Adsorption/desorption study of antibiotic and anti-inflammatory drugs onto bioactive hydroxyapatite nano-rods

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**Abstract**

The use of high doses of antibacterial and anti-inflammatory drugs for patients with bone diseases, associated to implants or bone filling, can develop adverse effects; and consequently, it promotes to think new strategies to avoid this problem. In this work, it has been described the adsorption/release (or desorption) behavior of two drugs, ciprofloxacin (CIP) and ibuprofen (IBU), onto hydroxyapatite (nano-HA) at 37 °C. Through Ultraviolet-Visible (UV-Vis) spectroscopy, the concentrations of both drugs in adsorption, kinetic and desorption processes were obtained. The Fourier Transformed-Infrared (FT-IR) spectroscopy, Zeta-potential (ζ-potential), High-Resolution Transmission Electron Microscopy (H-TEM) and x-Ray Diffraction (xRD) were also used to characterize bare nanoparticles and those with adsorbed drugs. Five adsorption models (Langmuir, Freundlich, Sips, Temkin and Dubinin-Radushkevich) were used for describing the behavior of both active compounds. The adsorption processes (CIP/nano-HA and IBU/nano-HA) were better predicted by the Sips model than by the others. The kinetic adsorption data were processed, for both active agents, by application of Avrami’s model. Desorption/release process (of both drugs) was evaluated though Korsmeyer-Peppas (K-P) model. Owing to the predictability of these systems, we propose the use of these active ceramics as potential bone filler for improving the treatment against bacterial bone infections and to avoid its associated inflammatory process.

1. Introduction

For controlling infection in a certain tissue the antibiotic must reach its therapeutic concentration. This concentration depends on the type of antibiotic, bacterium strain and also the target tissue. Highly irritated tissues are easily reached by the antibiotics; however, bone has low perfusion [1–5]. Ciprofloxacin (CIP) is a fluoroquinolone (see Fig. 1A) widely used in treatment of bone diseases and other infections [6,7] and it has a large penetration in tissues [8]. It has very high activity against aerobic Gram negative bacilli and it is one of the quinolones with highest activity against Pseudomonas aeruginosa [9,10]. Osteomyelitis is associated with bone loss and this issue is generally addressed by bone grafting [11,12]. Hence, this schedule of doses and time administration of CIP can generate adverse effects [8]. Castro et al. have reported implantable systems based on phosphates for in-situ release of CIP [13]. For this reason, the development of a system with the possibility for releasing CIP into the affected tissue and also for filling the loss of bone mass [11] would decrease its adverse effects.

Inflammatory processes are frequently associated to infection states. For addressing this issue associated to bone infection, such as osteomyelitis, besides antibiotic administration, an anti-inflammatory therapy must also be considered [14]. In this sense, IBU (see Fig. 1B) is one of the most commonly used drugs for joint pain and inflammation [15,16].

Inorganic nanoparticles often exhibit multiple functions in medicine; for example, as filling materials, because of their good mechanical properties. Prevention and treatment of diseases such as osteomyelitis, periodontitis and infections associated with orthopedic implant surgery have been the central issue in the antimicrobial and anti-inflammatory fields. Hydroxyapatite has been used as a drug carrier due to its chemical composition, which is comparable to the bone and teeth mineral phase, and also for its mechanical properties [17–19]. Additionally, it has been used for the sustained release and long-term delivery of several drugs such as anti-inflammatory and antibiotics [20–22]. Hydroxyapatite of nanometer size peaks our attention because of its improved biocompatibility properties, such as those necessary for bone grafting, and its mechanical properties compared with micro-hydroxyapatite [23].

The development of systems with two delivery-phases (first fast and second sustained) for in situ release are shown as a good alternative for
many drugs. These delivery systems could provide a combination of these two types of drug release with the properties of osteoconduction, osteointegration and resorbability of nanoparticles [24]. In this context, it has been reported the development of nano-hydroxyapatite scaffold which were capable of realizing bone regeneration additionally to antimicrobial effects [17,25,26]. Due to that fact, Placente et al. [27] have reported the development of a biocompatible drug delivery system based on nano-hydroxyapatitite which had the comparable features than used in this work. In this case, the nanoparticles were loaded with CIP and IBU in similar concentrations than those used in the current work. Those concentrations showed a lack of toxicity in rat primary osteoblast culture after 48 h incubation and those loaded nano-HAs also showed high stability and local supply of antibiotic and anti-inflammatory agents and predictable release behavior in concordance with the results obtained in current work.

HA nanoparticles used in this work have provided an excellent platform for the adsorption of CIP. This process is directed by the electrostatic interactions between the PO₄³⁻ and Ca²⁺ ions of the nanoparticle surface and the molecules of CIP with charge. The functionalization of the nano-HA by means of the amino acid L-Arginine (L-ARG) results in a nanoparticle with positive net charge [28]; thus, this charge has generated electrostatic interactions with IBU molecules under defined conditions which are negatively charged.

The bone remodeling cause modifications in the pH environmental conditions into the injured zone [29], and this fact affects to the actors involved in this process, in this case: CIP and nano-HA and IBU and nano-HA. These pH variations, naturally triggered, can influence on the ionization of CIP and also IBU, and consequently modify the surface potential of nano-HA, adsorption and the release processes of both drugs. Therefore, in this work the behavior of adsorption/desorption of CIP and independently IBU at different pH values onto nano-HA have been assessed. Five isotherms models have been used for analyzing the behavior of the adsorption: Langmuir [30], Freundlich [31], Sips [32], Temkin [33] and Dubinin-Radushkevich [34]. The application of these five models was extensively described in literature; its fundamental characteristics are summarized in Section 2 of ESM. The obtained parameters and correlation coefficients of the interaction between CIP and nano-HA and also those between IBU and nano-HA, were best predicted by Sips isotherm model and it is shown that the adsorption depends on the pH of the medium. The adsorption kinetic process for each drug has been discussed and also the Avrami’s kinetic parameters [35]. Finally, Korsmeyer-Peppas model [36,37] has been used to assess the desorption process for both drugs independently. For this reason, this exhaustive study of adsorption/desorption behavior of both drugs onto nano-HA is an advance towards our horizon: the implantable therapeutic bioceramics.

2. Experimental

2.1. Materials

Hexadecyl-trimethyl ammonium bromide (CTAB, Mw = 364.48 g mol⁻¹, 99% Sigma), a poly(propylene glycol) (PPG, Sigma-Aldrich, Mw = 425 g mol⁻¹, δ = 1.004 g cm⁻³ at 25°C), calcium chloride (CaCl₂, Mw = 91 g mol⁻¹, 99% Sigma), sodium phosphate (Na₃PO₄, Mw = 148 g mol⁻¹, 96% Sigma), sodium nitrite (NaNO₂, Mw = 69 g mol⁻¹, 97%), ciprofloxacin (CIP, Mw = 331,346 g mol⁻¹, ≥ 98% Sigma), ibuprofen (IBU, Mw = 206.29 g mol⁻¹, ≥ 98% Sigma) and L-arginine (L-ARG, Mw = 174.20 g mol⁻¹, ≥ 98% Sigma) were used without further purification. For solutions preparation, only triple-distilled water was used.

2.2. Methods

2.2.1. Synthesis of naked nano-HA and their characterization

The synthesis of nano-HAs and their characterization were previously reported [29,35,39]. In the current work, synthesis of nano-rods was carried out by means of a modification of the method reported by Liu et al. [40] and their characterization was carried out by High-Resolution Transmission Electron Microscopy (H-TEM), x-Ray Diffraction spectroscopy (xRD), zeta potential (ζ) and Fourier Transform Infrared Spectroscopy (FT-IR).

2.2.2. High-Resolution Transmission Electron Microscopy (H-TEM)

Rod-like nanoparticles were observed using High-Resolution Transmission Electron Microscopy (H-TEM). The microphotographs were taken using a ZEISS Libra 200 FE OMEGA transmission electron microscope operated at 200 kV with magnification of 1,000,000×. Observations were made in a bright field. Powdered samples were placed on carbon supports of 2000 mesh. Selected-area electron diffraction (SAED) patterns were extracted from the Fourier transform (FFT) of HTEM microphotographs by digitalized image processing.

2.2.3. Powder x-Ray Diffraction (xRD)

Powder x-Ray Diffraction (xRD) data were collected with a Philips PW 1710 diffractometer with Cu Kα radiation (λ = 1.5418 nm) and graphite monochromator, operated at 45 kV, 30 mA and 25°C. The mean crystalline size (δ) of the particles was calculated from xRD line broadening measurement using the Scherrer equation [38,41]:

\[
\delta = \frac{0.89\lambda}{\beta\cos\theta}
\]

(2.A)

where λ is the wavelength (Cu Kα), β is the full width at the half maximum of the HA (211) line and θ is the diffraction angle. The fraction of crystalline phase (Xc) of HA powders was evaluated by the following equation [38,41]:

\[
X_c = 1 - \frac{I_{300}}{I_{211}}
\]

(2.B)

where I_{300} is the intensity of (300) diffraction peak and I_{112/200} the intensity of the hollow between (112) and (300) HA diffraction peaks corresponding to Miller plane family of HA (JCPDS file #09-0432). The estimated uncertainties are about 20% [38].
2.2.4. Fourier Transformed Infrared spectroscopy (FT-IR)

The FT-IR spectra were obtained in 4000–400 cm\(^{-1}\) wavelength range by a NICOLET Nexus 470 spectrophotometer, with a 2 cm\(^{-1}\) resolution and 64 scans per minute using an AVATAR smart diffuse reflectance accessory. The samples were performed at 1% weight/weight in KBr (FT-IR grade, Aldrich Chemical Co.); therefore, 1.5 mg of either oven-dried sample and 150 mg of KBr were homogenized thoroughly in an agate mortar with precaution taken to avoid the moisture. Each record was done in triplicate. For the background a micro sample cup of pure KBr was prepared.

2.2.5. Zeta potential (ζ-potential)

Zeta potential of the bare nanoparticles and those nanoparticles with adsorbed active principles was measured by means of a Zeta sizer Nano ZS90 (Malvern Instruments, Malvern, UK). The Software of the instrument provides the zeta potential from electrophoretic mobilities (μE), using the Henry equation [42].

2.2.6. Ultraviolet-visible spectroscopy (UV–Vis)

CIP and IBU adsorbed (or desorbed) onto nano-HA were measured with a UV–vis-NIR scanning spectrophotometer (Varian Cary 100 Bio) provided with a temperature controller (UV09005M013), using a 1 cm path length rectangular quartz cell. Adsorption and desorption experiments were carried out in 5 ml glass-tubes with round bottom immersed in a thermostatic bath at 37 °C. Hence, 3.5 mg of nano-HA were incorporated into 4 ml of aqueous solutions of the antibiotic and the anti-inflammatory drug (in independent experiments) with concentration ranges of 0.0022 to 0.033 mM. There were used different pHs, L-ARG was firstly adsorbed onto nano-rods and then the interaction with IBU was assessed. Adsorbed concentrations of IBU were measured by UV–Vis spectroscopy as it was mentioned in Section 2.2.6. Additionally, De Palma et al. have reported the enhancement of anti-inflammatory effects of IBU when a combination with L-ARG is produced [50]. The used concentration of L-ARG was established with modifications form the relation phosphate group: L-ARG (1:6) reported by Wang et al. [51]. The C\(_0\) concentration range of IBU in those three mentioned media was from 0.0145 to 0.145 mM. Data of adsorption process were fitted to five mentioned adsorption models and Avrami’s model was used for analyze the kinetic behavior. Desorption process was carried out such as that for CIP, and finally the results were fitted by korsmeyer-Peppas model.

2.2.7. Elemental analysis (EA)

The analysis of the nano-HA with adsorbed arginine was carried out with the elemental analyzer, Exeter Analytical CE-440 (UK). This technique was used to obtain the percentage of N corresponding to L-ARG absorbed onto nano-HA. Due to that nanoparticles and IBU molecules have negative charge; L-ARG was used to increase the interaction between nano-rods and IBU. To obtain the percentage of N, samples at pH 6 were prepared and they were repeated in three independent experiments. The used pH for the absorption of L-ARG onto nanoparticles was 6 because at this pH it has the most amounts of negatively charged at working pHs, L-ARG was firstly adsorbed onto nanorods and then the interaction with IBU was assessed. Adsorbed concentrations of IBU were measured by UV–vis spectroscopy as it was mentioned in Section 2.2.6.

2.2.8. Adsorption, kinetic and desorption processes and their assessment by means of models

The adsorption process of CIP was carried out by incubation of 3.5 mg of nano-rods into solutions of the antibiotic for 7 days (10,080 min) at 37 °C. Concentrations of CIP solutions (C\(_0\)) were from 0.0018 to 0.033 mM. There were used different media: phosphate saline solution (PBS), pH = 7.4; tridistilled water (w), pH = 6; and acetate buffer (AcB), pH = 4.2. After incubation time, the supernatant of each solution was measured by UV–Vis spectroscopy at 275 nm, and the maximum adsorbed concentration, was obtained. Then, the behavior of adsorption in each medium was assessed by different models. It is important to remark that the adsorption kinetic studies and desorption at pH 4.2 were carried out for CIP during 1440 min because of nanoparticles of hydroxyapatite start to degrade at that time [39]. Owing to the adsorption process of CIP onto nano-HA depends on the time, from adsorption experiments the kinetic behavior was evaluated using Avrami’s model.

Desorption experiments were carried out in those mentioned media at same temperature during 10,080 min with exception of samples at pH 4.2 such as it was explained above. Briefly, each sample with CIP adsorbed is incubated into those different media and then the presence of CIP desorbed at certain time is measure by UV–Vis spectroscopy. Three independent experiments have been developed for each experimental point. Finally, the results were fitted through korsmeyer-Peppas model.

The adsorption process of IBU was carried out using same conditions than those used for the CIP adsorption study; however, to increase the interaction between nano-HA and IBU, being both structures negative charged at working pHs, L-ARG was firstly adsorbed onto nano-rods and then the interaction with IBU was assessed. Adsorbed concentrations of IBU were measured by UV–Vis spectroscopy as it was mentioned in Section 2.2.6. Additionally, De Palma et al. have reported the enhancement of anti-inflammatory effects of IBU when a combination with L-ARG is produced [50]. The used concentration of L-ARG was established with modifications form the relation phosphate group: L-ARG (1:6) reported by Wang et al. [51]. The C\(_0\) concentration range of IBU in those three mentioned media was from 0.0145 to 0.145 mM. Data of adsorption process were fitted to five mentioned adsorption models and Avrami’s model was used for analyze the kinetic behavior. Desorption process was carried out such as that for CIP, and finally the results were fitted by korsmeyer-Peppas model.

2.3. Loaded and release efficacy

The percentages of adsorbed concentration and the maximum released for both drugs nano-HA has been established as follows:

\[
L_{AP} \% = \left( \frac{[AP] - [AP]_f}{[AP]_i} \right) \times 100
\]  

(2.C)

\[
R_{AP} \% = \left( \frac{[AP] - [AP]_f}{[AP]_i} \right) \times 100
\]  

(2.D)

\[
[AP]_f = [AP] - [AP]_i
\]  

(2.F)

where \(L_{AP}\)\% is the percentage of the active principle (CIP or IBU) loaded onto nano-HA and \(R_{AP}\)\% is the released one. \([AP]_i\) and \([AP]_f\) are the initial and final supernatant concentration of active principle where nano-rods of HA are incubated. \([AP]_i\) and \([AP]_f\) are the adsorbed and released concentration of active principle.

2.4. Statistical analysis

All experiments were conducted at least three times. All values were informed as the mean value ± standard deviation (SD). Statistical analysis was carried out through the one-way analysis of variance (one-way ANOVA). Student’s 𝑡-test and probability values below 0.05 (< 0.05) were considered significantly different.

3. Results And Discussion

3.1. Physical and Chemical characteristics of nano-HA

The obtained hydroxyapatite nanoparticles were inspected by means of H-TEM (Fig. 2). Their diameter ~8 nm and their length ~47 nm are in agreement with previous reported values [29,38,52]. Chemical features of nano-HA were studied by XRD, ζ-potential and by FT-IR. Nano-HA corresponds to poorly crystallized hydroxyapatite (HA) distinguished by a non-stoichiometric composition [35,53]. In Fig. S1A it is shown that the main peaks correspond to small HA particles and this fact is in concordance with the sizes shown by H-TEM in Fig. 2.
All synthesized samples correspond to crystalline materials with all peaks that could be indexed to hexagonal HA. Microcrystalline structure was also evidenced by the evaluation of the selected-area electron diffraction (SAED) patterns that can be extracted from the Fast Fourier transform (FFT) of high resolution TEM microphotographs, Fig. 2a and b, by digitalized image processing. Several single-crystal spot electron diffraction (ED) images, as that reported in Fig. 2c were obtained. The ED representation displays a perfect hexagonal pattern characteristic of HA unit cell, which can be indexed as (002) planes after measurements of inter-planar distances, d = 0.345 nm, Fig. 2d.

It is known that the presence of different charged groups onto surface of nanoparticles confers electric properties, which combined with their size, have great influence on biointegration properties [54]. Therefore, the presence of those groups has been verified by FT-IR and the charge balance of particles, in the three studied media, was obtained by ζ-potential. These last measurements were highly influenced by pH of the medium; therefore, their range was from −13.48 ± 0.03 mV, for naked nanoparticles in PBS, to −21.12 ± 0.10 mV in buffer acetate. These results, and those for nanoparticles with adsorbed drugs, have been summarized in Table 1. The negative charge on the surface of nano-particles is conferred by the presence of phosphates groups; however, Ca²⁺ on the surface diminishes their negative potential because of their positive charge. FT-IR spectrum of bared nanoparticles is shown in Fig. S1B of ESM. In this figure vibrational bands of phosphate groups, stretching of −OH groups are marked. Briefly, phosphate absorption bands are shown at 1036 (ν3), 602 (ν2), 563 (ν4) cm⁻¹, the region between 3000 and 3700 cm⁻¹ is related to the O–H stretching, While the area at 3440 cm⁻¹ reflects the water adsorbed onto the surface of nano-materials.

The bioactivity, biocompatibility (lack of toxicity) and osteo-conductive properties of HA nano-rods used here have been wide investigated and reported in previous works of our research team [27,38,52,53,55].

### 3.2. Adsorption behavior assessment of CIP and, independently, IBU onto nano-HA exposed to different pH and ionic strength conditions. Adsorption Models

The description of each model used for assessment of adsorption behavior of both active principles onto the surface of nano-HA is addressed in Section 2 of the ESM.

In order to determine the mechanism involved in CIP adsorption, experimental results were fitted to the five mentioned adsorption models. All parameters of the equations are governed by experimental conditions such as temperature, pH and ionic strength. On the right (from A to E) of Fig. 3, a comparison of different fitting plots of the adsorption experimental results of CIP, is shown. That same figure, but on the left (from F to J), shows those corresponding to absorption of IBU. In Table 2 are summarized the parameters derived from the fits, standard deviations and their respective coefficients of determination, R².

Before proceeding to the fitting of the experimental data, at first glance, certain patterns can be observed from the obtained plots. The first of these is the effect of pH: in the case of CIP, there is a greater similarity in the plots for the two lowest values of pH. While for IBU, this similarity occurs for the two highest values of pH. The other notable pattern is related to the shape of the plots: bearing on CIP, the adsorption is more gradual, while IBU adsorption presents a large initial slope followed by a plateau area. This pattern is more noticeable at the highest pH values.

### Table 1

ζ-Potential values of rod-like and loaded hydroxyapatite nanoparticles.

<table>
<thead>
<tr>
<th>System</th>
<th>Media</th>
<th>pH</th>
<th>ζ Potential ± SD (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nano-HA</td>
<td>PBS</td>
<td>7.4</td>
<td>−13.4 ± 1.2</td>
</tr>
<tr>
<td>Nano-HA</td>
<td>Water</td>
<td>6</td>
<td>−18.6 ± 1.7</td>
</tr>
<tr>
<td>Nano-HA</td>
<td>Acetate buffer</td>
<td>4.2</td>
<td>−21.1 ± 1.8</td>
</tr>
<tr>
<td>Nano-HA/CIP</td>
<td>PBS</td>
<td>7.4</td>
<td>−15.4 ± 1.4</td>
</tr>
<tr>
<td>Nano-HA/CIP</td>
<td>Water</td>
<td>6</td>
<td>−11.04 ± 1.4</td>
</tr>
<tr>
<td>Nano-HA/CIP</td>
<td>Acetate buffer</td>
<td>4.2</td>
<td>−4.09 ± 0.8</td>
</tr>
<tr>
<td>Nano-HA/L-ARG</td>
<td>Water</td>
<td>6</td>
<td>−6.01 ± 0.7</td>
</tr>
<tr>
<td>Nano-HA/L-ARG/IBU</td>
<td>PBS</td>
<td>7.4</td>
<td>−14.22 ± 0.8</td>
</tr>
<tr>
<td>Nano-HA/L-ARG/IBU</td>
<td>Water</td>
<td>6</td>
<td>−20.43 ± 0.9</td>
</tr>
<tr>
<td>Nano-HA/L-ARG/IBU</td>
<td>Acetate buffer</td>
<td>4.2</td>
<td>−9.54 ± 1.2</td>
</tr>
</tbody>
</table>
Fig. 3. Graphical representations of the proposed fitting models (dotted lines) to our experimental adsorption values of CIP (A–E) and IBU (G–J) at different pH: 4.2 (squares), 6 (circles) and 7.4 (triangles).
The concentration of CIP adsorbed onto nano-particles is highly dependent of pH. On one hand, this is due to the changes in surface potential conditions of nano-particles when pH varies and on the other hand, because of the ionization properties of CIP at different pH. CIP has bi-cyclic skeleton and two main ionizable groups (see Fig. 1A): the carboxylate group (COO−) shows negative charge when it ionizes and the amine group (NH) of the aliphatic ring become positive when it ionize. The carboxylate group has a pKa of 5.76 and the cyclic amine 8.68 [56]. Consequently, this molecule exhibit four species depending of pH values: neutral (CIP), anion (CIP−), cation (CIP+), and Zwiterion (CIP±). At working pH range, there is a prevalence of zwiterion species and a minor percentage of CIP positive-charged. For this reason, we will focus on those two species and also because CIP must be positive charged for interacting to nanoparticles.

The highest equilibrium concentration of CIP adsorbed (q.e) onto nano-HA was 0.0226 (mmol/g) per gram of nano-particles pH 6 and the lowest was 0.0042 (mmol/g) at pH 4.2. The minimal inhibitory concentration (MIC) of antibiotics depends on the type of antibiotic (action mechanism/pharmacodynamics) and the strain of bacteria. Osteomyelitis, a bone infection associated with the second type osteomyelitis, these three types of bacterial strains must be considered [57]. Escherichia coli (0.0015–0.00302 mM), Pseudomonas aeruginosa (4.5e−5–0.00151 mM), and Escherichia coli (6.035e−5–9.053e−5 mM) [58]. Therefore, these are the concentrations which must to reach the target site in an infection process. Whether one gram of our material is included in a bone defect caused by one of these strains, as filler, it is clear to notice that an appropriate amount of CIP (within those ranges of concentration) will be available for treating that infection. This makes our systems ideal candidates as bone filler to avoid or minimize bone infections.

In order to determine the adsorption mechanism of CIP, experimental data were fitted to five different models. Langmuir model was the first used due to its simplicity. It explains very well the adsorption behavior of CIP at pH 7.4, but at 4.2 and 6 the determination coefficients are lower than those reported for Sips model. Thus, at pH 7.4 a monolayer is probably formed at the beginning of the adsorption process, but this behavior is not evidenced into the other two pH values. Such as we have mentioned in Section 2 of ESM, Sips model is a combination of Langmuir and Freundlich adsorption models because it incorporates some features of both models. We hypothesized that adsorption process at pH 7.4, fits well with Sips model, probably, due to that a monolayer is first formed and for this reason, the Langmuir portion of the Sips equation explains this behavior and contributes to the high value of the determination coefficient into Sips model. The adsorption process at pH 7.4 is also well fitted by Temkin model (with R² lower than Sips model). This is probably due to that Temkin model is used for explaining the adsorption behavior in reversible a process. Additionally, we propose the use of D-B model to study the adsorption processes due to the lack of homogeneity of the nano-rods surface. This model has been applied for studying porous systems [34,59]. However, D-B model does not explain well the adsorption behavior of CIP onto nano-HA; consequently, we hypothesized that despite of the lack of homogeneity of the surface of our nanoparticles, their surfaces cannot be compared to those of porous systems. Thus, the behavior of adsorption of CIP onto our synthesized nanoparticles is better explained by Sips adsorption model for all studied pH. The adsorption process is explained by Sips model. This means that neither the surface of nanoparticles is homogeneous (but it is not resemble a porous system) nor the adsorption sites are energetically equivalent. Therefore, the lack of surface homogeneity of nanoparticles and pH sensibility of CIP are two highlighted points for their application as drug carrier.

Bare nano-rods have negative charge which varies from −13.4 to −21.1 mV while the pH decreases, and the positive charge of CIP is increasing its value in same way (while pH decreases).

At pH 6, 36.24% of CIP molecules are such as (CIP+) and the other 63.55% with both (acid and basic) ionized groups (CIP ±). This behavior could be analyzed such as it was reported by Chen et al. [60]. At this pH we found the highest concentration of CIP adsorbed and this fact is possibly due to that the balance of negative and positive charges onto nanoparticles is compensated with the range of charges of CIP. This means that CIP can interact with the negative and the positive charge of nano-rods (PO4−3 and Ca2+, respectively) which increase their concentration onto their surface. This behavior, has been reported as a clinically interaction problem when CIP is administrated in combination of antibiotics or sulcrate at same time [8].

In the adsorption process carried out at pH 7.4 it is shown that three adsorption models (Langmuir, Sips, and Temkin) have very close coefficients of determination of linear regression (R²). Consequently, due to that one of the models is the Langmuir one; we hypothesized, that the adsorption process could be related with the formation of CIP monolayers onto surface of nano-HA at this pH. The ζ-potential at pH 7.4 does not vary significantly between bare and CIP adsorbed
nano-HA, this behavior could be as a consequence of: the 92.44% of molecules of CIP has both ionizes groups (−NH+ and −COO−) (CIP ±) and would form a monolayer exposing the negative groups with a similar distribution than those of phosphate groups of hydroxyapatite nano-rods. Another possibility is also considered: when the zwiterion specie of CIP is absorbed the net balance of charge absorbed added to nanoparticles is negligible.

The adsorption of CIP molecules onto nano-HAs depends on pH. Bare nanoparticles at low pH (4.2) have the highest negative value of ζ-potential; however, at this pH the 97.29% of CIP molecules have ionized amine group which generates a positive charge (CIP+) and, therefore, a positive increase of ζ-potential is evidenced. This behavior indicates that positive charged CIP is adsorbed onto nanoparticles. Therefore, the interaction of CIP molecules and nano-rods by electrostatic forces, decrease the negative charge of nano-rods from −21.1 to −4.09 mV. The net charge of nanoparticles is negative; however, on their surface also positive zones provided by Ca2+ ions are presented. The abruptly decreases of negative charge of nano-rods is due to the interaction with the CIP+. Therefore, between positive charged zones (Ca2+) of nano-rods surfaces and the adsorbed molecules of CIP+ the decrease of interaction (attractive electrostatic strengths) with more molecules of CIP positive charged, is evidenced. Consequently, the concentration of CIP adsorbed onto their surface is the lowest at this pH.

The LAdv% and Raadv% for CIP depends on pH values. Thus, at pH 6 LAdv% was 68.2 ± 11%; at pH 7.4, 25.84 ± 7% and at pH 4.2, 9.03 ± 6% at 37°C. These findings are in agreement with the results reported by Li et al. [61].

To carry out the adsorption process of IBU onto nano-HA, it was necessary to adsorb L-ARG at first. The adsorption of this aminoacid was confirmed by ζ-potential, EA and FT-IR. The value of ζ-potential was −18.6 mV for bare nanoparticles at pH 6 and for those nanoparticles loaded with L-ARG was −6.01 mV at same pH. This decrease of negative charge of nanoparticles is indicative of interaction between the surface of particles and the aminoacid. These results are shown in Table 1.

L-ARG has a pH dependent behavior; therefore, it has two positive charges (two amine groups) and one negative (carboxylate) at all used pH in this study [62]. The percentage of this specie varies from 98.15 for pH 7.4 to 99.39 for pH 6. By mean of EA was determined that the percentage of nitrogen (N), which it was approximately 6% of the total elements. The adsorption of L-ARG used for IBU adsorption was carried out at pH 6. By means of FT-IR it has been also detected the presence of L-ARG onto the surface of nano-HA. Peaks between 1400 and 1420 cm−1 is ascribed the presence of −NH2 group which was previously reported by Zhao et al. [63]. In Fig. 4 is shown the FT-IR spectra of nano-HA and nano-HA loaded with L-ARG.

L-ARG adsorption process was studied by means of Avrami's model. The mechanism of adsorption and then desorption of IBU at pH 4.2 was erratic and poor; and for this reason a brief commentary we will mentioned below. Thus, we carefully carried out the analysis of adsorption process and desorption (see Section 3.4) at pH 6 and 7.4. Langmuir, Sips and D-B adsorption models fit very well with the experimental data for pH 6 and 7.4; however, slight difference among their determination coefficients is evidenced. For this reason, we propose that adsorption behavior is driven by the formation of a monolayer of IBU onto surface of nanoparticles which is explained by Langmuir model. Additionally, by mean of analysis of D-B model, we could infer that adsorption of L-ARG (previously to IBU adsorption) generate a mechanism of adsorption of IBU which could be compared with that produced onto a porous surface. Additionally, the adsorption of IBU onto nano-HA (for both cases) is better explained by Sips model (which include the Langmuir model) due to that the determination coefficients of Sips model are higher than the other two models.

The LAdv% and Raadv% for IBU also depends on pH values. Thus, at pH 6, LAdv% was 81.2 ± 10%; at pH 7.4, 91.79 ± 12% and at pH 4.2, 0.8 ± 0.5% at 37°C. It is important to remark that the pKa of IBU is 4.85; therefore, at pH 4.2, it has 81.77% of their molecules in neutral form and for this reason, the interaction of IBU molecules with positive charged L-ARG adsorbed onto nano-HA surface was poor. Owing to that fact, the adsorption process at pH 4.2 was inefficiency and erratic. This behavior is corroborated by the lack of variability of ζ-potential between L-ARG-loaded nanoparticles (−6.01 ± 0.7 mV) and those “loaded” with IBU (−9.54 ± 1.2 mV).

Above pH 6, all molecules of IBU are negative charged; therefore, the interaction with the surface of nano-HA positively charged due to the L-ARG loaded, is favored. Thus, the concentration of IBU adsorbed at pH 6 and pH 7.4 is increased. By means of ζ-potential is evidenced the increase of negative charge of nano-HAs loaded with L-ARG when IBU is adsorbed. At pH 6 the ζ-potential values of loaded L-ARG nanoparticles was −6.01 and when IBU is adsorbed at this pH, ζ-potential values decrease to −14.22 mV and −20.43 mV for pH 7.4.

The pharmacokinetics behavior of IBU was reported by Davies [64]. In that work the author shown that a concentration between 0.12 and 0.16 mM of IBU is reached to bloodstream of a patient after the intake of a tablet of 400 mg and consequently, this concentration is available to act on target tissue. Considering our system, the release at pH 7.4 will be 0.0368 mmol (−7.6 mg) of IBU per gram of nano-HAs. Therefore, 3.8 g of IBU-loaded nano-HA will be necessary to reach a therapeutic concentration at the site of affected tissue. These 3.8 g should be implanted into the bone defect as a filler material with therapeutic and bioactive properties due to that inflammatory processes generate the inhibition of collagen synthesis and increase the activity of osteoclasts which stimulate bone resorption [14].

3.3. Kinetic adsorption behavior assessment of CIP and, independently IBU, onto nano-HA. Effect of pH and ionic strength conditions

The kinetic adsorption process was studied by means of Avrami's model. The description of this for both active principles is addressed in Section 2.2.3 of the electronic supplementary material (ESM).

Fig. 5 shows the dependence with time of concentration CIP (Fig. 5A) and IBU (Fig. 5B) adsorbed onto nano-HA. The adsorption equilibrium for both drugs is almost reached at 1440 min for all studied pH. The results of these processes, parameters such as the order of the kinetic process (n) and the constant (Kav) for each study (for both drugs), are analyzed and summarized in Tables 4 and 5 for CIP and IBU, respectively. Considering the adsorption process of CIP, neither the values of Kav nor n have not extremely dependence with pH, but the adsorbed concentrations are different depending on pH. The adsorption
study for both, CIP and IBU, at pH 4.2 was carried out until 1440 min due to above this time nano-HAs start to dissolve [39] at this pH and the results could be misinterpreted. Conversely, for IBU it is shown that the $K_{av}$ at pH 7.4 is more than double of this constant at pH 6 which could imply that the adsorption process is been developed by different mechanism. However, this model does not allow us to know what type of mechanism is involved into the adsorption (Table 3). Additionally, it could be hypothesized that, for CIP, the adsorption process could be heterogeneous [35,65]. The $n$ for IBU shows similar values at those studied pH. As we mentioned previously at pH 4.2 the adsorption process of IBU is erratic due to the low interaction between IBU and L-

![Fig. 5. Kinetic adsorption studies for A-CIP, and B-IBU. Avrami's model at 37 °C. Triangles pH 7.4; circles pH 6; squares pH 4.2.](image)

### Table 3
Values of the parameters and $R^2$ of the five models used to analyze the adsorption process for IBU.

<table>
<thead>
<tr>
<th>Model</th>
<th>Adsorption media</th>
<th>$K_L$ (mmol/g)</th>
<th>$q_{mon}$ (mmol/g)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Langmuir</td>
<td>pH 4.2</td>
<td>7.427 ± 0.934</td>
<td>0.0108 ± 0.0007</td>
<td>0.94544</td>
</tr>
<tr>
<td>pH 6</td>
<td>6.090 ± 0.68847</td>
<td>0.126 ± 0.00347</td>
<td>0.991866</td>
<td></td>
</tr>
<tr>
<td>pH 7.4</td>
<td>28.59 ± 2.319</td>
<td>0.127 ± 0.00338</td>
<td>0.98629</td>
<td></td>
</tr>
<tr>
<td>Freundlich</td>
<td>pH 4.2</td>
<td>0.2658 ± 0.0486</td>
<td>0.010 ± 0.00038</td>
<td>0.94741</td>
</tr>
<tr>
<td>pH 6</td>
<td>0.284 ± 0.04592</td>
<td>0.10792 ± 0.00396</td>
<td>0.95753</td>
<td></td>
</tr>
<tr>
<td>pH 7.4</td>
<td>0.1266 ± 0.01785</td>
<td>0.1245 ± 0.01785</td>
<td>0.98363</td>
<td></td>
</tr>
<tr>
<td>Adsorption media</td>
<td>$n$</td>
<td>$K_f$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adsorption media</td>
<td>$1/\alpha_s$</td>
<td>$b$ (l/(mmol)$^{-1}$)</td>
<td>$q_m$ (mmol/g)</td>
<td>$R^2$</td>
</tr>
<tr>
<td>Sips</td>
<td>pH 4.2</td>
<td>0.5429 ± 0.0698</td>
<td>1.70823 ± 0.43251</td>
<td>0.94054</td>
</tr>
<tr>
<td>pH 6</td>
<td>1.4697 ± 0.15688</td>
<td>17.728 ± 6.1259</td>
<td>0.1138 ± 0.00276</td>
<td>0.99076</td>
</tr>
<tr>
<td>pH 7.4</td>
<td>0.2312 ± 0.3664</td>
<td>1.0693 ± 0.4199</td>
<td>0.2408 ± 0.0456</td>
<td>0.98912</td>
</tr>
<tr>
<td>Temkin</td>
<td>pH 4.2</td>
<td>121.02 ± 18.7815</td>
<td>0.002 ± 0.00032</td>
<td>0.95011</td>
</tr>
<tr>
<td>pH 6</td>
<td>72.823 ± 2.667</td>
<td>0.02536 ± 0.00252</td>
<td>0.97749</td>
<td></td>
</tr>
<tr>
<td>pH 7.4</td>
<td>87.65857 ± 9.540</td>
<td>0.01371 ± 0.00179</td>
<td>0.98202</td>
<td></td>
</tr>
<tr>
<td>Adsorption media</td>
<td>$B$</td>
<td>$b_0$ (mmol/g)</td>
<td>$R^2$</td>
<td></td>
</tr>
<tr>
<td>D-B</td>
<td>pH 4.2</td>
<td>0.1363 ± 0.0281</td>
<td>0.0102 ± 0.0005</td>
<td>0.94048</td>
</tr>
<tr>
<td>pH 6</td>
<td>0.1639 ± 0.00961</td>
<td>0.1174 ± 0.00182</td>
<td>0.99479</td>
<td></td>
</tr>
<tr>
<td>pH 7.4</td>
<td>0.0524 ± 0.0092</td>
<td>0.1262 ± 0.0031</td>
<td>0.96157</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4
Kinetic adsorption parameters of CIP onto nano-HA obtained using Avrami equation at 37 °C.

<table>
<thead>
<tr>
<th>Adsorption media</th>
<th>$K_{av}$ (min$^{-1}$)</th>
<th>$n$</th>
<th>$q_t$ (mmol/g)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 4.2</td>
<td>0.0018 ± 0.001554e-4</td>
<td>1.58473 ± 0.19899</td>
<td>0.00105 ± 3.13655e-5</td>
<td>0.98257</td>
</tr>
<tr>
<td>pH 6</td>
<td>0.00199 ± 1.85776e-4</td>
<td>1.26275 ± 0.12901</td>
<td>0.0059 ± 2.2792e-4</td>
<td>0.99346</td>
</tr>
<tr>
<td>pH 7.4</td>
<td>0.00196 ± 1.529262e-4</td>
<td>1.3865 ± 0.13597</td>
<td>0.00217 ± 7.78049e-5</td>
<td>0.99201</td>
</tr>
</tbody>
</table>

### Table 5
Kinetic adsorption parameters of IBU onto nano-HA obtained using Avrami equation at 37 °C.

<table>
<thead>
<tr>
<th>Adsorption media</th>
<th>$K_{av}$ (min$^{-1}$)</th>
<th>$n$</th>
<th>$q_t$ (mmol/g)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 4.2</td>
<td>0.02186 ± 0.001326</td>
<td>0.39903 ± 0.02372</td>
<td>0.00087 ± 0.00005</td>
<td>0.95877</td>
</tr>
<tr>
<td>pH 6</td>
<td>0.00113 ± 8.29284e-4</td>
<td>0.74058 ± 0.06603</td>
<td>0.115456 ± 0.00153</td>
<td>0.99768</td>
</tr>
<tr>
<td>pH 7.4</td>
<td>0.00398 ± 7.1175e-4</td>
<td>0.89407 ± 0.15238</td>
<td>0.12984 ± 0.02962</td>
<td>0.98917</td>
</tr>
</tbody>
</table>

### Table 6
Desorption studies for CIP. Parameters of Korsmeyer –Peppas model in different media at 37 °C.

<table>
<thead>
<tr>
<th>Desorption studies for CIP. Parameters of Korsmeyer –Peppas model in different media at 37 °C.</th>
<th>$K$ (min$^{-1}$)</th>
<th>$n$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 4.2</td>
<td>9.22545e-4 ± 1.4798e-5</td>
<td>0.1658 ± 0.019413</td>
<td>0.9970</td>
</tr>
<tr>
<td>pH 6</td>
<td>9.8719e-4 ± 6.56887e-5</td>
<td>0.2045 ± 0.007831</td>
<td>0.9901</td>
</tr>
<tr>
<td>pH 7.4</td>
<td>0.00158 ± 1.85661e-4</td>
<td>0.19921 ± 0.02652</td>
<td>0.9921</td>
</tr>
</tbody>
</table>
ARG onto the surface of nano-rods, and for this reason kinetic process cannot be analyzed satisfactorily. However, the obtained numbers are shown in Table 5 for supporting this consideration.

### 3.4. Desorption/release behavior assessment of CIP and, independently, IBU from nano-HA in different pH and ionic strength conditions. Desorption models

The CIP and IBU release from nanoparticles has been evaluated through Korsmeyer-Peppas model. We based its election on: it is an easy analysis method and can be applied to systems with the features of our nano-rods which have a cylindrical shape and they are not swellable. The description of models and equations are shown in Section 2.3 of ESM. Fig. 6 (6A: CIP and 6B: IBU) shows the release behavior of both drugs indicating the Q in mmol of drug released per gram of nano-HA and also at the right of each fitted curve is displayed the percentage of each drug released ($R_{Ap\%}$) which was calculated with Eq. (2). The $R_{Ap\%}$ values for CIP were: at pH 6, 26.9 ± 7%; at pH 7.4, 56.27 ± 9%. All parameters; constants, order and coefficient of determination are shown in Table 7. It is worth noting that, quantitatively, at pH 6 more concentration of CIP is released compared to the others two cases, but only the 27% of CIP adsorbed onto nano-rods is released at this pH during 10,080 min (7 days). Therefore, in this case, CIP-loaded nano-rods are working such as drug carrier with sustained release properties and as a reservoir host of CIP which could be released during the bone remodeling process (for example: By means of variation of pH naturally triggered in this process). This concentration is higher than that necessary to inhibit the growth of the mentioned bacteria strains. At others pHs, the concentration of CIP released from nano-HAs is also enough, and possibly, an infection could be treated by application in situ of CIP-loaded nanoparticles. At pH 4.2 the highest percentage of CIP is released ($R_{Ap\%}$) related to the CIP absorbed. This could be due to the ionization of CIP and also by early stages of degradation of nano-HA. At pH 7.4 the percentage of released CIP is 56% which could be due to the low solubility of CIP in this medium.

Peppas and coworkers have reported a set of studies which the shape and size of the structure of release system plays an important role in the process [66,67]. In those works, the authors reported that the value of $n$ is highly influenced by the heterogeneity of the structure of the nano-rods from which the drug is released. This means that the value of $n$, due to we are working with rods or cylinders is affected by the size distribution, long and diameter. Thus, a value of $n = 0.43$ for a released drug from spherical matrix could become into 0.3 or less if the heterogeneity of the particles increase. They also report that small particles have importance in the first steps of the release and then, the rest of the process is influenced by bigger particles. We hypothesized that this factor is playing an important role in our samples and for this reason; the value of $n$ does not reach 0.4. However, the limits of the values of $n$ can be modified depending on the particle size distribution. Thus, by considering our results, we cannot assume linearly that the release of both active principles follows the Fick’s model.

The $R_{Ap\%}$ values for IBU were: at pH 6, 94.4 ± 11%; at pH 7.4, 5.7 ± 1%, at pH 4.2, 1.05 ± 1%. All parameters; constants, order and coefficient of determination are shown in Table 7. We mentioned above that the adsorption process at pH 4.2 was erratic and therefore the release process was not reliable (the determination coefficients are not good) (see Table 7). The 94% of IBU loaded onto nano-HA was released at pH 6. This behavior is opposite to that for CIP (which 27% of loaded drug is released). However, for both cases the released drugs reach higher values than those obtained into the others media. At pH 7.4, 6% of the loaded IBU is released. Therefore, at this pH, the system behaves as a reservoir of drug which should increase the release percentage when pH turns into 6. We propose that, if the system is implanted as a bone filling material and considering the naturally triggered pH changes into an affected zone, the IBU should increase it release percentage when pH change from 7.4 to 6.

### 4. Conclusion

In this work has been studied the adsorption and release behavior of two active principles (CIP and IBU), used for the treatment of bone infection diseases, onto synthesized nano-rods of hydroxyapatite. The active principles were adsorbed directly (CIP) or by means of L-ARG as a linker (IBU) following a pH-responsive behavior. Experimental data (for IBU and CIP) were fitted to five adsorption models, and the adsorption process was better explained by Sips model than by the others. The adsorption kinetic for both drugs was fitted by Avrami’s model. The release of both drugs from nano-HA was in their therapeutic-range concentration and follows a pH-responsive release. It is shown that the highest concentration of CIP was adsorbed at pH 6, but their released percentage was the lowest. Contrary, the highest concentration of IBU was adsorbed at pH 7.4 but their percentage released was the lowest at pH 6. Owing to that the value of $n$ in korsmeyer-Peppas model is lower than 0.45 for both drugs, it is hypothesized that the polydispersity of

<table>
<thead>
<tr>
<th>Desorption medium</th>
<th>$K$ (min$^{-1}$)</th>
<th>$n$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 4.2</td>
<td>2.464 ± 0.1381</td>
<td>0.084 ± 0.0092</td>
<td>0.8214</td>
</tr>
<tr>
<td>pH 6</td>
<td>0.0295 ± 0.00179</td>
<td>0.147 ± 0.0078</td>
<td>0.9923</td>
</tr>
<tr>
<td>pH 7.4</td>
<td>0.0054 ± 0.00106</td>
<td>0.266 ± 0.03041</td>
<td>0.9721</td>
</tr>
</tbody>
</table>

Table 7 Desorption studies for IBU. Parameters of Korsmeyer–Peppas model in different media at 37°C.
nano-rods plays an important role in release performance. Finally, these systems would have an essential quality to fulfill the requests of new treatment strategies related to bone defects reconstruction and additionally, it is expected a reduction of the risk of toxicity-related side effects of these active agents.

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Appendix A: Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.msec.2019.01.098.

References


