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Synthesis of Nano Hydroxyapatite: Application in Drug Delivery of Sulfasalazine

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Abstract

In this work we synthesized Nano hydroxyapatite (HAp) by Sol-gel method, Then we functionalized hydroxyapatite nanoparticle by use of 3-Aminopropyl trimethoxysilane (APTMS) , to improve the loading and control release of sulfasalazine drug bonded to APTMS. The drug release patterns from Sulfasalazine loaded HAp nanoparticles at pH value 8 For 6h, Sulfasalazine loaded functionalized HAp nanoparticles (Sulfasalazine loaded HAp-APTMS) at pH value 8 as in the intestine for 48h. Moreover, the functionalized HAp showed relatively slower release rate of sulfasalazine compare with non functionalized HAp. because the strong ionic interaction between NH_2 group in sulfasalazine in HAp-APTMS. On other side, the functionalized HAp loaded more drug than pure HAp. The synthesized nanoparticles and functionalized HAp characterized by Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), Fourier transform infrared (FT-IR) and UV/Vis analysis techniques. Then the obtained material was studied in the simulated body fluid (SBF) to this investigated storage and release properties.

1. Introduction

Since mesoporous MCM-41 was introduced as a drug storage release system for the first time [1], mesoporous materials have drawn much attention for their excellent properties such as high surface area, tunable pore size and relatively large pore volume [2]. So far, many functionalized nanoparticles materials have been synthesized to further increase the ability of drugs loading, transfer them to the target organism and then release in a desirable period of time [3-6]. Although mesoporous drug carriers are experiencing a booming development, most of them are based on silica

or doped-silica materials; while, the overuse of silica may raise the possibility of silicosis, chronic bronchitis, and even pulmonary cancer [7]. So it is still necessary to synthesize more biocompatible materials to loading the drugs. Hydroxyapatite (HAp), with the molecular formula of $Ca_{10}(PO_4)_6(OH)_2$, has a dominant position in the composition of bone and teeth in amniotes including human. Furthermore, it has been widely studied as important biocompatible materials because of its chemical similarity to the natural Ca phosphate mineral present in a biological hard tissue [8-11]. Owing to its bioactivity, biodegradability and osteoconductivity properties [12-13], HAp has been widely used in many medical applications. For example, it can be used as a material for metallic implant coating or for bone cavity fillings [14-15]. Several methods have been reported for the synthesis of mesoporous hydroxyapatite nanoparticles (mHAp), such as mechanochemical synthesis [16,17], combustion preparation [18], and various techniques of wet chemistry, such as direct precipitation from aqueous solutions [19], electrochemical deposition [20], sol-gel procedures [21-24], hydrothermal synthesis [25-26], emulsion or micro-emulsion routes [27], ultrasonic route [28], and etc.

Sulfasalazine (SSN) (Fig. 1), 2-Hydroxy-5-[(E)-2-{4-[(pyridin-2-yl) sulfamoyl] phenyl} diazen-1-yl]-benzoic acid is a sulfa drug, a derivative of mesalazine 5-aminosa;cylic acid abbreviated as 5-ASA), used primarily as an anti-inflammatory agent in the treatment of in flammatory bowel disease as well as for rheumatoid arthritis and ulcerative colitis [29-30].

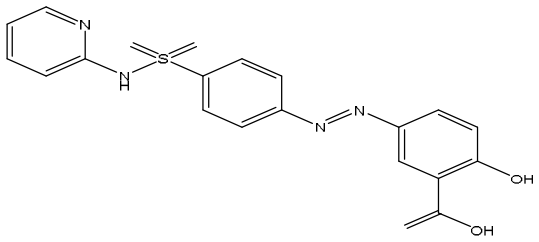

Fig. 1. Structure of Sulfasalazin

In this work, we present a produse hydroxyapatite nanoparticles with wet chemical synthesis, The drug storage and release kinetic in simulated body fluid (SBF) was investigate on the nonmodified and APTMS (Fig 2). modified HAP this system using sulfasalanine (SSN) as a model drug.

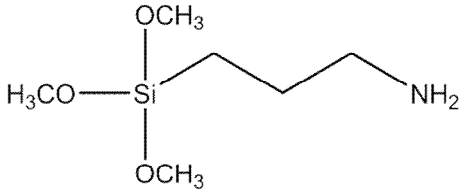

Fig. 2. Structure of APTMS

2. Experimental procedure

2.1. Materials and methods

All the chemical substance contains: 3-Aminopropyl trimethoxysilane (APTMS), Suflasalazine, Ethanol, Methanol, NH4H2PO4, Ca(NO3)2.4H2O, Acetone, were purchased by sigma Aldrich.

2.2. Preparation hydroxyapatite nanoparticles

Hydroxyapatite nanoparticles was synthesized according to the method described in the paper[31]. Briefly, 1.67 M $Ca(NO_3)_2.4H_2O$ solution in ethanol (pH 11) was added at a constant rate of 4 ml/min using a peristalatic pump into the 1M $NH_4H_2PO_4$ solution under severe stirring room temperature. After the white precipitate solution was observed, this is aged for 24h at room temperature. The gel

achieved after aging course was filtered and washed several times by using double distilled water to remove NH_4^+(aq) and NO_3^- (aq) ions. moreover, the gel was washed several times by using acetone and filterd. The Product obtained by this process was then dried at 80 °C for 12 h in an oven and the dried powder was calcined at 600 °C for 5 h.

2.3. Chemical synthesis of APTMS-functionalized hydroxyapatite nanoparticles

Amino functionalized HAp was prepared by this method: 1g of calcined HAp was added to 50 ml of dry toluene in a 250 ml flask. Then 1 ml of 3-Aminopropyl trimethoxysilane (APTMS) was added and stirred for 24h . the product was filtrated, washed by toluene and ehanol and dried in 70 °C.

2.4. Drug loading

Sulfasalazine was dissolved in 50 ml sodium hydroxide 0.1 M. And 0.5g of modified HAp was soaked in a sulfasalazine solution in a closed vial under static condition for 24h. The solid product was filtered off, and washed three times and dried at room temperature for 24h. The amount of adsorbed drug was estimated spectrophotometrically from absorbance values before and after the adsorption.

2.5. Drug Release

Due to the stability consideration, the long time in vitro drug release study was only performed using purified water as the release. Sulfasalazine release system was obtained by the addition of 200 mg SSN@HAp sample in 100 ml simulated body fluid (SBF) in a beaker at 37 ˚C under static conditions. (the simulated body fluid has an ionic composition similar to the human body plasma). replaced immediately with fresh SBF into the system which was accounted for when calculating the amount release. Samples were collected at different time points and the released sulfasalazine concentration in the solution was measured by UV/Vis spectrometer at wavelength of 359 nm.

3. Characterization

X-ray diffraction (XRD) analysis of the mHAp sample was carried out by using Philips PW 1800, X-ray diffractometer using Ni-filtered Cu Kα radiation.

The surface nanoparticles of the sample was obtained by Scanning electron microscopy (SEM), by means a LEO-1455VP by an energy dispersive X-ray spectroscopy (EDX).

The UV-Vis absorbtion spectra were measured with a Shimadzu UV-Vis scanning spectrometer.

Fourier transform infrared (FTIR) spectrum were obtained using on Shimadzu 460 spectrometer. The Sample was prepared in KBr pellets for FTIR analysis.

4. Result and discussion

The XRD pattern of pure HAp nanoparticles obtained after calcination at 600 ˚C is shown in Fig.3. According to this figure, there is no peak attributable to possible impurities, which indicates the final product is of high purity. All possible peaks can be indexed to pure hexagonal phase of HAp (*P63mc*) with lattice parameter of a = 4.5460 A˚ From XRD data, the crystallite diameter (D_c) of HAp nanoparticles was calculated to be 30 nm using the Scherer equation as below:

$D_c = K\lambda/\beta\cos\theta$ Scherer equation

where β is the breadth of the observed diffraction line at its half intensity maximum, K is the so-called shape factor, which usually takes a value of about 0.9, and λ is the wavelength of X-ray source used in XRD.

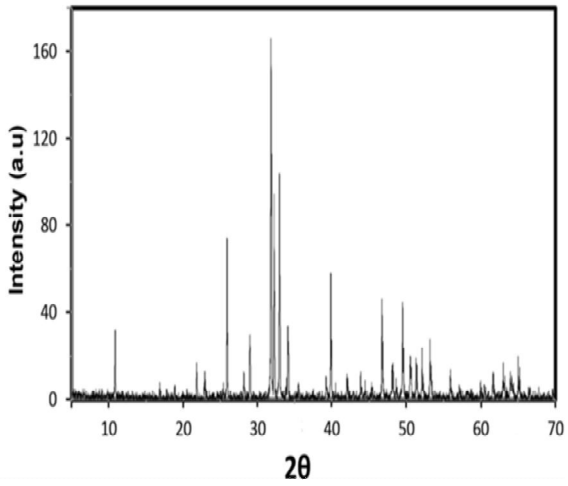

Fig. 3. XRD pattern of nano hydroxyapatite

The particle size of the as-synthesized pure nano-HAp is also measured using scanning electron microscopy (SEM) According to Fig.4 the morphologhy of HAp particles is sphere-like nanostructures with particle size of ~40-50 nm.

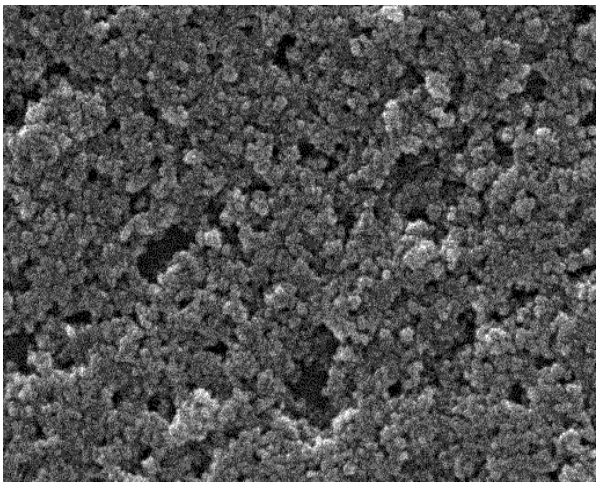

Fig. 4. SEM image of the synthesis HAp

Fourier Transform Infrared Spectroscopy (FTIR) for investigated samples are collected. Characteristic and adsorption bands appear there: for υ_4 PO_4^{3-} at 603 and 567 cm^{-1}; for υ_3 PO_4^{3} at 1081 and 1041 cm^{-1} ; the υ(OH) stretching vibration is observed at 3420 cm^{-1} in Fig.5.a.

Fig.5.b showed the FTIR spectrum of Hap functionalized with APTMS. The intensity of υ(OH) in 3420 cm^{-1} is weak, because the interaction NH_2 group with OH group of HAp.

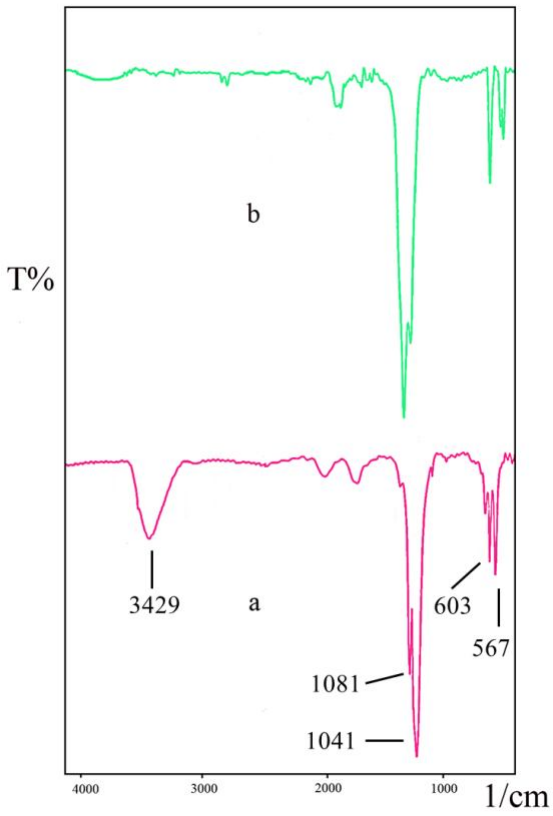

Fig. 5. FT-IR Spectra of (a) pure HAp (b) APTMS-functionalized Hap

4.1. Drug release profile

Fig. 6. shows the curves of the percentage of drug release as function of time. It can be seen that the total amount of the drug released after 48h was 13% in the case of sample SSN@HAp and 100% in the case of the sample SSN@HAp-APTMS.

The drug release patterns from SSN@HAp nanoparticles at pH value 8, Sulfasalazine loaded functionalized HAp nanoparticles (SSN@HAp-APTM) at pH value 8 as in the intestine, Fig. 6 a, b respectively.

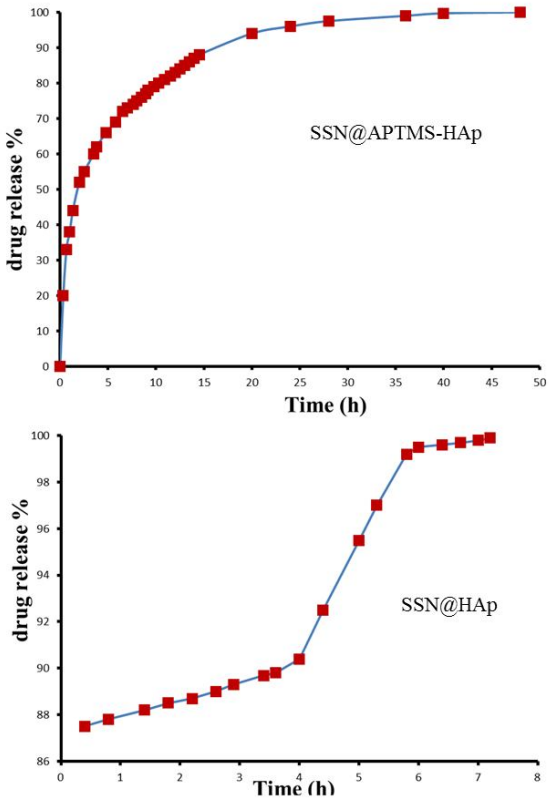

Fig. 6. SSN release by HAp in pH 8 and APTMS-functionalized HAp in pH 8.

Sulfasalazine loaded HAp shows immediate releas of sulfasalazine up to 87%, sulfasalazine-HAp-NH_2- shows up to 30% release at the bginning. This is because the interaction between sulfasalazine molecules with OH group in hydroxyapatite particle in weaker than that of sulfasalazine with NH_2- group in SSN@HAp-NH_2-. At the initial brust release in both can for this reason: by this initial brust release the drug molecule is brought close to the surface.

5. Conclusion

In this work, we studied the amine-modified and nonmodified hydroxyapatite nanoparticles for the adsorption and desorption properties of the Sulfasalazine delivery. The result showed modification of the surface of the HAp with amino groups lead towards better carriers drug in simulated body fluid (SBF). And the release profile of SSN@HAp-APTMS showed steady slow drug release for 48h.

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