Molybdocene dichloride intercalation into zirconium phosphate nanoparticles

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ABSTRACT

Molybdocene dichloride (Cp_2MoCl_2), a metallocene dichloride currently being evaluated as potential anti-cancer drug, has been intercalated into zirconium phosphate, an inorganic layered nanomaterial. Hydrolysis of the chloride ligands appears to occur, leading to the formation of the monocation species ($[Cp_2Mo(H_2O)(OH)]^+$), which intercalates into the ZrP layers by ion exchange. IR spectroscopy, X-ray powder diffraction (XRPD), UV−Vis spectrophotometry, scanning electron microscopy with energy dispersive X-ray spectroscopy, NMR spectroscopy, and thermogravimetric analysis were used to confirm the presence of the metallocene between the layers of zirconium phosphate. The XRPD data indicates that a new intercalated phase with an expanded interlayer distance of 11.0 Å was obtained; thermogravimetric analysis indicates up to 64% loading in the context of molar ratio (0.64 mol per ZrP formula unit) or 32% exchange capacity. Further evidence for intercalation was obtained from ^{31}P MAS NMR experiments. IR spectroscopy confirms the presence of the cyclopentadienyl bearing metallocene in the layers.

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1. Introduction

The development of targeted drug delivery systems is currently one of the most important areas of drug research. Presently, many of the drugs designed, discovered or already in use for therapeutics are limited by their poor solubility, high toxicity, high dosage, aggregation, nonspecific delivery, *in vivo* degradation, and short circulating half-lives [1]. Previous reports have shown that nanoparticles can overcome these limitations, particularly by the enhanced permeability and retention effect shown by cancer cells toward nanoparticles presenting a passive mechanism for selective delivery of drugs to tumor cells [2]. Before such efforts can advance, detailed chemical characterization of the drug-immobilized nanoparticles are needed to better guide the understanding of cell- and in-vivo studies.

Our current interest is in using the tetravalent metal phosphate, zirconium phosphate (zirconium bis(monohydrogen orthophosphate) monohydrate ($Zr(HPO_4)_2 \cdot H_2O$, α-ZrP) as drug carrier for potential anticancer agents such as metallocene dichlorides, in particular molybdocene dichloride (MDC). ZrP has been extensively studied and reported as an inorganic layered nanomaterial (ILN) [3]. The use of ILNs as potential drug carriers has been growing since the discovery of their ability to encapsulate bioactive compounds and control their release via a chemical switch [3–5]. α-ZrP is one of the best characterized ILNs with an interlayer distance of 7.6 Å and a layer thickness of 6.6 Å (Fig. 1) [6].

In α-ZrP the zirconium atoms in each layer nearly align in a plane with bridging phosphate groups located alternately above and below the metal atom plane. Six oxygen atoms from different phosphate groups octahedrally coordinate the Zr atoms while each phosphate group is bonded to three different Zr atoms. The fourth oxygen atom of the phosphate group is protonated and it is

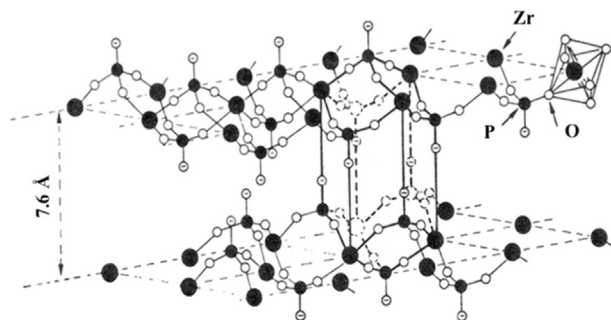**Fig. 1.** A portion of the $Zr(HPO_4)_2 \cdot H_2O$, α-ZrP structure showing the relationship between adjacent layers. A cavity is shown in heavy outline and the negatively marked circles are oxygens bonded to exchangeable protons. Adapted from Ref. [6].

pointing towards the interlayer space and can be deprotonated via an ion exchange reaction [6].

This arrangement creates a zeolitic cavity per formula unit encapsulating a water molecule stabilized by hydrogen bonding with the hydroxy groups of the phosphates inside the cage. These zeolitic cavities interconnect to each other by entrances with a diameter of 2.61 Å. Due to this narrow space just a few cations can diffuse through, limiting the direct ion-exchange capacity of this material [6].

Our laboratory developed an approach that overcomes the difficulty of direct intercalation in α-ZrP [7]. In this approach a highly hydrated phase of ZrP (containing six molecules of water per formula unit, known as the θ phase) is used to achieve direct intercalation of large species without the need of a preintercalation process (Fig. 2A). The highly hydrated phase of the layered ZrP is an acidic ion exchanger that has been used for the immobilization of several photo- [7], bio- [5] and redox-active compounds [8]. The applications for these materials can range from being used as catalysts [9], electron-transfer systems [7,8], drug carriers [5], and modified electrodes [8]. In addition, ZrP can be modified by manipulating certain variables in its synthesis to obtain a material with nanoscale dimensions [10], suitable for additional applications in the areas of nanotechnology and drug delivery. θ-ZrP was chosen to intercalate MDC (Fig. 2B) to demonstrate its use of these nanoparticles as anticancer drug delivery agents.

Since the discovery of titanocene dichloride's antitumor properties in 1979 [11], investigations of other related potential metallocene-based drugs begun to emerge due to the lower toxicity exhibited in comparison with the known anticancer drug cisplatin (*cis*-Pt(NH$_3$)$_2$Cl$_2$). The metallocenes of general formula Cp$_2$MX$_2$ (Cp = cyclopentadienyl; M = Ti, V, Nb, and Mo; X = halides and pseudohalides) have shown antitumor activity against a wide variety of tumor cells [12–14]. Contrary to titanocene dichloride which is unstable in water and looses its Cp ligands to produce insoluble species at physiological pH, MDC maintains its Cp ligands in water and accumulates in significant amounts in the cellular nuclei [15], which provoked our interest in the intercalation of MDC in ZrP.

The mechanism of action of MDC is not yet clear in terms of the cellular uptake, distribution, and damage in the tumor cells [12]. The Mo metal in MDC, being a softer metal than Ti, prefers coordination to softer ligands, such as thiols, over phosphate, amino, and carboxylate groups. Therefore, this compound would be more attracted to thiol-containing biomolecules as a cellular target. In addition, MDC has a poor aqueous solubility that limits its utility as an antitumor drug.

To overcome the drawbacks of low solubility in water, instability, and non-localized release of MDC we decided to study the direct intercalation of this anticancer drug into θ-ZrP. Direct intercalation of metallocenes into θ-ZrP has not been previously reported in the literature with exception of the direct intercalation of ferrocene for use as a redox agent in our own effort [8]. Here, we report the successful direct intercalation of MDC into ZrP producing an expanded intercalated phase. In addition, we present an extensive characterization of the drug-immobilized nanomaterial that indicates that the chemical integrity of the cyclopentadienyl rings of MDC are preserved in the MDC-intercalated material and that MDC can be released with a pH stimulus.

2. Experimental section

2.1. Materials

Zirconyl chloride octahydrate ($ZrOCl_2 \cdot 8H_2O$, 98%), was obtained from Aldrich and used without further purification. Phosphoric acid (H_3PO_4, 85% v/v) and bis(cyclopentadienyl) molybdocene dichloride (99%) were obtained from Fisher Co. Nanopure water was obtained using a Barnstead purification train (18 MΩ·cm). All other reagents were of spectroscopic grade and were used without further purification.
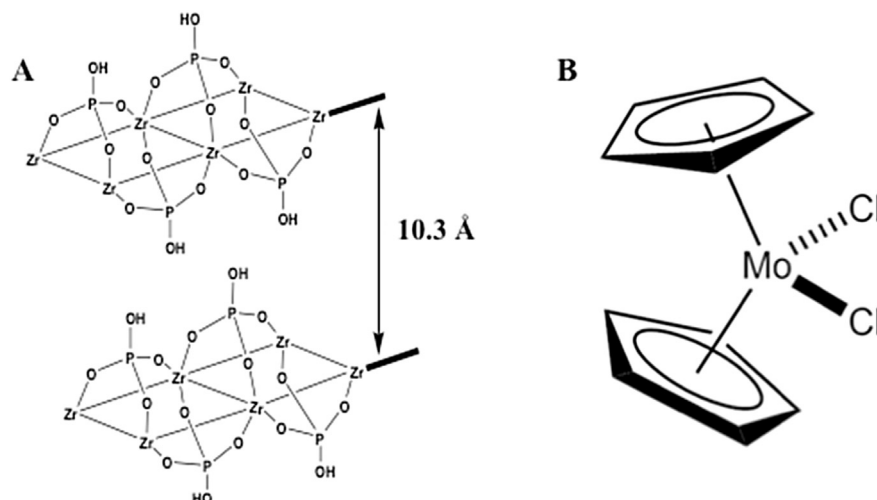

Fig. 2. Structure of (A) $Zr(HPO_4)_2 \cdot 6H_2O$, θ-ZrP and (B) molybdocene dichloride (MDC).