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Original Article

Evaluation of Ibuprofen Release from Gelatin /Hydroxyapatite /Polylactic Acid Nanocomposites

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Abstract

Gelatin-hydroxyapatite-polylactic acid (PLA) nanocomposites were synthesized using five different formulations. The nanocomposites were loaded with ibuprofen and the amount of drug in the carriers was determined. X-ray diffraction (XRD) analysis was conducted before and after drug loading to ensure the presence of ibuprofen on the nanocomposites. Drug delivery was evaluated in phosphate buffered saline (PBS) solution at pH 7.4 and 37°C. The results of XRD analysis showed acceptable synthesis of hydroxyapatite and the composites, confirming the loading of ibuprofen onto the synthesized nanocarriers. The results showed that maximum drug loading (58.2%) was recorded for sample D (30% gelatin, 40% nHA and 30% PLA), and minimum loading was recorded for sample E (30% gelatin, 30% nHA and 40% PLA). The maximum percentage of drug release over the course of 72 h (95.8%) was for nanocomposite D (30% gelatin, 40% nHA and 30% PLA). The minimum percentage of drug delivery (77%) was for nanocomposite E (30% gelatin, 30% nHA, 40% PLA), which contained the maximum PLA content.

Keywords: Nanocomposites, Hydroxyapatite, Polylactic acid, Ibuprofen, Release profile, Drug delivery

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1. Introduction

Simultaneous bone repair and drug delivery technology is an interdisciplinary challenge and is the subject of important research affecting human health. As an important source of calcium phosphate, hydroxyapatite has been recently investigated as potential material for drug delivery systems due to its physical and chemical properties and biological characteristics [1, 2]. This type of drug delivery system can release a therapeutic agent using a bioactive matrix [3].

Producing calcium phosphate through a chemical method is costly and the lack of

accurate stoichiometric results during production means it will contain a high percentage of impurities. An alternative method for production of hydroxyapatite is to extract it from natural sources. Researchers have tried to produce this material from natural sources such as the skin of crustaceans [4] and eggshells [5]. Liu et al. [6] placed bovine serum albumin (BSA) in calcium deficient hydroxyapatite nanoparticles (CDHA) to produce BSA nanocarriers.

Lian et al. [7] used HA nanoparticles to carry antibiotic drugs (vancomycin). Palazzo et al. [8] used cylindrical microporous apatite for the delivery of ibuprofen-lysine and hydrocortisone Na-succinate, both of which are anti-inflammatory agents. Liu et al. [9] reported bone cement containing antibiotic gentamicin. Joosten et al. [10] used HA cement as a bone-filler containing vancomycin to treat osteomyelitis. Haghbin [11] also investigated drug delivery and drug loading of gentamicin in apatite cement. Calcium phosphate cement can be utilized as a matrix for the delivery of antibiotic, antitumor and anti-inflammatory drugs.

Although hydroxyapatite offers advantages such as the similarity of its chemical properties to bone composition, its high biocompatibility and its ability to make highly acceptable bonds with bone [12], the weak mechanical behavior of this bioceramic greatly limits its medical and dental applications [13]. Attempts have been made to develop new materials as alternatives to bone. Many composite materials that show biocompatibility and the ability to integrate with bone have been developed for medical applications [14, 15] and hydroxyapatite/polymer composites have attracted attention [16-18].

Various biocomposites in nature synthesize an organic matrix with a mineral compound [3]. These composites provide the mechanical properties required for their applications such as for bone, teeth or cells of living organisms [19, 20]. Numerous biodegradable polymers have been developed for use in medical applications. Among these, PLA has attracted attention because it offers biodegradability, biocompatibility, elasticity when heated and non-toxicity after hydrolysis [21].

Gelatin is derived from collagen and has good biological properties [22]. Because the main organic portion of hard tissue is made of collagen, it has potential medical applications. Among the advantages of gelatin are ductility and high efficiency, which can facilitate manufacturing [22]. Its delivery properties such as swelling make it a potential hydrogel for drug delivery systems. Its lack of antigenic properties and its biodegradability, biocompatibility and commercial availability at relatively low cost are other advantages of this cellular biodegradable material [23, 24]. Gelatin is extensively used in drug delivery, wound healing and as adhesive bandages [25, 26]. As for other natural polymers, it can only be used in soft tissue because it has an unacceptable modulus of elasticity and lacks the mechanical strength necessary for hard tissue.

A three-component composite of gelatin, hydroxyapatite and PLA shows greater potential as the advantages of the three

components can be integrated [27]. These include the bioactivity and bone repair of hydroxyapatite, the morphology and ductility of gelatin and the excellent biodegradability of PLA [17, 18]. Dual hydroxyapatite/gelatin and hydroxyapatite/PLA composites have been synthesized by researchers and their properties and abilities as drug carriers have been investigated. The present study synthesized a triple composite of hydroxyapatite/gelatin/PLA and examined its potential use as a drug delivery carrier for ibuprofen. This composite can be applied for bone repair and possibly for targeted drug delivery in the future.

2. Materials and Methods

2. 1. Materials

Polylactic acid (3251D injection grade) was obtained from Nature Works. Gelatin (type B bovine) was obtained from Aldrich. The solvents used (chloroform, hexane and acetic acid with purities of over 99.9%) and the NaCl, KCl, KH_2PO_4 and Na_2HPO_4 for construction of PBS were purchased from Merck. Ibuprofen with a chemical composition of $C_{13}H_{18}O_2$ and molar mass of 206.3 was also purchased from Merck.

2.2. Synthesis of Gel/Nha/PLA Nanocomposites

In our previous work [29], we synthesized and characterized a gel/nHA/PLA nanocomposite. In the current study, the nanocomposites were loaded with ibuprofen and the release profiles were determined. Synthesis proceeded as follows. Cattle bone was used as the biological source of the hydroxyapatite. First, the spongy parts of the bone, bone morrow, meat and fat remnants were removed from the high-density parts of the bone. These high-density parts were heated to burn and remove the organic compounds. The color of the resulting material was black from the carbon produced during burning. To remove the carbon, the black ash was heated for 60 min at 800°C in the air, which produced a white powder composed of natural hydroxyapatite [30].

In the second stage, gelatin was dissolved in acetic acid and then stirred for 3 h at room temperature. The hydroxyapatite powder was added to it and they were stirred in a water bath at 36°C for 8 h. The PLA granules were stirred in chloroform for 3 h at room temperature. After the PLA had dissolved in the chloroform, the solution was added to the gelatin and hydroxyapatite mixture and suspended in acetic acid and stirred. The resulting mixture was stored in the water bath for 36 h to produce a jelly-like material. This gel was stored at room temperature and then placed in an oven at 60°C for 24 h to dry out. The nHA/PLA/gel nanocomposite was synthesized using the different formulations shown in table 1.

2.3. Characterization of Nanocomposites

In order to ensure the production of the composite and drug loading onto the nanocarrier, phase analysis was conducted using an XRD device (Bruker; model D8-Adcance) using CuK_α radiation with a

Table 1. Compositions and maximum drug release from different nanocomposites.

	A	B	C	D	E
٪ Gelatin	30	30	30	30	30
٪ nHA	70	60	50	40	30
٪ PLA	0	10	20	30	40
Maximum drug released (%)	87.2±3.4	81.3±4.6	92.1±2.8	95.8±2.1	77±3.4

wavelength of 1.5418 Å at a range of 2θ = 5-70 and a step size of 0.01. The morphology of the nanocomposites was evaluated using field emission scanning electron microscopy (FESEM; model S-4160; Hitachi; Japan).

2.4 Ibuprofen Loading and Release in Nha/Gel/PLA Nanocomposites

First, 40 mg of ibuprofen was dissolved in 10 ml of hexane (4 mg/ml) and then 100 mg of nHA/PLA/gel nanocomposite was suspended for 24 h in this solution. After 24 h, the powder was separated from the solution by centrifugation and the resulting material was dried at room temperature for 48 h. Ibuprofen absorption onto the nanocomposites was estimated indirectly by calculating the difference in ibuprofen concentration in the hexane solution before and after drug loading. The drug loading percentage was calculated as:

Drug loading percentage = $(X - Y)/X \times 100$

Where X and Y represent initial and final drug concentrations, respectively.

In-vitro ibuprofen release from the nHA/PLA/gel nanocomposite was done in 10 ml PBS solution at pH 7.4 and 37°C. The ibuprofen absorption intensity was measured at specific time intervals. The amount of released ibuprofen was evaluated using a visible-ultraviolet spectrophotometer at a wavelength of 264 nm (model 1650 PC; Shimadzu; Japan). The drug release profiles were repeated three times, and the cumulative drug release percentage as a function of time was recorded.

3. Results and Discussion

To investigate drug loading onto the nanocomposites, XRD tests were prepared for the pure ibuprofen drug as shown in figure 1. The sharp peaks seen in this figure are evidence of the regular crystalline structure of this drug.

The XRD patterns for ibuprofen-loaded samples A-E are shown in figure 2. Additional peaks can be observed in the structure, showing evidence of the presence of ibuprofen.

As seen, the sharp peaks corresponding to ibuprofen in figure 1 had become weak, indicating that the crystallinity of the drug had decreased. Another reason for the decrease in intensity of the diffraction peaks could be attributed to the decrease in the size of drug crystallites in the structure after loading and solvent extraction.

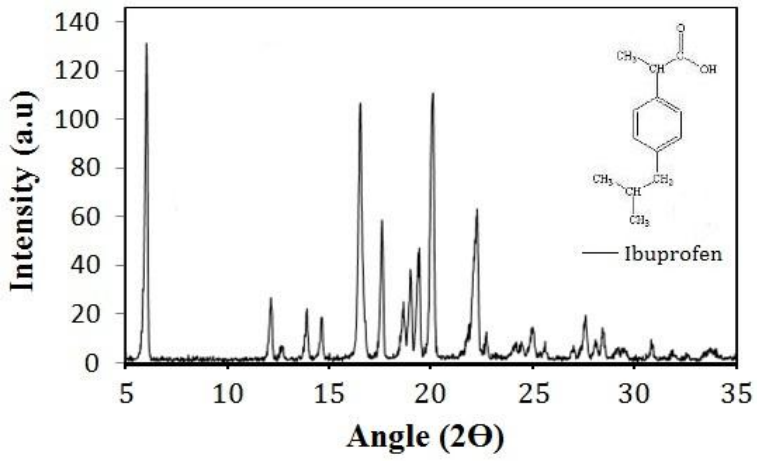

Figure 1. XRD pattern of ibuprofen.

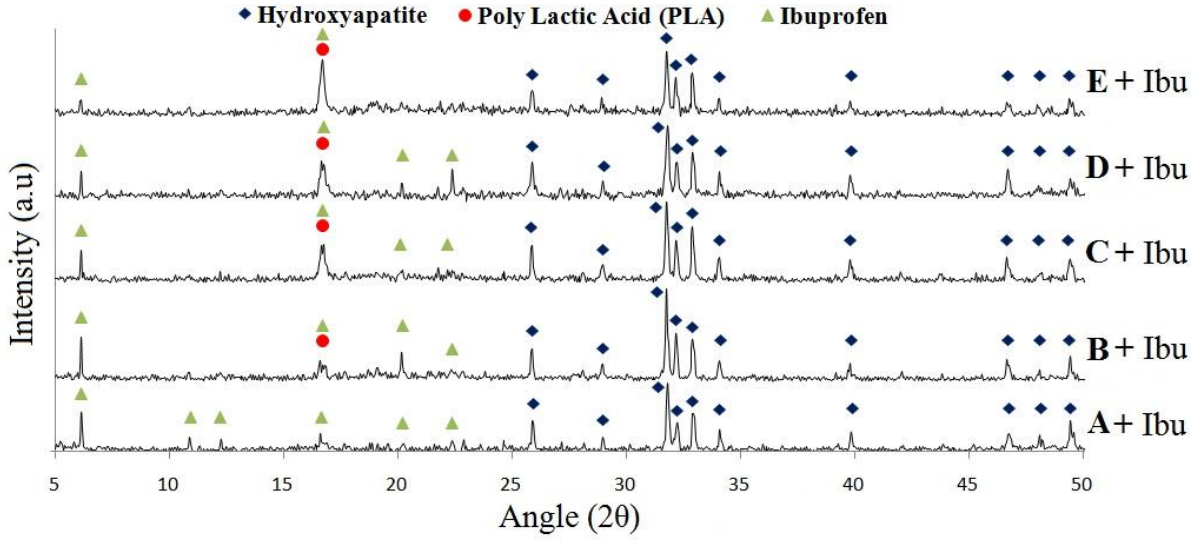

Figure 2. XRD patterns of gel/nHA/PLA nanocomposites loaded with ibuprofen.

Figure 3(a) shows the FE-SEM images of HA particles at different magnifications. The morphology of the particles is spherical, which provided the highest specific surface area. The size of the prepared hydroxyapatite particles is on the nanometer scale and the relatively homogeneous and narrow (uniform) distribution of the particle size can be clearly seen in these images. Electron microscope images of the 30% gelatin and 70% nHA binary composite are shown in Figure 3(b). It can be observed that the gelatin, which is a polymer, covers the surface of the hydroxyapatite nanoparticles. It can be also stated that the porosities of the hydroxyapatite nanoparticles are largely saturated and their surfaces covered by this polymeric material.

Figure 3(c) shows the 30% gelatin/30% nHA/40% PLA ternary composite and represents the surface coverage and

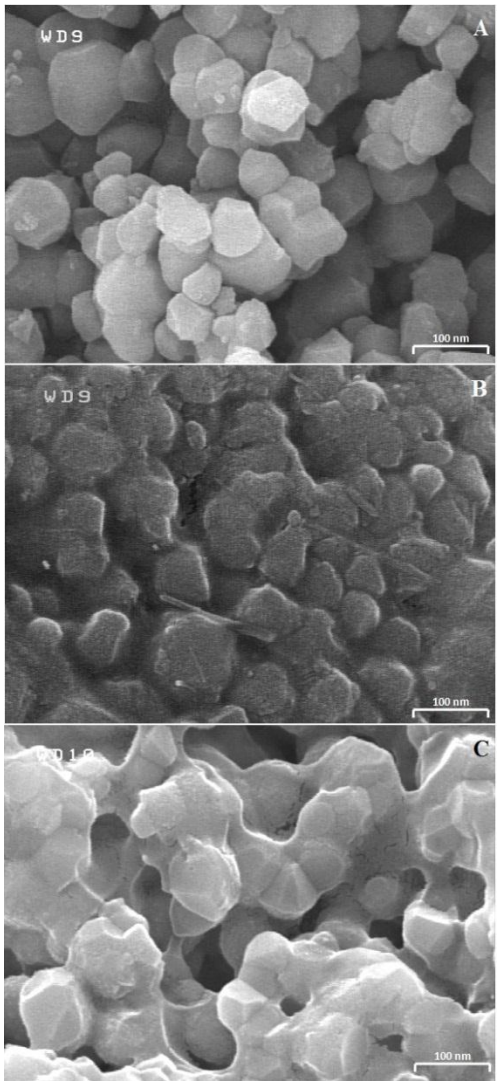

Figure 3. Electron microscope images of the microstructure of: (a) hydroxyapatite nanoparticles; (b) 30% gel/70% nHA binary composite (c) 30% gel/30% nHA/40% PLA nanocomposite (sample E).

nanoparticle porosities of the polymer. This indicates that the composite particle size was still within the nanometer scale. The shapes also verified accumulation and clustering to some extent.

Figure 4 shows the level of drug loading versus the level of PLA present in the nanocomposite. The percentage of ibuprofen loading was calculated for the different formulations. As observed, the maximum percentage of drug loading in sample D with 30% PLA was 58.2%.

It is evident that an increase in the percentage of PLA up to 30% improved drug loading; however, a further increase sharply decreased drug delivery. The reason for the improvement in the loading of the nanocomposites up to 30% PLA relates to the increase in pores as well as the improvement in the surface binding of the drug with the nanoparticles. Above 30% PLA, the accumulation and clustering of the particles increased and this agglomeration decreased loading. It suggests that PLA concentration up

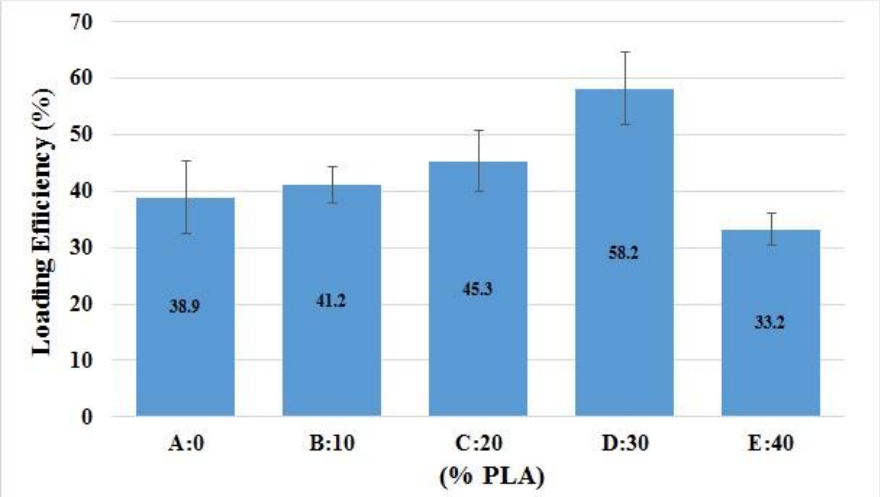

Figure 4. Loading efficiency of nanocomposite as a function of PLA weight fraction.

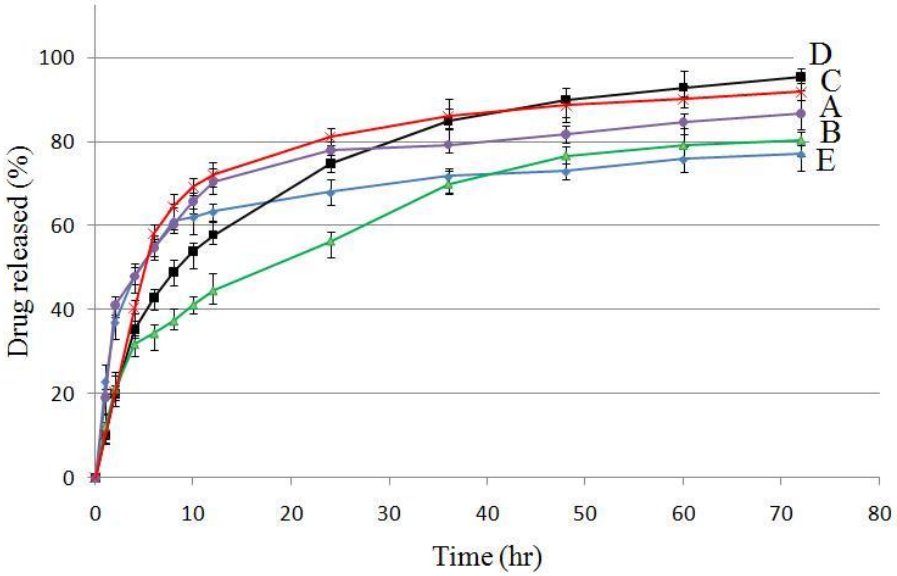

Figure 5. Drug release profile for nanocomposites having different compositions. Data represents mean ± S.D. (n = 3).

to 30% has a significant effect on the drug encapsulation efficiency.

Figure 5 shows the drug release curves. The sample drugs were normally delivered in two steps. In the first step, the drug was rapidly released. For this step, the slope of the curve was sharp and a high percentage of the drug was released. In the second step, the slope of the curve decreased and the drug was delivered at a slower rate. In other words, despite the type of sample or formulations, the ibuprofen delivery rate was particularly fast at the beginning, which was likely controlled diffusion of the drug delivery system. At first penetration passage was short, which resulted in a steeper slope for the concentration and fast drug delivery. Over time, the penetration passage lengthened which resulted in a

decrease in the slope of the drug concentration and decreased the drug delivery rate.

The advantage of initial fast drug delivery is that, under some conditions, fast delivery of a high concentration of drug is preferred; for example, at the beginning of wound healing. In these cases, high initial drug delivery can alleviate pain; thereafter, the slow and gradual delivery of the drug gradually improves the condition. The drug delivery profiles follow a controlled release mechanism.

The lowest and highest rates of ibuprofen delivery in the first step of 12 h were for samples C (45%) and B (71%), respectively. The final delivery percentage for the samples is shown in table 1. As shown, the maximum drug release was for sample D (30% gel/40% nHA/30% PLA). A 10% increase in PLA decreased the drug release. When the PLA reached 20%, drug delivery from the ternary nanocomposite occurred faster than for the dual nanocomposite. The percentage of drug delivery by the nanocomposite was the maximum value for 72 h. Nanocomposite E (30% gel/30% nHA/40% PLA), which contained the highest percentage of PLA, released only 75% of the loaded drug in 72 h.
During drug loading and delivery, the ibuprofen molecules are adsorbed onto the surface of the porous materials to saturation and then released through controlled diffusion. In fact, when ibuprofen is absorbed by the surface, OH^- groups on the surface must attach to the hydrogen bonded with ibuprofen carboxyl groups.

4. Conclusion

Nanocomposite systems which are based on hydroxyapatite nanoparticles, PLA and gelatin can be designed and applied as efficient tools for controlled drug delivery of therapeutic agents like ibuprofen. In the current study, ibuprofen was loaded onto a nanocomposite as a therapeutic agent or mediator and a long delivery time was observed. The maximum drug loading percentage in the nanocomposite with 30% PLA (sample D) was 58.2%. It was observed that the 30% gel/30% nHA/40% PLA nanocomposite had the lowest delivery percentage (77%), while the 30% gel/40% nHA/30% PLA nanocomposite had the highest delivery percentage (95.8%) in 72 h. The results of drug loading and release using more than 30% PLA in this nanocomposite did not produce the desired results and even caused a drop in the ibuprofen loading and release percentages. It can be concluded that, in addition to bone repair and the reconstructive applications of these nanocomposites related to hydroxyapatite nanoparticles, they also have the potential for drug delivery, especially for bone repair and reconstruction.

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