Nanomed Res J 7(4):360-369, Autumn 2022

RESEARCH ARTICLE

Preparation of titania-hydroxyapatite composite nanoparticles as a potential drug carrier for ibuprofen

Fatemeh Ghourchian[1], Leila Torkian[2,3], Negar Motakef Kazemi[4,*]

[1] *Department of Nanochemistry, Faculty of Pharmaceutical Chemistry, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.*

[2] *Department of applied Chemistry, Islamic Azad University, South Tehran Branch, Tehran, Iran*

[3] *Research Center of modeling and Optimization in Science and Engineering, Islamic Azad University, South Tehran Branch, Tehran, Iran.*

[4] *Department of Medical Nanotechnology, Faculty of Advanced Sciences and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.*

ARTICLE INFO

Article History:
Received 19 Aug 2022
Accepted 06 Oct 2022
Published 01 Nov 2022

Keywords:
Titanium dioxide
Hydroxyapatite
Composite
Nanoparticles
Ibuprofen

ABSTRACT

Objective(s): Hydroxyapatite (HAP) is a natural calcium apatite mineral. Hydroxyapatite has many applications in the field of biomedicine and drug delivery systems. Titanium dioxide (TiO2) is becoming increasingly important because of its potential use in new medical treatments. In this study, titania-hydroxyapatite composite nanoparticles were evaluated as a potential drug carrier for ibuprofen.

Methods: Titania-hydroxyapatite composite nanoparticles (titania-HAP) with two different titania weight ratios (30 and 70 wt%) were prepared and loaded with ibuprofen (IBU) as a pain reliever drug. The composites were studied as potential carriers of ibuprofen by X-ray diffraction (XRD) patterns, field emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM) analyzes, energy dispersive X-ray spectrum (EDX), Fourier transform infrared spectroscopy (FTIR), and thermal gravimetric analysis (TGA). Cytotoxicity of nanocomposites was investigated in vitro on osteosarcoma cell line by MTT assay.

Results: The functional groups were investigated by FTIR. Size and morphology of samples were evaluated by FESEM and TEM. Chemical composition and phase formation were confirmed by EDX and XRD patterns. Based on the results of MTT assay, no significant effect was observed on MG-63 cell lines. TGA was used to determine the amount of ibuprofen loaded on the composites.

Conclusions: Ibuprofen release was studied in neutral and acidic simulated media by ultraviolet–visible (UV-visible) spectroscopy and the results showed better sustained release of the drug in acidic medium. Titania-hydroxyapatite composite nanoparticle can have a good potential for medicinal applications.

How to cite this article
Ghourchian F., Torkian L., Motakef Kazemi N. Preparation of titania-hydroxyapatite composite nanoparticles as a potential drug carrier for ibuprofen. Nanomed Res J, 2022; 7(4): 360-369. DOI: 10.22034/nmrj.2022.04.006

INTRODUCTION

HAP ($Ca_{10}(PO_4)_6(OH)_2$) is a calcium phosphate-based bio-ceramic with a composition similar to bone tissue. Due to the outstanding characteristics of biocompatibility, low toxicity and osteoconductive properties, HAP materials have attracted considerable attention in medical and biotechnological applications [1, 2] and absorb biomaterials [3]. Its common forms have low mechanical strength, which limits their use as independent materials. Therefore, various reinforcements have been used to improve the overall strength of HAP materials, including ceramic particles such as titania and its alloys, alumina and zirconia [4]. These modified composites have shown excellent properties that are good candidates for implant coatings [5, 6], bio-functional scaffolds, bone substitutes [7], tissue

* Corresponding Author Email: *motakef@iaups.ac.ir*

repairs [8], gene [9], bone engineering applications [10], dentistry [6, 11], protein delivery [12], antitumor [13], and drug carriers [14, 15]. In recent years, hydroxyapatite polymer composite has been used for various applications. The use of composites based on nano hydroxyapatite, highly porous structures, large surface area and light weight also provides the unique advantage of nanotechnology. The ability to adjust the particle size, composition and morphology of hydroxyapatite composites makes them suitable candidates for the delivery of various pharmaceutical compounds.

In recent decades, titanium dioxide particles have been widely used in medical technology due to their availability, biocompatibility, antimicrobial nature, mechanical strength, and chemical stability. Many interesting works have been published on the use of titanium dioxide nanoparticles to improve the physical and chemical properties of organic materials [16], dental products [17, 18], biosensors [19], drug delivery [20], medicine [21, 22], and biomedical applications [23]. Studies have confirmed that nanostructured titanium dioxide stimulates the formation of osteoblast-like hydroxyapatite [24] and the role of hydroxyapatite-titania composites in bone fusion [25]. Recently, titanium dioxide polymer composite has been used for different purposes. One of the important applications of titanium dioxide nanocomposite is the controlled drug release against pathogens [26].

Ibuprofen is widely used to treat pain or inflammation as a non-steroidal anti-inflammatory drug. IBU is the insoluble drug model and is mostly used for sustainable release studies [27, 28]. A wide range of reports have been reported for small bowel mucosal fractures [29] and gastrointestinal problems [30, 31] due to the short half-life of ibuprofen (~ 1-3 hours) [32]. Therefore, modifying the release rate of ibuprofen by loading it into polymer systems has been studied in a wide range of studies [33]. Sustained release formulations allow slow and controlled release of the drug, reduce toxicity, side effects and improve drug bioavailability in body fluids. The aim of this work was to prepare titania-hydroxyapatite composites with ibuprofen and evaluate the possibility of using composite materials as drug delivery systems. Here we report the synthesis of hydroxyapatite nanoparticles and titanium-loaded hydroxyapatite composites at 30 and 70 wt%. Then, ibuprofen was used as a model analgesic drug and loaded into composite nanoparticles.

MATERIALS AND METHODS

Materials and apparatus

Ibuprofen was provided from Hakim pharmaceutical company, Tehran, Iran. Calcium hydroxide ($Ca(OH)_2$), ethanol 96% (C_2H_6O) and phosphoric acid were purchased from Merck Company (Darmstadt, Germany).

The samples were identified using XRD, FESEM-EDX, TEM, and FTIR analyzes. TGA analysis combined with UV-visible spectroscopy was used to check drug loading. *In vitro* release of samples was evaluated by UV-visible spectroscopy. The toxicity of nanoparticles was assayed by MTT method. Tetrazolium dye MTT was added to the extracts, and since MTT is only reduced by living cell mitochondrial dehydrogenase, the amount of formazan produced during this reaction is related to the number of living cells. Formazan is a purple-blue precipitate that then dissolves in isopropyl alcohol, and the optical density (OD) of the resulting purple solution is related to the number of living cells.

The X-ray diffraction spectra of the powder were recorded at room temperature by Philips PW1800/00 diffractometer operating at 40 kV and 30 mA, Cu (Kα = 1.54056 Å). The morphology and size of nanostructures were investigated by FESEM (Zeiss-SIGMA VP, Germany) with gold coating and TEM (Zeiss, EM10C-100 KV, Germany). FTIR spectra were recorded in the 400-4000 cm^{-1} spectrum with Shimadzu FTIR-8400S. Thermal analyses were performed using differential scanning calorimetry method with METTLER TOLEDO TG1 system. UV-visible spectroscopy was recorded using a UV-Visible spectrophotometer PG Instrument T80.

Preparation of HAP powder

Hydroxyapatite nanoparticles were prepared by neutralization method [34]. To prepare 9.95 mmol of hydroxyapatite, 7.4 g of calcium hydroxide was first dissolved in deionized water (100 ml). Then, 4.11 ml of phosphoric acid (85%) was gradually added to the calcium hydroxide suspension under a constant magnetic stirrer for 1 hour. The resulting suspension was kept for 2 days in a closed container at 50-70° C while stirring. The mixture was then centrifuged, isolated, and suspended again in deionized water (at 100° C for 1 hour). The obtained sample was filtered and dried in an oven at 110 ± 5 °C for 5 hours. Finally, the resulting powder was manually ground in a mortar and then calcined at 800 °C for 0.5 hour. The formation of hydroxyapatite nanoparticles was confirmed by FTIR, FESEM, TEM and XRD spectroscopy.

Preparation of titania-hydroxyapatite composites (titania-HAP)

30 wt% titania-HAP composite: 1.4 g of hydroxyapatite powder was dissolved in 1.4 mL of ethanol (96%). Then 60 mL of colloidal solution of titania nanoparticles (anatase form, 1%) was added to the solution. The sample was subjected to a magnetic stirred for 2 days in a closed container. Finally, the obtained sample was filtered and dried at room temperature.

70 wt% titania-HAP composite: 0.8 g of hydroxyapatite powder was dissolved in 0.8 mL of ethanol (96%). 120 mL colloidal solution of titania nanoparticles (anatase form, 1%) was added to the solution and the sample was magnetically stirred in a closed container for 2 days. The obtained sample was filtered and dried at room temperature. The prepared composites were characterized by XRD and EDX analyses.

Preparation of ibuprofen-loaded composites

Drug-specific ratios of nanoparticles (1:1 and 2:1) were selected. For this purpose, precise amounts of nanoparticles were added to ibuprofen ethanol solutions with solubility of ibuprofen in ethanol 25g/ml and the mixtures were magnetically stirred for 48 hours. The resulting mixtures were centrifuged (10 minutes), isolated and the drug-loaded composites were dried in an oven.

Cell culture

MG-63 (NCBI C555) cell lines were obtained from Pasteur Institute, Tehran, Iran. Cells were grown in a culture flask containing RPMI medium containing 10% FBS. The flask was incubated at 37 °C in an atmosphere of 90% humidity and 5% CO_2. The culture medium was changed every 3-4 days.

Extraction

Sample extracts were prepared in accordance with EN ISO 10993-5 regulation. The nanoparticles were incubated at 37 °C and then 1 mL of culture media were added to each sample (0.1 g of sample per 1 mL of culture medium). The supernatants were collected after 3 and 7 days of incubation at 37 °C and added to MG-63 culture media. Culture medium (RPMI) incubated in empty wells was used as negative controls.

Cytotoxicity evaluation test

MG-63 cell lines were seeded in 96 well culture plates with a ratio of 1×10^4 cell to well in 100 µl culture media. Next, the media were removed from the wells and replaced with 90 µl of each sample extract, supplemented with 10 µl of FBS, and the well plates were incubated for 24 hours at 37 °C to ensure that the cells attachments to the well plates. Then, 100 µl MTT reagent solution in PBS (0.5 mg/ml) was added to each well. The cells were incubated with MTT reagent for 24 hours at 37 °C. The MTT reagent and medium were removed and the purple formazan precipitate was dissolved in isopropanol by continuous shaking for 15 minutes. Finally, the amount of soluble formazan in the solution was calculated by optical density (OD) measurement at the wave length of 545 nm using a micro plate reader (ELISA MicroPlate Reader StatFax 2100, USA). The cell viability (%) relative to controls (without nanoparticles) was calculated by equation (1):

Equation (1):

$$Toxicity\% = (1 - \frac{mean \text{ OD of sample}}{mean \text{ OD of control}}) \times 100$$

$$Viability\% = 100 - Toxicity\%$$

In vitro ibuprofen release

First, the hydrophobic ibuprofen drug was incorporated into the composites by physical adsorption for 48 hours at room temperature. 10 mg of each drug-free nanoparticles (HAP nanoparticles, 30% and 70 wt% titania-HAP composites) were placed in 2 mL glass vials. 2 mL of simulated body fluids (SBFs with pH 7.4, and pH 4.7) were added to each vial and these vials were analyzed as the blanks. Sample vials were prepared by adding 10 mg of drug-loaded nanoparticles (ibuprofen-loaded HAP nanoparticles and both ibuprofen-loaded titania-HAP composites) to 2 mL of simulated body fluid solutions in glass vials. Samples and blanks were stirred in closed vials at 37 °C and measurements were performed by cumulative release method. A small number of centrifuged vials was removed from the medium after 0.5, 1, 2, 4 and 6 hours, and the fresh simulated fluids (SBFs at pH 7.4 and pH 4.7) with equivalent volume were replaced immediately. The concentration of ibuprofen released in the samples was measured by UV-Visible spectroscopy at the maximum wavelength of 222 nm (each sample was measured three times).

Characterization

The structure and morphology of synthesized hydroxyapatite nanoparticles were confirmed by FTIR, XRD, FESEM and TEM analyses and matched with the HAP observed by literature [34].

RESULTS AND DISCUSSION

XRD

The crystalline phases of the nanocomposites were identified by XRD analysis. The main diffraction peaks of hydroxyapatite from the (002), (211), (112), (300) and (202) in the range 25–35° (2θ) were according to JCPDS standard card number 009-0432 [35]. The average crystal size of 34.97 nm for hydroxyapatite particles was estimated by Debye Scherrer equation (base on the crystal plane of (211)), and confirmed the successful synthesis of nano-sized particles (Fig. 1 a). Titanium dioxide (titania) nanoparticles were loaded into HAP using a simple impregnation method. Fig. 1 b shows the XRD patterns of 70 wt% titania-hydroxyapatite sample. The diffraction peaks at 2θ are 24.8°, 37.3°, 47.6°, 53.7°, 55.1° and 62.2° for the crystal plates (101), (004), (200), (105), (211) and (204) that agreed with JCPDS card number 21-1272 and confirmed the crystalline phase of TiO_2 anatase [34]. X-ray diffraction results confirmed the crystal structure.

Electron microscope

Fig. 2 shows TEM and FESEM images of the prepared hydroxyapatite particles, which confirm the spherical nanostructure and uniform size of the hydroxyapatite nanoparticles. The average particle diameter is 50-70 nm.

The sample composition was analyzed by EDX analysis. As shown in Fig. 3 a and b, the energy dispersive X-ray spectra of 70 and 30 wt% titania-hydroxyapatite composites show peaks of calcium (Ca), phosphorus (P), and titanium (Ti) which confirmed the presence of hydroxyapatite with different contents of titanium.

FTIR

FTIR spectra of pure hydroxyapatite nanoparticles and drug-loaded titania-HAP composites are shown in Fig. 4. Compared to the FTIR spectra of hydroxyapatite nanoparticles, the ibuprofen-loaded of 30 and 70 wt% titania-HAP composites showed adsorption bands of ibuprofen around 1500-1600 cm^{-1} (C=C and C=O), (Fig. 5 b and c) [36]. The peaks related to stretching vibration of COO^- moiety of IBU were also observed at 1383 cm^{-1}. A broad band at 3000-3500 cm^{-1} was assigned to O-H stretching vibration

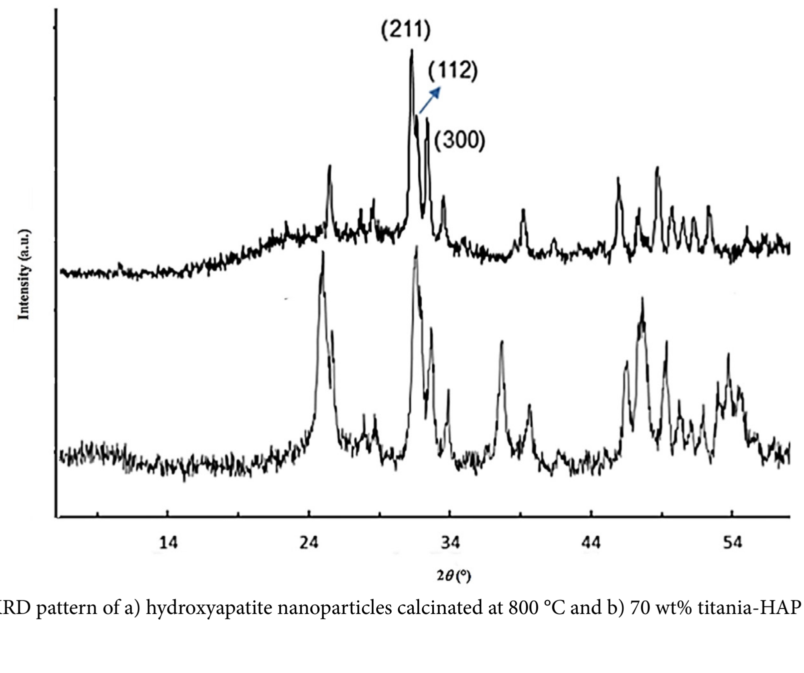
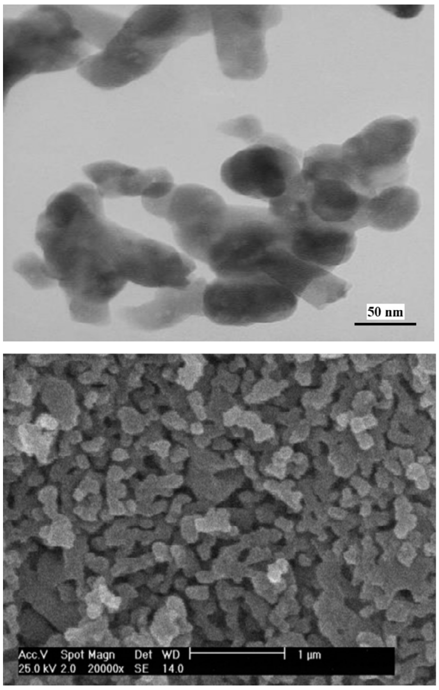


Fig. 1. XRD pattern of a) hydroxyapatite nanoparticles calcinated at 800 °C and b) 70 wt% titania-HAP composite.

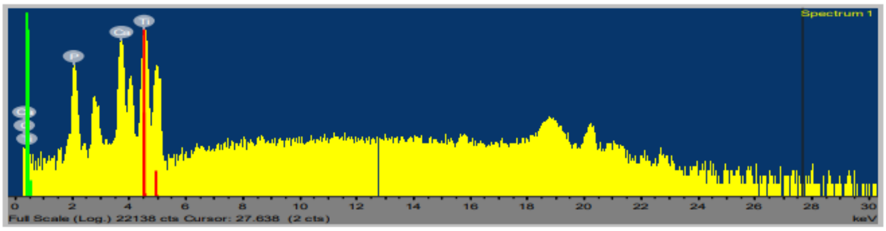


a

b

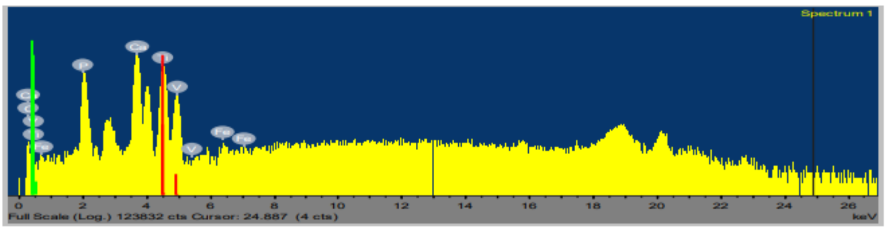
Fig. 2. a) TEM and b) FESEM images of hydroxyapatite nanoparticles.

a)



Elem.	Line	Mass[%]	3sigma	Atomic[%]	Intensity[cps/mA]	Formula	Mass[%]	Molecule[%]
15 P	K	6.42	0.16	5.33	213.82	**P2O5**	**14.71**	8.01
20 Ca	K	16.49	0.21	10.59	957.5	**CaO**	**23.07**	31.79
22 Ti	K	37.3	0.24	20.05	1892.81	**TiO2**	**62.23**	60.2
O		39.79	0.28	64.03				

b)



Elem.	Line	Mass[%]	3sigma	Atomic[%]	Intensity[cps/mA]	**Formula**	**Mass[%]**	Molecule[%]
15 P	K	11.5	0.19	9.32	448.46	**P2O5**	**26.63**	14.1
20 Ca	K	29.09	0.28	18.21	1605.37	**CaO**	**41**	55.1
22 Ti	K	19.14	0.23	10.03	870.44	**TiO2**	**32.37**	30.34
O		39.64	0.31	62.17				

Fig. 3. EDX spectroscopy of a) 70 wt% titania-HAP and b) 30 wt% titania-HAP composites.

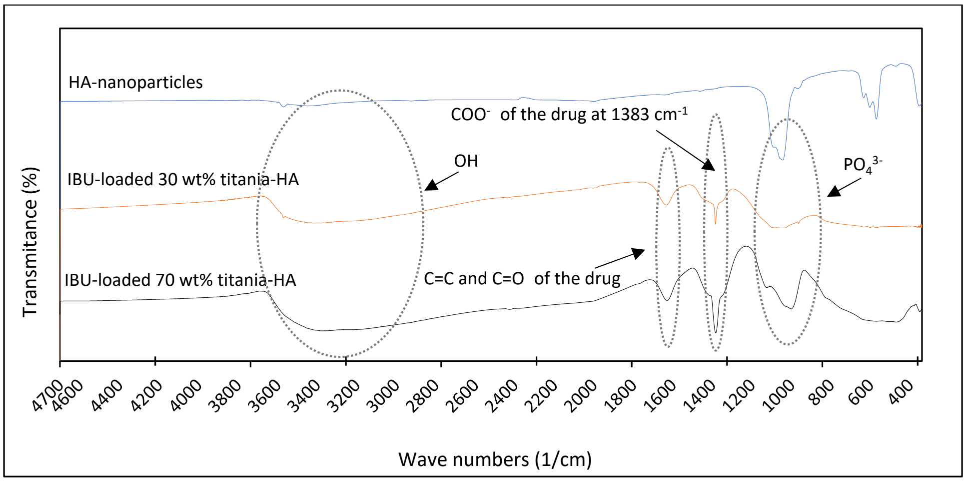


Fig. 4. FTIR spectra of hydroxyapatite nanoparticles and ibuprofen-loaded 30 and 70 wt% titania-HAP composites.

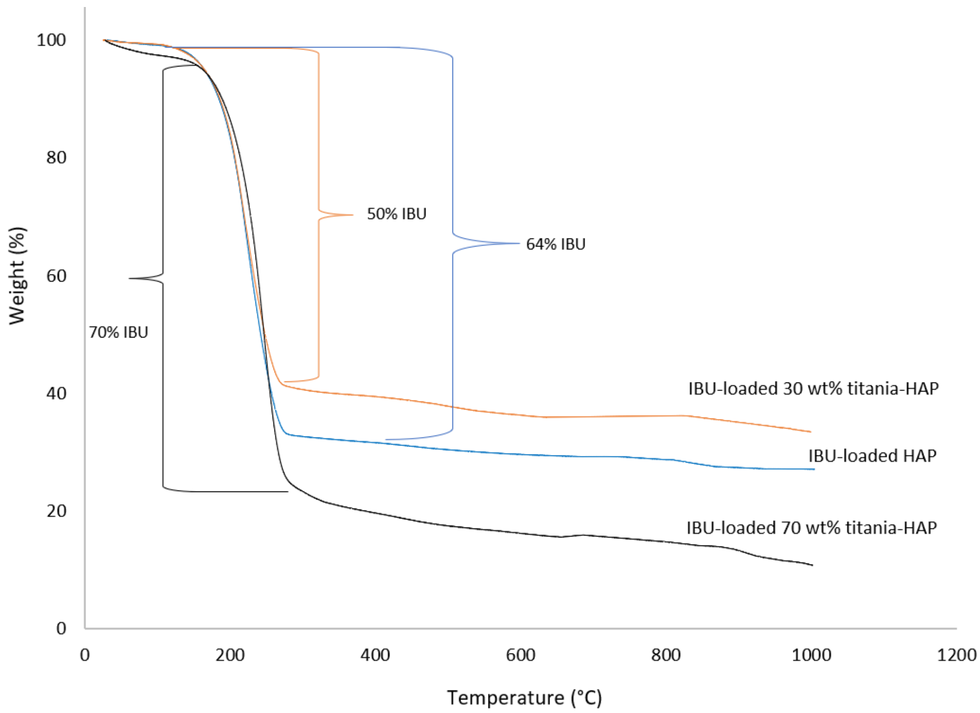


Fig. 5. TGA diagram of IBU-loaded HAP and IBU-loaded titania-HAP composites.

of O-H in titania-HAP composites. The peaks related to the bending and stretching vibrations of hydroxyapatite phosphates groups (PO_4^{3-}) are seen at 950-1100 cm^{-1}. FTIR spectra of both drug-loaded titania-HAP composites show the characteristic peaks corresponding to the bending modes of the titania surface hydroxyl groups at 1631 cm^{-1} and the bending and stretching modes of Ti–O–Ti in the 400-700 cm^{-1} [37, 38]. Based on FTIR results, the functional groups were confirmed qualitatively.

TGA

Thermogravimetric analysis of ibuprofen loaded composites is shown in Fig. 5. The samples were heated from ambient temperature to 1000 °C in a nitrogen atmosphere at a constant rate of 10 °C/min. According to the degradation temperature of ibuprofen (250 °C), major weight loss at 150-

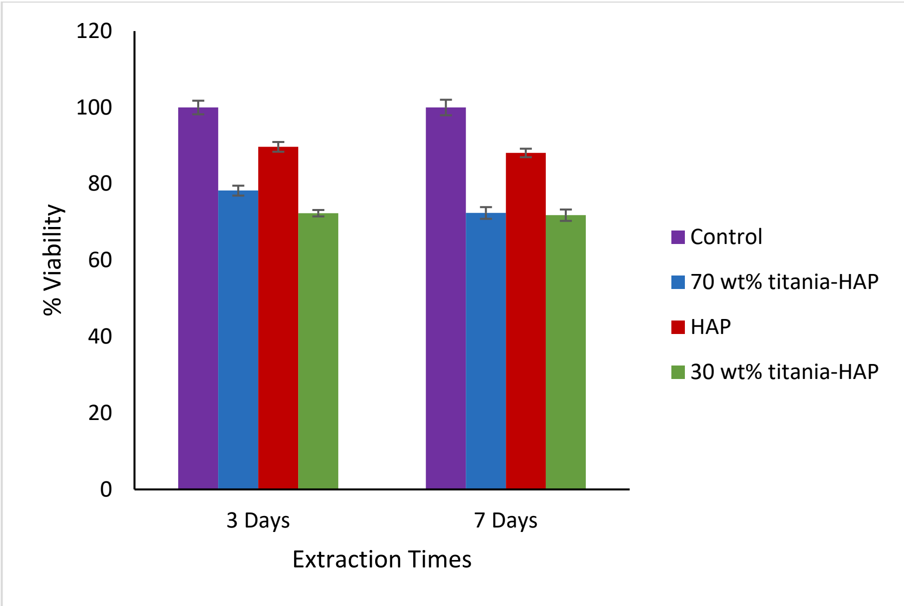


Fig. 6. Cytotoxicity of HAP, titania-HAP composites and control samples at two different extraction times using MTT assay test.

300 °C is attributed to the complete evaporation of loaded ibuprofen (40). The major weight loss for ibuprofen-loaded 30 wt% titania-HAP composite started at 170 °C and ended at 270 °C (50% weigh loss) and was attributed to drug loading of about 50%. As shown in the TGA curve, the loading percentage of ibuprofen-loaded 70 wt% titania-HAP and ibuprofen-loaded HAP composites are 70% and 64%, respectively. Therefore, a higher titanium ratio in hydroxyapatite nanoparticles led to an increase in the loading capacity of the composite. This result is reported for the first time. Based on the results, ibuprofen loading of samples shows as following:

70 wt% tinania-HAP > HAP > 30 wt% tinania-HAP in acidic environment

In fact, in 30 wt% tinania-HAP sample, titanium occupies the active sites of hydroxyapatite and reduces drug absorption, but in 70 wt% tinania-HAP sample, titanium creates new active sites in addition to hydroxyapatite and increases drug absorption.

Evaluation of cytotoxicity

Cell viability was evaluated after exposure of MG-63 cell line with nanoparticle extracts using MTT assay method (Fig. 6). MTT assay was performed according to method Van Meerloo et al [39]. Nanoparticles at a concentration of 0.1g/ml (0.1 g sample per 1 mL of culture medium) were tested at two different extraction times (3 and 7 days). Based on the results, the cytotoxicity increased with the extraction times from 3 to 7 days. The extract on the third day of the composite containing 70 wt% titania showed less toxicity than 30 wt% titania-HAP composite, while the seventh day extract of both 30 and 70 wt% titania-HAP composites showed the same level of cytotoxicity. Although both titania-HAP composites showed less proliferation than hydroxyapatite nanoparticles, both composites have low overall toxicity (more than 70% of the cells remained). Therefore, the nanocomposites at the studied concentration did not show significant cytotoxic effects and changes in cell survival in both titania-HAP composites are not significant.

In vitro drug release

Ibuprofen release was investigated by immersing ibuprofen-loaded hydroxyapatite and its composites at 37 °C in pH 7.4 as simulated body fluids (SBF) and pH 4.7 as simulated acidic fluids (Fig 7). Ibuprofen concentration in the samples was measured at specific intervals after immersion using UV-Visible spectroscopy. As shown in Fig. 7 a, about 16% of ibuprofen was released in 30 minutes after immersion of IBU-loaded hydroxyapatite nanoparticles in SBF at pH 7.4. The drug release

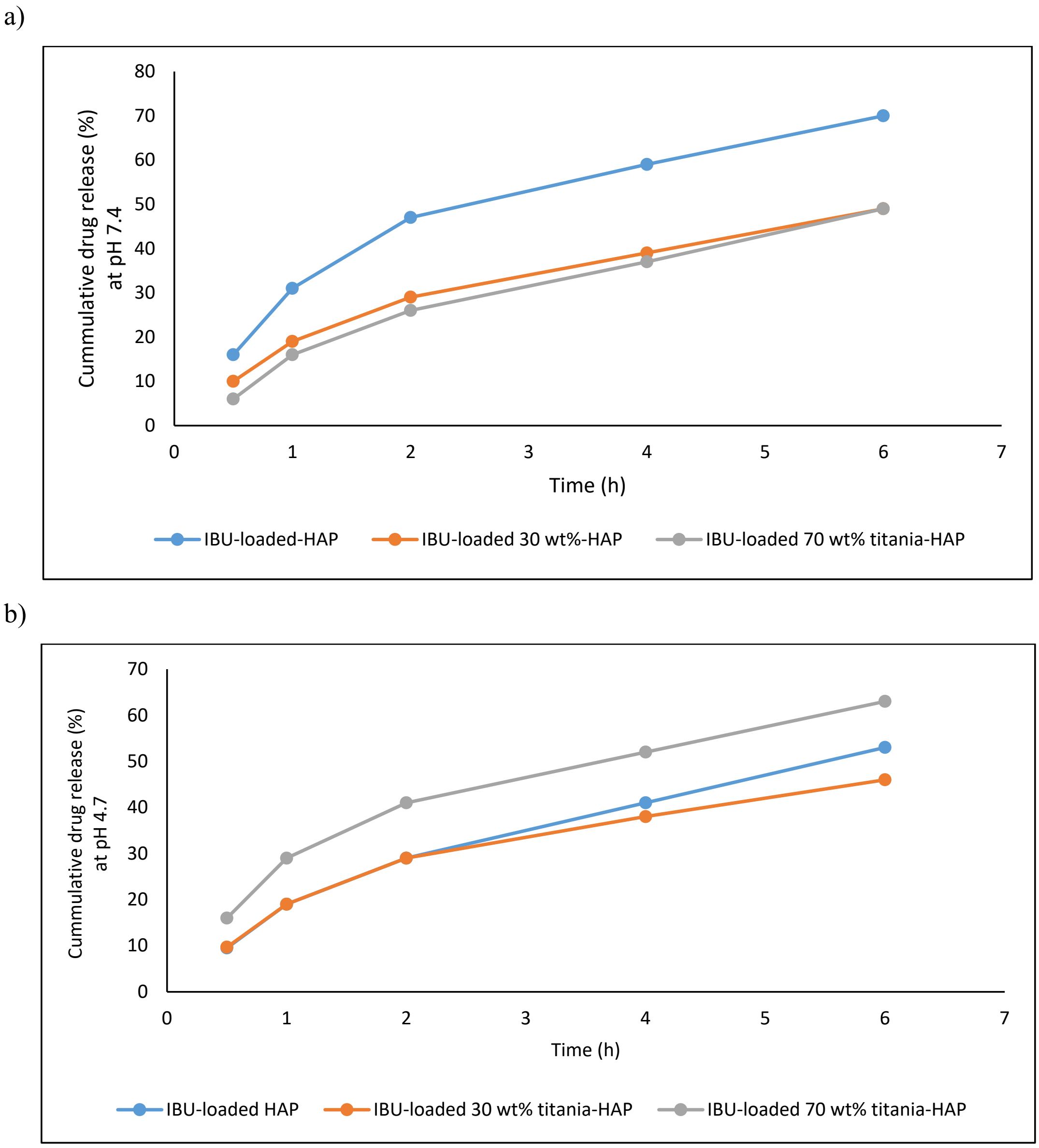


Fig. 7. Drug release behavior of ibuprofen-loaded hydroxyapatite and ibuprofen-loaded titania-HAP composites immersed in: a) SBF at pH 7.4 and, b) SBF at pH 4.7.

increased gradually and reached a maximum of 70% after 6 hours of immersion. Ibuprofen release from both 30 and 70 wt% titania-HAP composites had a very similar pattern, and about 5% of the drug was released in the first 5 minutes of immersion and reached to 60% after 6 hours.

Ibuprofen release from IBU-loaded hydroxyapatite nanoparticles in acidic medium (pH 4.7) was 10% after 30 minutes of immersion and reached a maximum of 53% after 6 hours. Ibuprofen release rate from 30 and 70 wt% tinania-HAP composites in acidic media was 46 and 63% after 6 hours of immersion. In general, IBU-loaded composites showed better sustained release behavior in acidic media. In fact, in the neutral environment, the presence of titanium has decreased the release percentage of two composite samples. While 70 wt% tinania-HAP composites have a higher percentage of drug, and the acidic environment has increased drug release because of ibuprofen functional group. Based on the results, ibuprofen release of samples shows as following:

HAP > 30 wt% tinania-HAP > 70 wt% tinania-HAP in neutral environment

70 wt% tinania-HAP > HAP > 30 wt% tinania-HAP in acidic environment

In neutral environment, the percentage difference of two samples of 30 and 70 wt% tinania-HAP is insignificant. Also, in acidic environment, the percentage difference of two samples of HAP and 30 wt% tinania-HAP is insignificant. According to the results, increasing the titanium percentage decreases the percentage of release in a neutral environment due to inhibition caused by the presence of titanium and increases the percentage of release in an acidic environment due to the more presence of drug in the sample and its functional groups effect.

CONCLUSION

The hydroxyapatite nanocomposite containing 30 and 70 wt% titania (titania-HAP composites) was synthesized. The titanium incorporation in the composites was confirmed by FTIR, XRD and EDX analyses. The nanocomposites were evaluated as nanocarriers for hydrophobic ibuprofen. FTIR results revealed the successful preparation of titania-doped hydroxyapatite composites. According to TGA analysis, the drug loadings were estimated to be 640, 500 and 700 mg/g of ibuprofen in HA, 30 and 70 wt% titania-HAP composites, respectively. The toxic potential of hydroxyapatite nanoparticles and titania-HAP composites on MG-63 cell line was investigated and the results of MTT assay showed no significant cytotoxic effects. The viability of MG-63 cells in the presence of 30 and 70 wt% titania-HAP composites was 72.3% and 78.23% respectively after incubation with third day extracts of the samples. Accordingly, 71.8%, 72.4% of the cells exposed to the seventh day extracts of 30 and 70 wt% titania-HAP composites. The kinetic study of ibuprofen release from drug-loaded composites in acidic medium showed slower release behavior compared to drug release in a simulated blood plasma fluid. Therefore, nanocomposite can be a good and economic potential for drug carriers to develop pharmaceutical applications.

CONFLICT OF INTEREST

There is no conflict of interest

REFERENCES

1. Pu'ad, N.M., et al., Synthesis method of hydroxyapatite: A review. Materials Today: Proceedings, 2020. 29: p. 233-239. https://doi.org/10.1016/j.matpr.2020.05.536
2. Odar, M. and C. Motakef-Kazemi, Nanohydroxyapatite and its polycaprolactone nanocomposite for lead sorbent from aqueous solution. Nanomedicine Research Journal, 2020. 5(2): p. 143-151. https://doi.org/10.24200/amecj.v4.i03.145
3. Haider, A., et al., Recent advances in the synthesis, functionalization and biomedical applications of hydroxyapatite: a review. Rsc Advances, 2017. 7(13): p. 7442-7458. https://doi.org/10.1039/C6RA26124H
4. Abbas, Z., et al., Toughening of Bioceramic Composites for Bone Regeneration. Journal of Composites Science, 2021. 5(10): p. 259. https://doi.org/10.3390/jcs5100259
5. Jung, J.-H., et al., Hydroxyapatite-coated implant: Clinical prognosis assessment via a retrospective follow-up study for the average of 3 years. The Journal of Advanced Prosthodontics, 2018. 10(2): p. 85-92. https://doi.org/10.4047/jap.2018.10.2.85
6. Bordea, I.R., et al., Nano-hydroxyapatite use in dentistry: A systematic review. Drug metabolism reviews, 2020. 52(2): p. 319-332. https://doi.org/10.1080/03602532.2020.1758713
7. Szcześ, A., L. Hołysz, and E. Chibowski, Synthesis of hydroxyapatite for biomedical applications. Advances in colloid and interface science, 2017. 249: p. 321-330. https://doi.org/10.1016/j.cis.2017.04.007
8. Du, M., et al., Recent advances in biomedical engineering of nano-hydroxyapatite including dentistry, cancer treatment and bone repair. Composites Part B: Engineering, 2021. 215: p. 108790. https://doi.org/10.1016/j.compositesb.2021.108790
9. Lee, M.C., et al., Development of novel gene carrier using modified nano hydroxyapatite derived from equine bone for osteogenic differentiation of dental pulp stem cells. Bioactive materials, 2021. 6(9): p. 2742-2751. https://doi.org/10.1016/j.bioactmat.2021.01.020
10. Shi, H., et al., Hydroxyapatite based materials for bone tissue engineering: A brief and comprehensive introduction. Crystals, 2021. 11(2): p. 149. https://doi.org/10.3390/cryst11020149
11. Palmer, L.C., et al., Biomimetic systems for hydroxyapatite mineralization inspired by bone and enamel. Chemical reviews, 2008. 108(11): p. 4754-4783. https://doi.org/10.1021/cr8004422
12. Lara-Ochoa, S., W. Ortega-Lara, and C.E. Guerrero-Beltrán, Hydroxyapatite nanoparticles in drug delivery: Physicochemistry and applications. Pharmaceutics, 2021. 13(10): p. 1642. https://doi.org/10.3390/pharmaceutics13101642
13. Kojima, C. and K. Watanabe, Adsorption and desorption of bioactive proteins on hydroxyapatite for protein delivery systems. Journal of drug delivery, 2012. 2012. https://doi.org/10.1155/2012/932461
14. Bangera, M.K., R. Kotian, and N. Ravishankar, Effect of titanium dioxide nanoparticle reinforcement on flexural strength of denture base resin: A systematic review and meta-analysis. Japanese Dental Science Review, 2020. 56(1): p. 68-76. https://doi.org/10.1016/j.jdsr.2020.01.001
15. Gunputh, U.F., et al., Multilayered composite coatings of titanium dioxide nanotubes decorated with zinc oxide and hydroxyapatite nanoparticles: controlled release of Zn and antimicrobial properties against Staphylococcus aureus. International Journal of Nanomedicine, 2019. 14: p. 3583. https://doi.org/10.2147/IJN.S199219
16. Anaya-Esparza, L.M., et al., Use of titanium dioxide (TiO2) nanoparticles as reinforcement agent of polysaccharide-

based materials. Processes, 2020. 8(11): p. 1395. https://doi.org/10.3390/pr8111395
17. Wang, Q., et al., TiO2 nanotube platforms for smart drug delivery: a review. International journal of nanomedicine, 2016. 11: p. 4819. https://doi.org/10.2147/IJN.S108847
18. Kumar, N., et al., TiO2 and its composites as promising biomaterials: a review. Biometals, 2018. 31(2): p. 147-159. https://doi.org/10.1007/s10534-018-0078-6
19. Jafari, S., et al., Biomedical applications of TiO2 nanostructures: recent advances. International journal of nanomedicine, 2020. 15: p. 3447. https://doi.org/10.2147/IJN.S249441
20. Neta, I., et al., Nanostructured titanium dioxide for use in bone implants: a short review. Cerâmica, 2020. 66: p. 440-450. https://doi.org/10.1590/0366-69132020663802905
21. Hasanzadeh Kafshgari, M. and W.H. Goldmann, Insights into theranostic properties of titanium dioxide for nanomedicine. Nano-Micro Letters, 2020. 12(1): p. 1-35. https://doi.org/10.1007/s40820-019-0362-1
22. Ziental, D., et al., Titanium dioxide nanoparticles: prospects and applications in medicine. Nanomaterials, 2020. 10(2): p. 387. https://doi.org/10.3390/nano10020387
23. Sato, M., et al., Nanocrystalline hydroxyapatite/titania coatings on titanium improves osteoblast adhesion. Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials, 2008. 84(1): p. 265-272. https://doi.org/10.1002/jbm.a.31469
24. Oliveira, T.M., et al., Calcium phosphate-based bioceramics in the treatment of osteosarcoma: drug delivery composites and magnetic hyperthermia agents. Frontiers in Medical Technology, 2021: 26. https://doi.org/10.3389/fmedt.2021.700266
25. Mondal, S., S.V. Dorozhkin, and U. Pal, Recent progress on fabrication and drug delivery applications of nanostructured hydroxyapatite. Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, 2018. 10(4): p. e1504. https://doi.org/10.1002/wnan.1504
26. Venkatasubbu, G.D., et al., TiO2 nanocomposite for the controlled release of drugs against pathogens causing wound infections. Materials Research Express, 2018. 5(2): p. 024003. https://doi.org/10.1088/2053-1591/aaa936
27. Motakef-Kazemi, N., S.A. Shojaosadati, and A. Morsali, In situ synthesis of a drug-loaded MOF at room temperature. Microporous and mesoporous materials, 2014. 186: p. 73-79. https://doi.org/10.1016/j.micromeso.2013.11.036
28. Motakef-Kazemi, N., S.A. Shojaosadati, and A. Morsali, Evaluation of the effect of nanoporous nanorods Zn2 (bdc) 2 (dabco) dimension on ibuprofen loading and release. Journal of the iranian chemical society, 2016. 13(7): p. 1205-1212. https://doi.org/10.1007/s13738-016-0835-9
29. Goldstein, J., et al., Small bowel mucosal injury is reduced in healthy subjects treated with celecoxib compared with ibuprofen plus omeprazole, as assessed by video capsule endoscopy. Alimentary pharmacology & therapeutics, 2007. 25(10): p. 1211-1222. https://doi.org/10.1111/j.1365-2036.2007.03312.x
30. Che, X., et al., One-step preparation of ibuprofen fast-and sustained-release formulation by electrospinning with improved efficacy and reduced side effect. Pharmaceutical Development and Technology, 2020. 25(6): p. 659-665. https://doi.org/10.1080/10837450.2020.1728773
31. Mărginean, M.O., et al., Ibuprofen, a potential cause of acute hemorrhagic gastritis in children-a case report. The Journal of Critical Care Medicine, 2018. 4(4): p. 143. https://doi.org/10.2478/jccm-2018-0022
32. Mazaleuskaya, L.L., et al., PharmGKB summary: ibuprofen pathways. Pharmacogenetics and genomics, 2015. 25(2): p. 96. https://doi.org/10.1097/FPC.0000000000000113
33. Guerra-Ponce, W.L., et al., In vitro evaluation of sustained released matrix tablets containing ibuprofen: a model poorly water-soluble drug. Brazilian Journal of Pharmaceutical Sciences, 2016. 52: p. 751-759. https://doi.org/10.1590/s1984-82502016000400020
34. Gouveia, D., A.H.A. Bressiani, and J.C. Bressiani. Phosphoric acid rate addition effect in the hydroxyapatite synthesis by neutralization method. in Materials science forum. 2006. Trans Tech Publ. https://doi.org/10.4028/0-87849-423-5.593
35. Bouropoulos, N., A. Stampolakis, and D.E. Mouzakis, Dynamic mechanical properties of calcium alginate-hydroxyapatite nanocomposite hydrogels. Science of Advanced Materials, 2010. 2(2): p. 239-242. https://doi.org/10.1166/sam.2010.1092
36. Öner, M., et al., Ibuprofen release from porous hydroxyapatite tablets. Ceramics International, 2011. 37(7): p. 2117-2125. https://doi.org/10.1016/j.ceramint.2011.02.021
37. Singh, A., et al., Titanium dioxide doped hydroxyapatite incorporated photocatalytic membranes for the degradation of chloramphenicol antibiotic in water. Journal of Chemical Technology & Biotechnology, 2021. 96(4): p. 1057-1066. https://doi.org/10.1002/jctb.6617
38. Rashidzadeh, M., Synthesis of high-thermal stable titanium dioxide nanoparticles. International Journal of Photoenergy, 2008. 2008. https://doi.org/10.1155/2008/245981
39. Meerloo, J.V., G.J. Kaspers, and J. Cloos, Cell sensitivity assays: the MTT assay, in Cancer cell culture. 2011, Springer. p. 237-245. https://doi.org/10.1007/978-1-61779-080-5_20