulae Zn(KT)₂ was prepared, with low crystallinity degree.

Based on the elemental analysis, FT-IR spectroscopy, X-ray diffraction pattern and thermal analysis a general formula could be established for the synthesized compound.

The spectroscopic infrared experimental data suggests that the carboxylate of ketoprofen is co-ordinate to metal as bidentate bond.

The FT-IR spectrum, together with thermal analysis confirmed the absence of the co-ordination, respectively crystallization water.

The thermal investigation (studied by TG, DTG and DTA techniques) shows that the thermal decomposition process is one complex with simultaneous and/or competitive reactions. The final product of the thermal decomposition is ZnO, which through its percentage confirms the empirical formulae of the new complex prepared.

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Manuscript received: 29.06.2015