Inorganica Chimica Acta 468 (2017) 270–279

Contents lists available at ScienceDirect

Inorganica Chimica Acta

journal homepage: www.elsevier.com/locate/ica

Research paper

Poly(ethylene glycol)-modified zirconium phosphate nanoplatelets for improved doxorubicin delivery

Julissa González-Villegas [a], Yuwei Kan [b], Vladimir I. Bakhmutov [b], Aileen García-Vargas [c,d], Magaly Martínez [c,e], Abraham Clearfield [b], Jorge L. Colón [a,*]

[a] Department of Chemistry, P.O. Box. 23346, University of Puerto Rico, San Juan, PR 00931-3346, United States
[b] Department of Chemistry, Texas A&M University, P.O. Box 30012, College Station, TX 77842-3012, United States
[c] University of Puerto Rico Comprehensive Cancer Center, P.O. Box 363027, San Juan, PR, United States
[d] Department of Pharmacology and Toxicology, University of Puerto Rico-Medical Sciences Campus, P.O. Box 365067, Río Piedras, PR 00936-5067, United States
[e] Department of Pharmaceutical Sciences, University of Puerto Rico School of Pharmacy, P.O. Box 365067, San Juan, PR 00936-5067, United States

ARTICLE INFO

Article history:
Received 11 March 2017
Received in revised form 23 May 2017
Accepted 25 May 2017
Available online 26 May 2017

Special Volume: Protagonists in Chemistry
Dedicated to Professor Luis Echegoyen

Keywords:
Drug delivery
Nanoparticle
Zirconium phosphate
Doxorubicin
Anticancer drug
Surface modification
Poly(ethylene glycol)

ABSTRACT

Surface modification of doxorubicin (DOX) intercalated zirconium phosphate (ZrP) nanoparticles (DOX@ZrP) is proposed to improve the potential of this drug delivery system for cancer therapy. The surface of DOX@ZrP nanoparticles was modified with an amorphous layer of Zr(IV) followed by modification with monomethyl-poly(ethylene glycol)-monophosphate (m-PEG-PO_3) as a feature to increase the DOX@ZrP biocompatibility. $^{31}P\{^1H\}$MAS NMR data shows a new peak at −26 ppm corresponding to the PO_4^{3-} groups coordinated with Zr(IV) on the surface. Initial MTS cell viability assay reveals that m-PEG-PO_3/Zr(IV)/DOX@ZrP exhibits ~20% higher cytotoxicity than free DOX and the other ZrP materials when human prostate cancer PC3 cells are exposed for 48 h. m-PEG-PO_3 polymer coating of DOX@ZrP nanoparticles promise to have a strong impact on the targeting, distribution and degradation of the nanoparticles under physiological environment that should result in a more efficient chemotherapy agent than free doxorubicin.

1. Introduction

Inorganic layered nanomaterials (ILN) have become very attractive in the last decades [1]. The distinctive property of this type of materials lays on the strong covalent interactions of the atoms within an individual layer while at the same time they participate in weaker van der Waals interactions with the adjacent layers [2,3]. ILN have been used for many applications such as solar energy harvesting [4], pollutant absorbents [5,6], catalysis [7,8], electron transfer reactions [9,10], and nanomedicine [11,12]. There are different types of ILN: anionic, uncharged, and cationic [13–18]. Our research work focuses on the cationic-exchange ILNs, specifically on the layered tetravalent metal phosphate zirconium bis(monohydrogen orthophosphate) monohydrate ($Zr(HPO_4)_2{\cdot}H_2O$, α-ZrP) and the α-ZrP type of nanoparticles.

The zirconium phosphate crystal structure was first reported by Clearfield and Smith in 1968 by the single-crystal method and refined by Clearfield and Troup in 1977 [19,20]. According to the crystal structure, α-ZrP is constituted by zirconium atoms aligned in an imaginary plane with alternate phosphate groups above and below this plane. Three oxygens from each phosphate group are connected to three different Zr atoms, and the fourth is protonated and points away from the layer, either in the interlayer space or on the surface. This hydroxyl group can be deprotonated via an ion exchange reaction or via acid-base proton transfer. This structural arrangement provides a zeolitic cavity that is able to host only one water molecule per formula unit which is held in the crystal lattice by strong hydrogen bonds with the hydroxyl groups [21]. The interlayer distance of α-ZrP is 7.6 Å with each layer having a thickness of 6.6 Å [22]. A displacement of the water molecules in the lattice can occur during the intercalation process of a guest molecule. The zeolitic cavities interconnect to each other by entrances with a diameter of 2.61 Å, limiting the size of ionic species that can be incorporated by direct ion-exchange [17]. The charge field of the interlayer space can restrict the orientation and mobility of the

Abbreviations: DOX, doxorubicin; m-PEG-PO_3, monomethyl-poly(ethylene glycol)-monophosphate; ZrP, zirconium phosphate.

* Corresponding author.
E-mail address: jorge1962cr@gmail.com (J.L. Colón).

http://dx.doi.org/10.1016/j.ica.2017.05.057
0020-1693/

bound guest [22]. This material is highly stable at low pH and high temperature. Due to the versatility of this ILN an extensive list of uses and potential applications of α-ZrP have been reported such as ion exchange, sensors, polymer nanocomposites, antimicrobials, fire retardants, and non-soluble surfactants [18,23].

We have focused our interests in $Zr(HPO_4)_2{\cdot}6H_2O$ (θ-ZrP), the hydrated form of α-ZrP, which has six water molecules per formula unit and an interlayer distance of 10.3 Å, but maintains α-type layers. The θ-ZrP converts to the α-ZrP upon dehydration [24]. Using θ-ZrP for intercalation reactions while wet increases the number of compounds able to be intercalated, without a preintercalation step, compared to α-ZrP, as well as an increase in the number of potential applications of these materials. Nowadays the application of nanotechnology for delivery of chemotherapeutic drugs has become a promising area of research that aims to satisfy the necessity of side-effects inhibition. The acidic ion exchange capacity of θ-ZrP allows the incorporation of different species. During the past years, our collaborative efforts have been dedicated to the intercalation into ZrP of different anticancer drugs to develop potential drug nanocarriers to target cancer cells [25–28].

Doxorubicin (DOX) is one of the most widely used anticancer drugs with high efficiency [29]. DOX major mechanisms of action are the inhibition of Topoisomerase II and generation of radical oxygen species causing programmed cell death [30]. In spite of its effectiveness, DOX induces several acute side effects, such as cardiomyocytes degradation, due to the lack of selectivity to cancer cells [30]. Díaz et al. reported the first intercalation of DOX into zirconium phosphate layers (DOX@ZrP) as a potential drug delivery system [25]. An expansion of the interlayer distance to ca. 20 Å measured by X-ray powder diffraction (XRPD) was reported. A loading percent of 34.9% by weight of DOX into ZrP nanoparticles was achieved. Cellular studies showed a high bio- and hemocompatibility of ZrP nanoparticles, while DOX@ZrP intercalated nanoparticles showed an increment in cellular uptake and cytotoxicity in MCF-7 (breast cancer) and MDA-MB-231 (metastatic breast cancer) cell lines compared with free DOX [31]. In addition, the release of DOX was sustained for two weeks [25,31]. These results reveal the promissory anticancer potential of DOX@ZrP nanocarriers. The contact area between nanoparticles and the lipid bilayer is a crucial factor that influences the penetrating capability of these carriers [32–33]. ZrP nanoparticles are not spherical, instead they have a platelet shape which promises to be more suitable to reach the tumor endothelium and to improve contact area with the cell membrane [32]. Spherical nanoparticles tend to stay in the center of the capillary blood flow disrupting the extravasation process thorough the fenestrations, and consequently limiting their ability to recognize specific molecular markers on the tumor endothelium [34]. Although platelet-like ZrP nanoparticles modified with intercalants are attractive nanocarriers, there is interest to also modify the nanoparticle surface to add new capabilities, such as biocompatibility [35–36].

The high charge density of the surface of ZrP (up to one charge per 24 $Å^2$) is a feature of this material that can be exploited to modify the surface of this material [13]. The deprotonation (ion-exchange reaction) of hydroxyl groups and activation of the surface by means of the attachment of specific molecules open new possibilities to novel studies in the ILN area (Scheme 1). Different approaches of surface functionalization of α-ZrP for different applications have been recently reported by the Clearfield group [37–40]. The attachment of molecules to the surface may improve the biocompatibility of the ZrP nanoparticles [41]. Poly(ethylene glycol) (PEG) modification has become a widely used method to increase biocompatibility of nanoparticles [42]. Recently, NMR monitoring of PEG-modified DOX-intercalated ZrP showed partial intercalation of PEG chains into the interlayer space for sonicated samples [43]. Here we report the surface functionalization of DOX@ZrP nanoparticles with monomethyl-poly(ethylene glycol)-monophosphate (m-PEG-PO_3) for non-sonicated samples, which prevents intercalation of PEG chains. This ZrP surface PEGylation promises to improve biocompatibility of the nanoparticles [37].

2. Materials and method

2.1. Materials

Zirconyl chloride octahydrate ($ZrOCl_2{\cdot}8H_2O$, 98%) and phosphoric acid (H_3PO_4, 85% v/v) were obtained from Sigma-Aldrich. Doxorubicin hydrochloride 99% was obtained from Medchem Express Company. Monomethoxy-poly(ethylene glycol)-monophosphate (m-PEG-PO_3) 2000 MW was purchased from JenKem Technology. Ethanol was obtained from Alfa-Aesar. All reagents were used without further purification. Distilled water was obtained by using a Barnstead purification train (18 MΩ-cm).

2.2. Instrumentation

X-ray powder diffraction (XRPD) measurements were performed from 2 to 40° (in the 2θ axis) using a Bruker D8 Advance X-ray power diffractometer with Cu Kα radiation (λ = 1.5406 Å) with Bragg Brentano assembly and operated at a potential of 40 kV and a current of 44 mA. Bragg's law ($n\lambda = 2d_{hkl} \sin\theta$) was used to determine the interlayer distance in the ZrP layers considering the first order diffraction peak at the lowest diffraction angle, where λ is the wavelength of the X-ray source, d_{hkl} is the interlaminar distance between planes in the unit cell, and θ is the diffraction angle. Vibrational spectroscopy data were obtained using a Bruker-Tensor 27 FT-IR spectrometer with the OPUS Data Collection Program for the analysis. The $^{31}P\{^1H\}$, $^{13}C\{^1H\}$, and ^1H MAS NMR experiments were carried out with a Bruker Avance-400 solid-state NMR spectrometer (400 MHz for ^1H nuclei) equipped with a standard 2.5-mm MAS probe head. The standard single pulse (direct nuclear excitation, DE) and cross-polarization (CP) pulse sequences were applied for nuclei ^1H, ^{31}P, and ^{13}C at time delays needed for the full spin-lattice relaxation and the spinning rate was 7 kHz. The contact times of 2 and 6 ms were adjusted for ^{13}C and ^{31}P nuclei, respectively, to observe the regular proton-carbon and proton-phosphorus CP MAS NMR spectra. The external standards used for ^1H, ^{13}C, and ^{31}P NMR experiments were TMS and H_3PO_4 solution, respectively. Kinetics of proton-phosphorus cross polarization has been studied by variations in contact times between 100 and 10,000 μs used for the $^{31}P\{^1H\}$CP MAS NMR experiments. The kinetic data have been treated with a standard fitting computer procedure. Elemental analyses were performed using a four-spectrometer Cameca SX50 electron microprobe at a beam current of 20 nA and an accelerating voltage of 15 kV. All quantitative experiments employed wavelength-dispersive spectrometers (WDS). Analyses were carried out after standardization with pure elements. Thermogravimetric experiments were carried out on a TGA Q500 TA instrument. Samples were heated from room temperature to 1000 °C at a heating rate of 10 °C per minute under a mixture of air and nitrogen (90% air). Scanning electron microscopy (SEM) images were performed in a high-resolution field emission JEOL-JSM 7500F SEM to analyze powder samples previous deposited over ultrathin carbon film on lacey carbon support film, 400 mesh, copper was purchased from Ted Pella, Inc. The images were analyzed using scanning transmission electron microscopy (STEM) mode in bright field.

2.3. Zirconium phosphate synthesis

θ-ZrP was directly synthesized using the reflux method [44]. A 0.05 M $ZrOCl_2{\cdot}8H_2O$ solution was added dropwise to a 6 M H_3PO_4