Hindawi Publishing Corporation
International Journal of Chemical Engineering
Volume 2009, Article ID 273808, 5 pages
doi:10.1155/2009/273808

Research Article

The Kinetics of Ampicillin Release from Hydroxyapatite for Bones Regeneration

Giovanilton Ferreira da Silva,[1] José Sílvio Veras Albuquerque,[2] Caroliny Gomes de Oliveira,[1] Ricardo Emilio Ferreira Quevedo Nogueira,[2] and Andrea Lopes de Oliveira Ferreira[1]

[1] *Grupo de Pesquisa em Processos Biotecnológicos (GPBIO), Departamento de Engenharia Química, Universidade Federal do Ceará, Campus do Pici, Bloco 709, Pici, 60455-760 Fortaleza, Brazil*
[2] *Laboratório de Desenvolvimento de Materiais Cerâmicos (LDMC), Departamento de Engenharia Mecânica, Universidade Federal do Ceará, Campus do Pici, Bloco 720, Pici, 60455-760 Fortaleza, Brazil*

Correspondence should be addressed to Andrea Lopes de Oliveira Ferreira, andrea@ufc.br

Received 31 October 2008; Accepted 3 February 2009

Recommended by Eugénio C. Ferreira

Semisynthetic beta-lactam antibiotics are among the most used pharmaceuticals. Their use in veterinary and human medicine is in continuous expansion. There is a growing need for developing bioactive implants. Advantages of implantable drug delivery tools can include high release efficiency, precise dose control, low toxicity, and allow to overcome disadvantages connected with conventional methods. In this respect, hydroxyapatite (HA) is an elective material. It enables to produce architectures similar to those of real bones. Here we studied a kinetic model to describe ampicillin release from HA. In the course of adsorption experiment, ampicillin was dissolved, maintained at 30°C and shaken at 60 strokes/minute. Samples were withdrawn periodically for analysis and then returned to the mixture. Adsorbed amounts were measured by the difference of the concentration of the antibiotics before and after adsorption using UV adsorption at 225 nm. The aim of this work was to evaluate its application as ampicillin delivery carrier.

1. Introdution

Semisynthetic beta-lactam antibiotics are the most important class of antibacterial agents. Their use in veterinary and human medicine is in continuous expansion. Some examples of semisynthetic penicillins and cephalosporins are amoxicillin, ampicillin, cephalexin, cefadroxil, and cefazolin, among many others. They have in common the presence of the beta-lactam ring, responsible for their antimicrobial activity. They irreversibly inhibit the last step of the bacterial cell wall biosynthesis. The beta-lactam antibiotics can be described in terms of a beta-lactam nucleus with a side-chain (Figure 1).

Many different nuclei and side-chains are found in the antibiotics that are in use today. Different combinations of side-chains and nuclei form antibiotics with distinctive properties; for example, replacing the phenylacetic acid side-chain of penicillin G with D-phenylglycine (PG) results in the beta-lactam antibiotic ampicillin (Figure 2), which in contrast to penicillin G, is orally stable [1–4]. Ampicillin (6-[2-amino-2-phenylacetamide] penicillanic acid) is in the penicillin group of which penicillin proper was the first antibiotic to be used in therapy [5, 6]. It is one of most widely used semisynthetic beta-lactam antibiotics [7]. It has an estimated market of 20000 ton/year [5].

There is a growing need for developing bioactive implants, due biomaterials are biocompatible, resorbable, and present osteoconductive properties. It is known that the use of bone substances has many inherent disadvantages in practical applications, and it is linked to many surgical problems [8].

Advantages of implantable drug delivery tools can include high release efficiency, precise dose control, low toxicity, and allow to overcome disadvantages connected with conventional methods [9]. In this respect, hydroxyapatite (HA) is an elective material. It enables to produce

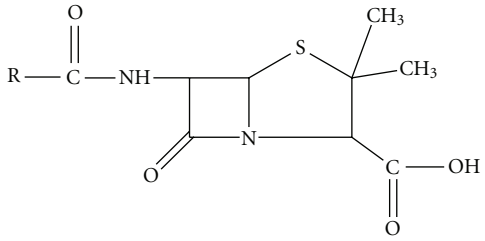

Figure 1: General structure of beta-lactam antibiotics.

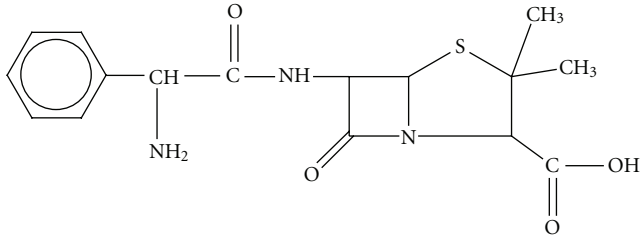

Figure 2: Structure of ampicillin, in highlighted: beta-lactam ring.

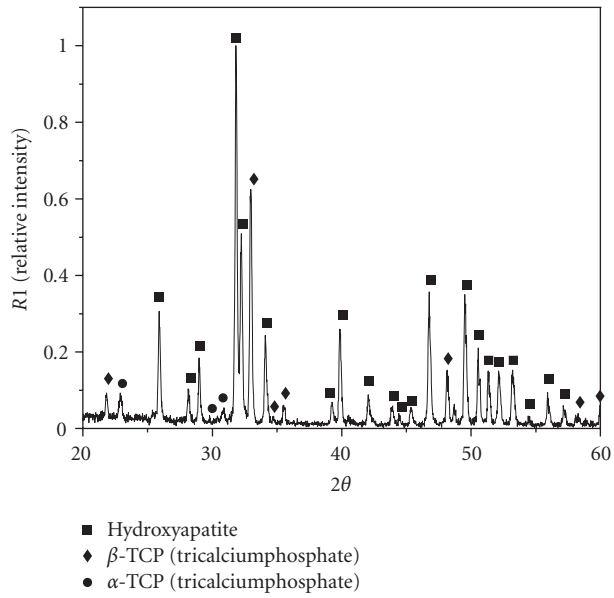

Figure 3: X-ray diffraction of HA pellet synthesized at 1350°C.

architectures similar to those of real bones. Here we studied a kinetic model to describe ampicillin release from HA. HA analogous to the mineral component of bones, its properties make it desirable as implant materials and delivery agents of drugs. This paper describes the ampicillin adsorption and release profiles of HA material. The aim of this work was to evaluate its application as ampicillin delivery carrier.

2. Materials and Methods

2.1. Materials. Ampicillin was from Aldrich Chem. Co., USA. All other chemicals were of laboratory grade from different commercial suppliers.

2.2. Antibiotic Loaded HA Samples. Ampicillin was used as drug molecules. HA were impregnated with 25 mM of antibiotic buffer solution at 30°C for 48 hours. Ampicillin adsorbed in the HA has been quantified by spectophotometric analysis. In the course of the release experiment, ampicillin was dissolved in phosphate buffer to make a stock solution, maintained at 30°C and shaken at 60 strokes/minute. Kinetic experiments to determine the amount of ampicillin adsorbed as a function of contact time were conducted by stirring. Samples were withdrawn periodically for analysis and then returned to the mixture. Adsorbed amounts were measured by the difference of the concentration of the antibiotics before and after adsorption using UV adsorption at 215 nm.

2.3. HA Synthesis. HA was synthesized by the aqueous precipitation method from CaO and H_3PO_4 as the reagents, and used as a carrier for charging ampicillin. In this study, apatite nanoparticles were produced by aqueous precipitation. The starting solution was 0.3 M H_3PO_4, 0.5 M $Ca(OH)_2$, and 1 M CH_3CHCO_2HOH. The pH value of the solution was

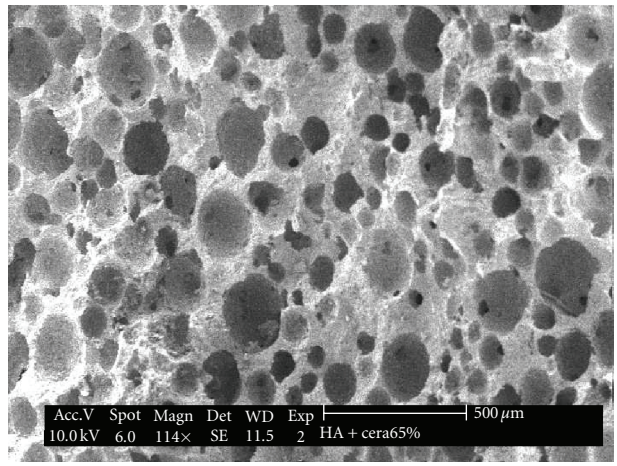

Figure 4: Scanning electron micrographs of a sample HA particle, after synthesis assay.

adjusted to pH 8 by NH_4OH additon. The suspensions were left overnight for ageing. The suspension was then vacuum filtered and washed in deionised water to remove NH_4OH. The powders were dried in an oven at 100°C overnight. HA powders and carnauba wax were synthesized in stirred 50°C. HA powders were then synthesize by means of uniaxial pressing (40 MPa) and convenient thermal treatments. The pellets were heated to 550°C to remove all wax. Pellets of cilinder shape were produced with size of 19 mm of diameter and 19 mm of length at 1350°C The powders were analyzed by X-ray diffraction (XRD) and then sintered. Sintered powders were again analyzed by XRD and scanning electronic microscopy (SEM) to assess the final phase composition [10]. Figures 3 and 4 show XRD and SEM of HA, porous of 100 ~ 300 μm were obtained.

2.4. Solubility Experiments. Solubility of ampicillin was determined following Gude et al. [11]. The samples were prepared gravimetrically. Glass-flasks with screw caps filled with the samples were immersed into a thermostated water bath and stirred. All samples were stirred for at least 4 hours. Subsequently, the mixture was allowed to settle. The samples were taken with syringes with an attached 0.2 μm filter to avoid entrainment of solids. The compositions of the liquid phases were analyzed by HPLC.

2.5. Analysis. Concentrations of ampicillin were determined using HPLC to analyze if antibiotic degraded during the assays: C18 column (Waters Nova-Pack, C18, 60 Å, 4 μm, 3.9 × 150 mm); eluent: 35% acetonitrile, 2‰ SDS (lauryl sodium sulphate), 10 mM H_3PO_4, 5 mM $K_2H_2PO_4$, with a flow of 1 mL/min at 25°C and $\lambda = 225$ nm.

2.6. Adsorption Performance. Ampicillin adsorption performance (AP) was defined as follows:

$$\%AP = \left(1 - \frac{C^*}{C^{\text{initial}}}\right) \times 100, \quad (1)$$

where C^* is the concentration of ampicillin (mM), and C^{initial} is the concentration of ampicillin at the beginning of the assays (mM).

3. Results and Discussion

3.1. Solubility Studies. The solubility of ampicillin was measured for pHs in the range 7.8–8.0, at 30°C. The selected range of pHs for the solubility studies was bracketed by stability of antibiotic. The obtained results, which are shown in Figure 5, are similar to the ones obtained by other authors [12–14].

Ampicillin solubility increases with the pH. This behavior can be explained by its determined values of the acid group pK (2.66) and amine group pK (7.24) and calculated of its isoelectric point (4.95). Hence, above the pH correspondent to its isoelectric point the number of ampicillin molecules with a neutral charge (which is the most insoluble form) decreases, leading to higher solubility values. This effect becomes more important for pH above 7.0.

Our aim is to perform the ampicillin adsorption with its separation from HA at 37°C, what implies to working at sorption conditions where the solubility of ampicillin (AMP) is the highest possible. Therefore, the obtained results indicate that for higher pH, the performance of the AMP release might be better. However, the best value for pH is a tradeoff between AMP solubility and temperature.

Temperature effects on the solubility of ampicillin were also evaluated by determining the compounds solubility at pH 7.0 and 8.0, at 37°C. The antibiotic solubility slightly increases at 37°C. Most of the solubility values at 37°C were around 11% higher than those at 30°C. However, operation of the system at 37°C implies too much lower adsorption rates than at 30°C. It is believed that an increase of 11% in the ampicillin solubility is not big enough to justify the operation at such a high temperature. Anyway, these experiments aimed at only indicating the range of adsorption conditions for studying the kinetic of the process.

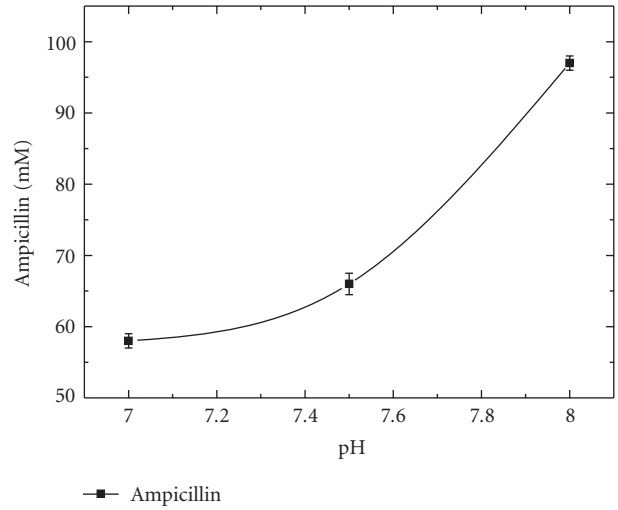

Figure 5: Solubility of ampicillin as function of pH at 30°C.

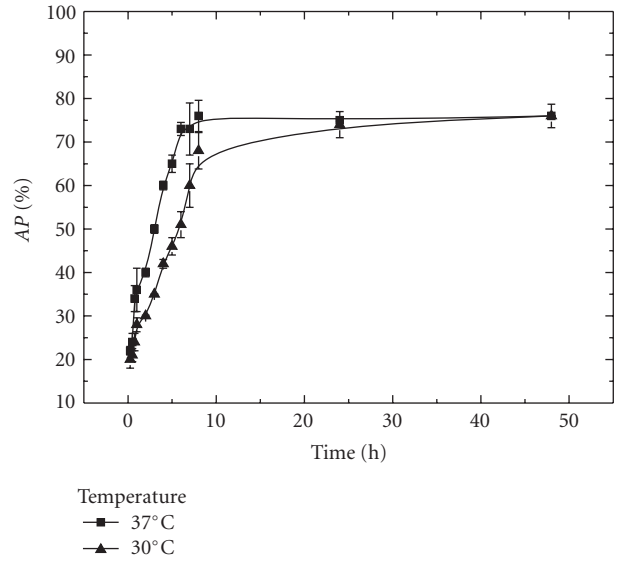

Figure 6: Equilibrium assays at $C^{\text{initial}} = 25$ mM, phosphate butter 10 mM , pH 7.5.

3.2. Equilibrium Time. In order to properly evaluate the adsorption process of ampicillin, the equilibrium curves were determined experimentally. Equilibrium time depends on adsorption rate, that is, affinity between antibiotic and HA, and temperature of assay. Figure 6 shows the results to ampicillin adsorption at 30 and 37°C, respectively. It can be observed that equilibrium state was obtained at 8 hours. At 37°C, adsorption rate higher than at 30°C can be explained due to increasing of mass transfer, because adsorption is not favor at higher temperatures.

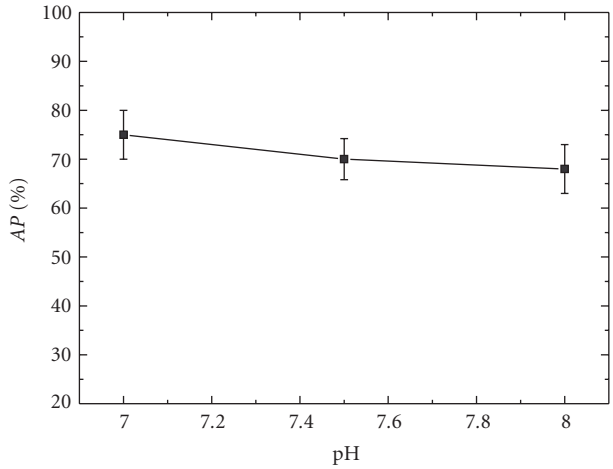

Figure 7: pH effect on adsorption ampicillin at 30°C.

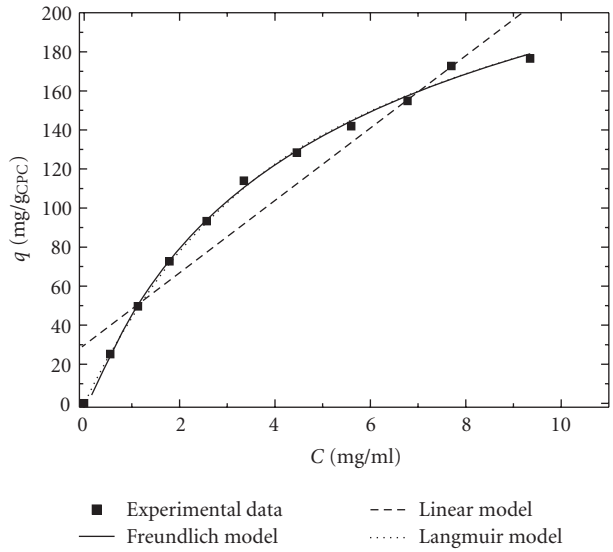

Figure 8: Adsorption isotherms of ampicillin on HA at 30°C, pH 7.0.

3.3. pH Effect at Adsorption Isotherms. Figure 7 shows the effect of pH at adsorption performance of ampicillin. It can be observed that *AP* decreases when pH increases. The amino group of ampicillin is not protoned when pH increases, so less antibiotic can be adsorbed on HA. Therefore, ampicillin adsorption slightly improves when pH decreases.

Adsorption isotherms of ampicillin on HA at 30°C and pH 7.0 were shown in Figure 8. The adsorption isotherms were further correlated to Linear, Freudlich, and Langmuir equations:

$$q = K_{\text{lin}}C,$$
$$q = K_{\text{Fr}}C^{n}, \quad (2)$$
$$q = \frac{q_m C}{K_{\text{Lan}} + C},$$

where q is the equilibrium adsorption capacity (mg/g_{HA}), C is the initial concentration of ampicillin (mg/mL), K_{lin}, K_{Fr}, and K_{Lan} are Linear, Freundlich, and Langmuir parameters, respectively. n is the Freundlich parameter related to the magnitude of adsorption driving force. q_m is the maximum adsorption capacity on HA. Some assumptions were made to adsorption model: the process operated under isothermal conditions, HA porosity was constant and homogeneous along as the particle.

The data plotted were fitted according to the Linear isotherms: $K_{\text{lin}} = 18.46 \pm 1.85$ and $R^2 = 0.958$. The Freundlich isotherms were $K_{\text{Fr}} = 52.51 \pm 3.48$, $n = 0.5688 \pm 0.036$, and $R^2 = 0.980$, and Langmuir isotherms were $q_m = 274.05 \pm 8.92$, $K_{\text{Lan}} = 4.99 \pm 0.34$, and $R^2 = 0.998$. The n value of Freundlich isotherm shows that adsorption process is favorable ($n < 1$) at 30°C. All parameters for the adsorption of ampicillin were obtained using the nonlinear least squares algorithm of Marquardt [15], with 95% confidence interval for the parameter estimates.

In the process of analysis of the models, as Freundlich isotherm and as Langmuir isotherm can represent the isotherm data of HA appropriately in the given concentration range. The Linear isotherm just presented good fitting at lower concentration. Note that a constant exist instead of zero in Figure 6, which is caused due to higher concentration of ampicillin. The best fit was obtained when Langmuir model ($R^2 = 0.998$) was used, although Freundlich model was also reached good fit.

4. Conclusions

Ampicillin solubility was studied at different pH. Higher pH improved ampicillin solubility. The equilibrium state was obtained at 8 hour of assay. At 37°C, adsorption rate was higher than at 30°C. The best results of adsorption performance were obtained when pH decreased (7.0). The relation between the adsorption capacity and the equilibrium solute concentration was analyzed. Linear, Freundlich, and Langmuir isotherms were used and provided good fit for data. The best model was achieved with Langmuir isotherm.

References

[1] M. Cole, "Penicillins and other acylamino compounds synthesized by the cell-bound penicillin acylase of *Escherichia coli*," *Biochemical Journal*, vol. 115, no. 4, pp. 747–756, 1969.

[2] M. A. Wegman, M. H. A. Janssen, F. van Rantwijk, and R. A. Sheldon, "Towards biocatalytic synthesis of β-lactam antibiotics," *Advanced Synthesis & Catalysis*, vol. 343, no. 6-7, pp. 559–576, 2001.

[3] A. Bruggink, E. C. Ross, and E. de Vroom, "Penicillin acylase in the industrial production of β-lactam antibiotics," *Organic Process Research & Development*, vol. 2, no. 2, pp. 128–133, 1998.

[4] M. P. A. Ribeiro, A. L. O. Ferreira, R. L. C. Giordano, and R. C. Giordano, "Selectivity of the enzymatic synthesis of ampicillin by *E. coli* PGA in the presence of high concentrations of substrates," *Journal of Molecular Catalysis B*, vol. 33, no. 3–6, pp. 81–86, 2005.

[5] R. A. Sheldon, F. van Rantwijk, L. M. van Langen, M. A. Wegman, L. Cao, and M. H. A. Janssen, "Biocatalysis and biocatalysis in the synthesis of β-lactam antibiotics," in *Synthesis of β-Lactam Antibiotics: Chemistry, Biocatalysis Process Integration*, A. Bruggink and P. D. Roy, Eds., pp. 102–149, Kluwer Academic Publishers, Dordrecht, The Netherlands, 2001.

[6] A. Pessina, P. Lüthi, P. L. Luisi, J. Prenosil, and Y.-S. Zhang, "Amide-bond syntheses catalyzed by penicillin acylase," *Helvetica Chimica Acta*, vol. 71, no. 3, pp. 631–641, 1988.

[7] S. Ospina, E. Barzana, O. T. Ramírez, and A. López-Munguía, "Effect of pH in the synthesis of ampicillin by penicillin acylase," *Enzyme and Microbial Technology*, vol. 19, no. 6, pp. 462–469, 1996.

[8] M. Yoshimura, W. Suchanek, T. Watanabe, B. Sakurai, and M. Abe, "Functionally graded $SrTiO_3$-$BaTiO_3$ thin films prepared by the hydrothermal-electrochemical method under flowing solution," *Journal of Materials Research*, vol. 13, no. 4, pp. 875–879, 1998.

[9] K. E. Uhrich, S. M. Cannizzaro, R. S. Langer, and K. M. Shakesheff, "Polymeric systems for controlled drug release," *Chemical Reviews*, vol. 99, no. 11, pp. 3181–3198, 1999.

[10] J. S. V. Albuquerque, J. V. F. Neto, J. I. L. A. Júnior, D. O. Lima, R. E. F. Q. Nogueira, and M. H. Prado da Silva, "Porous bioceramics produced with calcium phosphate nanoparticles," *Key Engineering Materials*, vol. 240–242, pp. 23–26, 2003.

[11] M. T. Gude, H. H. J. Meuwissen, L. A. M. van der Wielen, and K. Ch. A. M. Luyben, "Partition coefficients and solubilities of α-amino acids in aqueous 1-butanol solutions," *Industrial & Engineering Chemistry Research*, vol. 35, no. 12, pp. 4700–4712, 1996.

[12] M. B. Diender, A. J. J. Straathof, L. A. M. van der Wielen, C. Ras, and J. J. Heijnen, "Feasibility of the thermodynamically controlled synthesis of amoxicillin," *Journal of Molecular Catalysis B*, vol. 5, no. 1–4, pp. 249–253, 1998.

[13] E. S. J. Rudolph, M. Zomerdijk, K. Ch. A. M. Luyben, and L. A. M. van der Wielen, "Correlating the phase behaviour of semi-synthetic antibiotics and their precursors in water+1-butanol mixtures," *Fluid Phase Equilibria*, vol. 158–160, pp. 903–912, 1999.

[14] M. I. Youshko, L. M. van Langen, E. de Vroom, et al., "Penicillin acylase-catalyzed synthesis of ampicillin in "aqueous solution-precipitate" systems. High substrate concentration and supersaturation effect," *Journal of Molecular Catalysis B*, vol. 10, no. 5, pp. 509–515, 2000.

[15] D. W. Marquardt, "An algorithm for least-squares estimation of nonlinear parameters," *SIAM Journal on Applied Mathematics*, vol. 11, no. 2, pp. 431–441, 1963.