Materials Chemistry and Physics 148 (2014) 153–158

Contents lists available at ScienceDirect

Materials Chemistry and Physics

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

Mesoporous hydroxyapatite: Preparation, drug adsorption, and release properties

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HIGHLIGHTS

- Mesoporous HA was synthesized by a simple precipitation method without any template.
- The kinetics of adsorption followed the pseudo-second-order rate expression.
- Thermodynamics investigation showed that adsorption was spontaneous and endothermic.
- DOX-loaded HA showed a long-term, steady, and pH-controlled release rate.

ARTICLE INFO

Article history:
Received 20 March 2014
Received in revised form
25 June 2014
Accepted 20 July 2014
Available online 6 August 2014

Keywords:
Surfaces
Precipitation
Adsorption
Desorption

ABSTRACT

Mesoporous hydroxyapatite (HA) was synthesized through gas–liquid chemical precipitation method at ambient temperature without any template. Structure, morphology and pore size distribution of HA were analyzed via X-ray powder diffraction, scanning electron microscopy, transmission electron microscopy, high-resolution electron microscopy and N_2 adsorption/desorption. The chemotherapeutic agent doxorubicin (DOX) was used to investigate the drug adsorption and release behavior of HA. The kinetics of DOX adsorption on HA followed the pseudo-second-order rate expression. Adsorption isotherms at various temperatures were obtained, and the equilibrium data fitted the Langmuir model. The values of thermodynamic parameters (Gibbs free energy, entropy, and enthalpy changes) demonstrated that the adsorption process was spontaneous and endothermic. In vitro pH-responsive (pH = 7.4, 5.8) controlled release was investigated. DOX-loaded HA showed a slow, long-term, and steady release rate. The release rate at pH5.8 was larger than that at pH7.4. Consequently, the as-prepared mesoporous HA has potential applications in controlled drug delivery systems.

1. Introduction

Hydroxyapatite [HA; $Ca_{10}(PO_4)_6(OH)_2$] is a chemical analog of the bone tissue mineral component. In bones and teeth, HA is present in the needle-like nanocrystalline state and is embedded in the collagen matrix [1]. So, HA exhibits excellent bioactivity, biocompatibility, and high compressive strength [2]. Due to these properties, HA (mesoporous particles, hollow microspheres, etc.) has been widely applied to controlled delivery systems for proteins [3–5], drugs [6–10], and genes [11] in the past few decades. Piskounova and her colleagues had shown that HA was an excellent tool for delivery of the bone morphogenetic protein BMP-2 both in vitro and in vivo [12,13]. Brohede investigated the fast-loading slow-release biomimetic hydroxyapatite coatings on surgical implant with the antibiotics amoxicillin, gentamicin sulfate, tobramycin and cephalothin [14]. Also bisphosphonate delivery had been extensively investigated [15]. Forsgren successfully incorporated bisphosphonates and antibiotics (cephalothin) simultaneously into a biomimetic HA implant coating [16]. Chen used arginine-modified HA nanoparticles for DNA enzymes delivery which was therapeutic applied in a nasopharyngeal carcinoma model [17].

The goal of controlled drug delivery systems is to achieve a constant, controlled, and long-period release rate. However, obvious initial burst release and short-term release were often observed. Mizushima [18] suggested that modifying the manufacturing method of HA and adding other substances were useful to delay drug release. Wang [19] prepared Poly (lactide-co-glycolide) coated HA microspheres which showed significant slower drug release and lower initial burst than that of HA

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http://dx.doi.org/10.1016/j.matchemphys.2014.07.024
0254-0584/

microspheres. Li [20] synthesized alendronate functionalized HA, and the materials showed relatively slower release rate compared with HA. Recently, Steckel and his team [21] investigated that HA coatings deposited on TiO_2 coated fixation pins could exhibit bactericidal effects against staphylococcus aureus in agar medium for 6 days after loading with the antibiotics tobramycin for only 5 min. The same team also very recently showed that by optimizing the HA deposition process the tobramycin delivering HA coatings could deliver pharmaceutically relevant amounts of tobramycin over 12 days [22].

It is well known that biological performance of drug delivery systems are controlled by the drug adsorption/desorption (release) behaviors which depend on the structure, properties and the surrounded environment [23,24]. Compared to HA nanoparticles, mesoporous HA exhibited higher drug–loading capacity and enhanced drug release efficacy, which was due to its large surface areas and high pore volumes [25]. In this paper, we present a simple, modified precipitation method to synthesize mesoporous HA. Pure HA was obtained at ambient temperature without any template. Using a water-soluble anticancer drug, doxorubicin (DOX), as a drug model, the adsorption kinetics and thermodynamics of DOX on HA were investigated. Release profile exhibited that DOX-loaded HA had a slow, sustained, and pH-controlled release rate in the phosphate buffered saline (PBS).

2. Materials and methods

2.1. Materials

Calcium nitrate ($Ca(NO_3)_2{\cdot}4H_2O$), ammonium phosphate ($(NH_4)_2HPO_4$) and ammonia solution ($NH_3{\cdot}H_2O$) were purchased from Tianjin Guangfu Fine Chemical Research Institute (China). Anhydrous ethanol was obtained from Shanghai Zhenxing First Chemical Industry Factory (China). DOX was purchased from Yongnuo Pharmaceutical Factory. All reagents were of analytical purity and used without further purification.

2.2. Preparation of HA

HA was prepared via a gas–liquid chemical precipitation method. Ethanol solution of $Ca(NO_3)_2{\cdot}4H_2O$ (12.5 mmol, 50 ml) and aqueous solution of $(NH_4)_2HPO_4$ (7.5 mmol, 50 ml) with Ca/P ratio of 1.67 were prepared. As shown in Fig. 1, the ethanol solution of $Ca(NO_3)_2{\cdot}4H_2O$ was continuously stirred and placed in a bigger sealed beaker with the ammonia solution. The pH of $Ca(NO_3)_2{\cdot}4H_2O$ solution was adjusted to 10.5 ~ 11 using continuously generated NH_3 gas. Under vigorous stirring, the aqueous solution of $(NH_4)_2HPO_4$ was added dropwise to the $Ca(NO_3)_2{\cdot}4H_2O$ solution, and the resulting precipitates were stirred for another 2 h and stored for 24 h. The aged precipitate was washed by water and anhydrous ethanol and dried at 60 °C for 24 h.

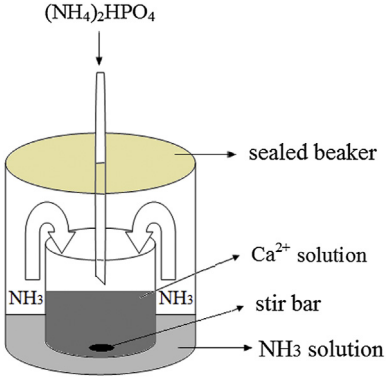

Fig. 1. Schematic representation of the experimental setting.

2.3. Characterization

Phase analysis was conducted via Purkinje XD-3 powder X-ray diffraction (XRD) with a Rigaku D/max-γ_A rotation anode X-ray diffractometer (CuKα, $\lambda = 0.15418$ nm). To examine the size and morphology of the as-synthesized samples, transmission electron microscopy (TEM) and high resolution transmission electron microscopy (HRTEM) images were obtained using a JEOL JEM-2100 high resolution electron microscopy with an accelerating voltage of 200 kV. Scanning electron microscopy (SEM) images were obtained using a HITACHI S4800 scanning electron microscope operated at 5 kV.

The specific surface area and pore size distribution were determined using MICROMERITICS ASAP2020M + C surface area and porosity analyzer with a degas temperature of 105 °C and an outgas time of 12 h according to the Brunauer–Emmett–Teller (BET) equation and the method of Barrett–Joyner–Halenda (BJH), respectively.

2.4. Adsorption of DOX

DOX solutions in various concentrations were freshly prepared.

To confirm the time needed for adsorption equilibrium establishment, adsorption kinetics was investigated. HA at 10 mg was mixed with 5 ml DOX solution (734 μg ml^{-1}). The resulting suspension was shaken (220r/min) in a water bath shaker (HQ45B, Wuhan Science and Technology Instrument Factory) at 301 K for 24 h. At certain time intervals, samples were withdrawn from the suspension and centrifuged. The concentrations of DOX in the supernatants, c_t, were then determined through ultraviolet–visible (UV–vis) spectroscopy (Purkinje TU-1901) at a fixed wavelength of 480 nm. The adsorption quality of DOX was defined as follows:

$$Q_t = \frac{(c_o - c_t)\cdot V}{m} \quad (1)$$

where Q_t is the amount of DOX adsorbed on HA (μg mg^{-1}), c_o and c_t are the initial and residual concentrations at time t of DOX, V is the volume of the DOX solution, and m is the mass of HA (mg).

For the determination of the maximum amount of DOX, 10 mg HA was impregnated with 5 ml DOX aqueous solution at different concentrations. The suspensions were shaken for 22 h at 296, 301 K, and 306 K. After equilibrium, the liquid and solid phases were separated by centrifugation, and the residual drug concentrations in the liquid phase were determined.

2.5. In vitro release of DOX

HA (0.1 g) was immersed in 40 mg ml^{-1} DOX solution and shaken for 22 h. The powders were isolated through filtration, washed with a small volume of water, and dried vacuum-dried to obtain the DOX-loaded HA.

In vitro release of DOX from HA was performed at 37 °C in PBS (pH = 7.4, 5.8). DOX-loaded HA (84.57 μg DOX/mg HA) at 25 mg was immersed in the release medium (10 ml). At predetermined time intervals, 6 ml buffer solution was obtained for UV–vis analysis, after which the same volume of fresh buffer solution was injected into the system.