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Bone implants modified with cyclodextrin: Study of drug release in bulk fluid and into agarose gel

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ABSTRACT

The aim of this work was to better understand the importance of the type of experimental setup used to monitor antibiotic release from functionalized hydroxyapatite implants. Microporous hydroxyapatite discs were prepared by sintering and subsequently functionalized with hydroxypropyl-β-cyclodextrin (HPβCD) polymer crosslinked with butanetetracarboxylic acid. On one hand, polymerization was performed within the implant after its impregnation with the monomers (CD-HA-M implant). On the other hand, a pre-synthesized HPβCD polymer was loaded and fixed onto the HA discs (CD-HA-P implant). Both types of implants were soaked with ciprofloxacin hydrochloride or vancomycin hydrochloride solution and dried at 37 °C. The DSC study highlighted that the cyclodextrin polymer could interfere with both drugs, due to the carboxylic groups carried by the crosslinks. Drug release was measured into phosphate buffered saline pH 7.4 in agitated vials, or into agarose gels to more realistically mimic *in vivo* conditions. Importantly, in all cases, drug release into agarose gels was much slower than into well-agitated phosphate buffer. Non-functionalized discs displayed faster drug release because no complex could be formed and/or due to the absence of the HPβCD polymer network hindering drug diffusion within the implant pores. In the case of ciprofloxacin hydrochloride, drug release from the CD-HA-M implants was faster than drug release from the CD-HA-P implants due to the different polymer structures resulting in different complexation strengths, whereas in the case of vancomycin hydrochloride the release patterns were similar because vancomycin hydrochloride was not included into the cyclodextrin. The agarose gel method seems more biorelevant and discriminatory than the vial method for drug release measurements from bone implants.

1. Introduction

Parenteral controlled drug delivery systems such as implants are steadily gaining in practical importance, allowing for optimized therapeutic effects. However, no regulatory standards have yet been established to assess the performance of these types of advanced drug delivery systems for quality control or during formulation development (Burgess et al., 2004). Various types of experimental setups have been reported in the literature, including the use of vials with or without agitation, dialysis membranes, the United States Pharmacopoeia (USP) apparatus 1 (paddle) and the USP apparatus 4 (flow through cell) (Burgess et al., 2004; Nastruzzi et al., 1993; Conti et al., 1995; Gido et al., 1993; Bain et al., 1999; Torrado et al., 2001). One major drawback of these frequently applied measurement techniques is the fact that the devices are exposed to bulk fluids, and this is not a realistic environment upon *in vivo* administration. Hence, the primary challenge is to design an *in vitro* drug release measurement technique that can simulate the real environment of the dosage forms after administration. Importantly, implants are designed to release drug into their vicinity: tissues with only very slowly moving extracellular fluids. To closely simulate this type of *in vivo* environment upon implantation, agarose gels have been chosen in this study because of their rheological properties and high water contents (Hoffman, 2002; Allababidi and Shah, 1998).

Bone infection (osteomyelitis) caused by bacteria introduced from trauma, surgery or implantation is a serious and possibly life-threatening condition. Difficulties in the treatment of osteomyelitis include poor antimicrobial drug distribution at the site of infection due to limited blood circulation in infected skeletal tissue and

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inability to directly address the biofilm of pathogenous growth on the implant surface. High systemic dosage of antibiotics to facilitate sufficient tissue and biofilm penetration is not preferable because of possible toxic side effects (Marculescu and Osmon, 2005). The use of controlled antimicrobial drug delivery systems in orthopedic combination devices offers an important adjunctive route to administer antibiotics for the prevention or treatment of osteomyelitis (Wu and Grainger, 2006; Frutos et al., 2009; Alkhraisat et al., 2009). Desired drugs can, thus, be *locally* released following implantation and at appropriate rates. Cyclodextrins have been recently studied as promising carriers for antibiotic drugs (Ferrari et al., 2008; Thatiparti and Recum, 2009). These cyclic oligosaccharides have a torus structure, with primary and secondary outwards oriented hydroxyl groups. As a consequence, cyclodextrins have a hydrophilic exterior and a hydrophobic cavity exhibiting the ability to form reversible complexes with a number of drugs. The latter can be released at pre-programmed rates upon drug–cyclodextrin complex incorporation into the implants (Bibby et al., 2000). The possibility of immobilizing the cyclodextrins onto medical devices was reported in many studies (Lepretre et al., 2009; Martel et al., 2002a; El Ghoul et al., 2007; Tabary et al., 2007; Blanchemain et al., 2008). The fixation results from a reaction of esterification between the cyclodextrin and a reticulating agent (often a polycarboxylic acid). The formed polymer is then grafted with covalent bonds on the medical device (e.g. on cellulose surfaces), or fixed by physical interactions around fibers (e.g. polyester-based, polypropylene-based), or is mechanically blocked inside the pores of the biomaterial (e.g. within porous polyvinylidene difluoride membranes). In this study, hydroxyapatite (HA) was used as implant matrix former and antibiotic carrier material for the treatment of bone infections, since their chemical composition is very similar to the bone mineral phase (Yu et al., 1992; Martin et al., 1998). The cyclodextrin polymer is immobilized in the cavity of the hydroxyapatite by mechanical blocking and physical interactions. The medical device, thus functionalized, is then impregnated in a solution of drug and subsequently dried.

The aim of this work was to better understand the importance of the experimental conditions used to monitor sustained release of drugs from bone implants. The agarose gel method was used to provide more realistic conditions during *in vitro* release measurements and was compared to the frequently used vial method.

2. Materials and methods

2.1. Materials

High-purity aqueous hydroxyapatite (HA) slurry (Hornez et al., 2007); 1,2,3,4-butanetetracarboxylic acid and sodium hypophosphite (NaH_2PO_2) from Aldrich Chemicals (St. Louis, USA); deuterium oxide (D_2O) from Euriso-Top (Saint-Aubin, France); Kleptose HPB® pharma grade (hydroxypropyl-β-cyclodextrin, MS = 0.65, HPβCD) from Roquette (Lestrem, France); ciprofloxacin hydrochloride from Bayer Schering Pharma (Berlin, Germany); vancomycin hydrochloride from Sandoz (Levallois-Perret, France); GenAgaroseose LE (agarose powder) from Genaxxon BioScience (Ulm, Germany); methanol HPLC grade (99.9%); acetonitrile HPLC grade (99.9%) from Fisher Chemical (Leicestershire, England); formic acid 98–100%; acetic acid 100% and aqueous ammonia 25–30% from Merck (Darmstadt, Germany).

Fig. 1 presents the chemical structures of the two antibiotic drugs (ciprofloxacin hydrochloride and vancomycin hydrochloride as well as of polyBTCA–HPβCD.

2.2. Methods

2.2.1. Implant preparation

The methods were inspired from our previous works (Lepretre et al., 2009; Martel et al., 2002a,b, 2006, 2005; El Ghoul et al., 2007; Tabary et al., 2007; Blanchemain et al., 2008). Briefly, the cylindrical and microporous HA discs (diameter: 15 mm, height: 2 mm, mass: 725 ± 40 mg, internal pore size: 1–10 μm) were prepared by sintering and subsequently functionalized with HPβCD polymer using 1,2,3,4-butanetetracarboxylic acid as cross-linker and sodium hypophosphite as catalyst. On one hand, polymerization was performed within the implant after its impregnation with the monomers (BTCA, catalyst and HPβCD) followed by a curing process (160 °C, 2 h). The CD polymer is formed from the reactant adsorbed onto the pore walls of the HA. On the other hand, a pre-synthesised CD polymer was loaded and fixed onto the HA discs under curing process (160 °C, 2 h). The pre-synthesis polymer, developed and prepared in our laboratory, diffused inside the pore of the HA before being fixed therein. In both cases, this treatment resulted in the fixation of cyclodextrin polymers (polyBTCA–HPβCD) within the porous structure of the HA discs by physical interactions with the support and mechanical fixation; after the polymerization process, the CD polymer was blocked in the pores of the HA as shown in our previous work (Lepretre et al., 2009). The structure of polyBTCA–HPβCD is shown in Fig. 1a. In accordance with the nomenclature used in a former paper (Lepretre et al., 2009), the modified implants issued from the two above mentioned methods will be called CD-HA-M (= monomer pathway) and CD-HA-P (= pre-polymer pathway) respectively. The cyclodextrin polymer contents in the implants, 7 wt%, were determined by thermogravimetric analysis (Lepretre et al., 2009). Both types of functionalized implants were loaded with antibiotic drugs by immersion into the following solutions: 2 g/L of ciprofloxacin hydrochloride and 10 g/L of vancomycin hydrochloride in water, under vacuum for 3 h and subsequent drying at 37 °C till constant weight. For reasons of comparison, also polyBTCA–HPβCD-free implants (non-functionalized implants) were loaded with the drugs.

2.2.2. Determination of the drug loading

HA implants were placed in 20 mL of methanol overnight at 37 °C, under shaking. The implants were then exposed to 10 mL of aqueous NaOH 0.1 N for 4 h at 37 °C, in order to remove the cyclodextrin polymers via hydrolysis, thus releasing the remaining antibiotics that resisted to the methanol desorption step. The amount of antibiotic was measured by HPLC analysis in the two solutions (methanol and aqueous NaOH).

2.2.3. In vitro drug release measurements

2.2.3.1. Drug release in agitated vials. Drug loaded implants were placed into 30 mL vials filled with 5 mL phosphate buffered saline pH 7.4 (0.01 M phosphate buffer containing 0.137 M sodium chloride and 0.0027 M potassium chloride, Sigma–Aldrich, Saint Quentin Fallavier, France). The latter were shaken by an orbital shaker plate system (Gerhardt Thermoshake, Königswinter, Germany) at 100 rpm and 37 °C. At pre-determined sampling time points, the release medium was completely replaced with fresh phosphate buffer. The amount of drug released was determined by UV–vis spectrophotometry at λ = 267 nm and 238 nm for ciprofloxacin hydrochloride and vancomycin hydrochloride, respectively.

2.2.3.2. Drug release into agarose gels. Agarose powder was dissolved in boiling water to prepare 180 mL of a 0.6% (w/w) agarose solution, which was sterilized by tyndallisation at 100 °C during 30 min on 3 consecutive days to prevent accidental microbial

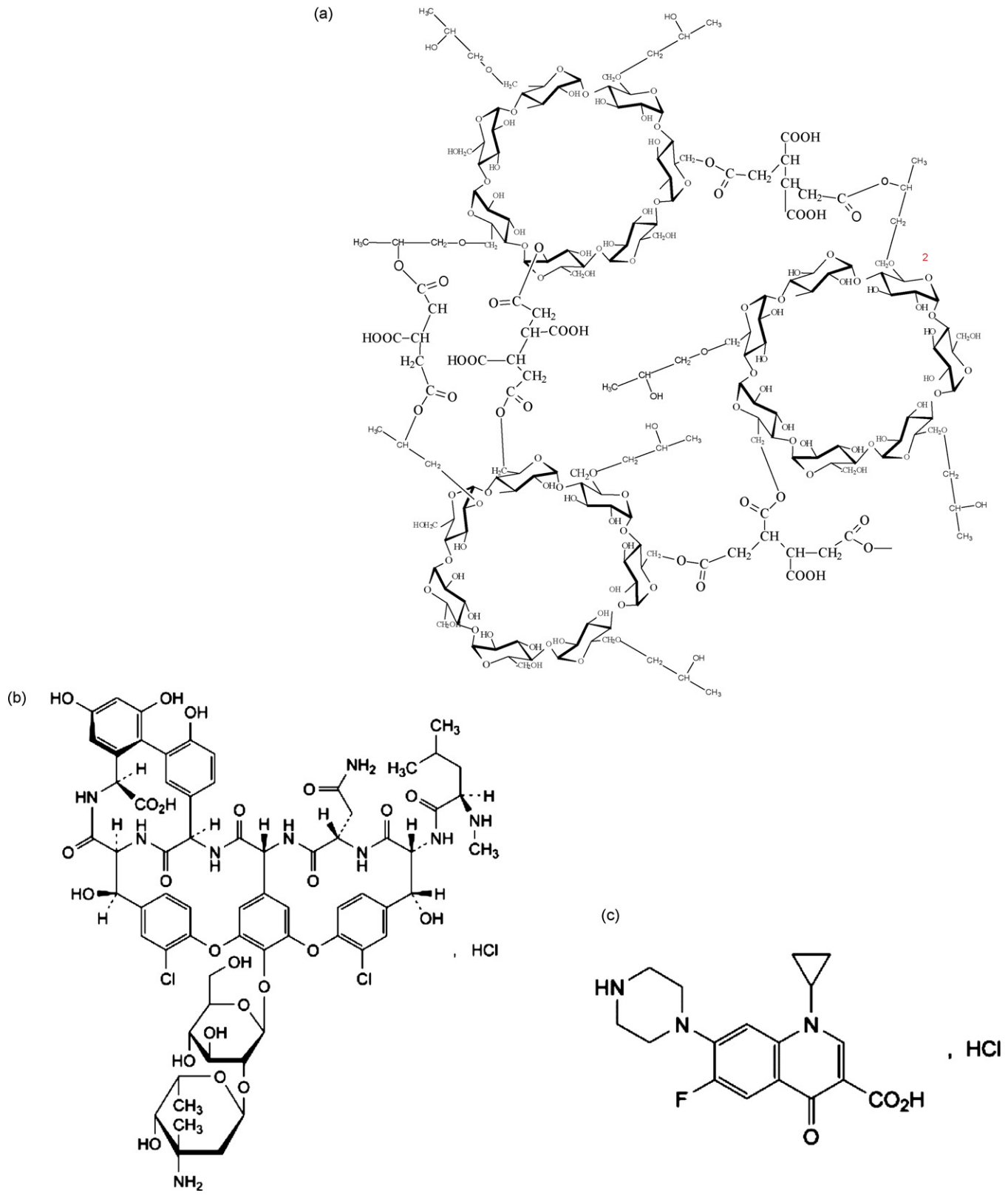

Fig. 1. Chemical structures of: polyBTCA–HPβCD (a); vancomycin hydrochloride (b) and ciprofloxacin hydrochloride (c).

growth during the observation period. The pH of the agarose solution was 7.0 ± 0.5 at 37 °C. The implant was placed into the agarose gel as follows: one third of the required agarose solution was cast into a Petri dish (19 cm in diameter) and allowed to solidify. Then, the implant was placed at the center of the gel and the remaining solution (40–45 °C) was carefully cast onto the first layer and allowed to solidify (Fig. 2). The Petri dish was then placed into a water-filled desiccator in an incubator (non-agitated) at 37 °C to prevent water evaporation during the experiments.

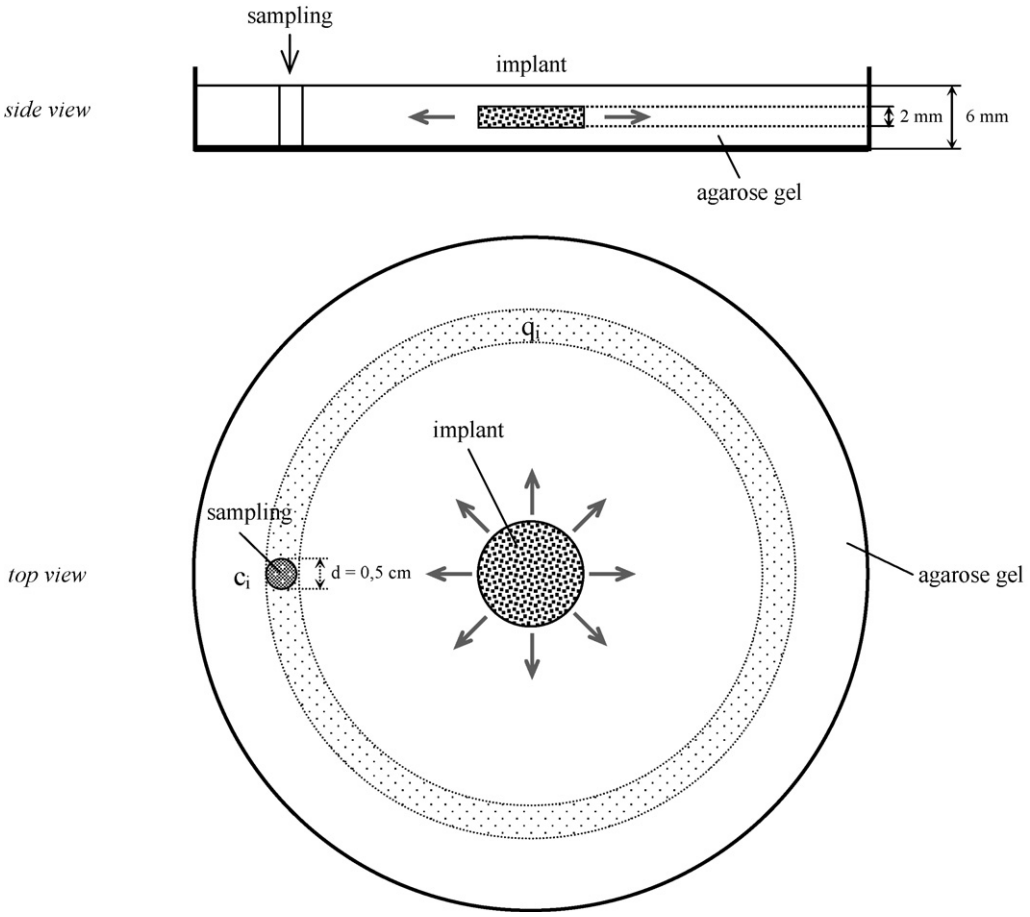

Fig. 2. Schematic illustration of the experimental setup used for drug release measurements into agarose gels (c_i = drug concentration in the withdrawn gel sample; q_i = drug amount in the corresponding concentric ring).

At pre-determined time points, cylindrical gel samples were removed at various distances from the implant using a glass tube of 5 mm in diameter. The samples were weighed and analyzed for their drug content by HPLC as follows: the gel was dissolved in the mobile phase at 100 °C (in the case of ciprofloxacin hydrochloride); or the gel was dispersed into the mobile phase using ultrasound during 5 min (in the case of vancomycin hydrochloride being thermolabile). The dispersions were filtered (0.45 μm) prior injection into the HPLC system. A Varian HPLC system (Les Ulis, France) was used consisting of a ProStar 230 pump, a ProStar 410 autosampler, a Prostar 325 UV–Vis detector, and the Galaxie software. The separation was performed on a Synergi Hydro-RP column (4 μm, 250 mm × 4.6 mm i.d.) at 30 °C. In the case of ciprofloxacin hydrochloride, the mobile phase was a 87:13 (v/v) mixture of an aqueous acetic acid solution (50 mL/L) and acetonitrile, and a flow rate of 1.5 mL/min was used. In the case of vancomycin hydrochloride, a gradient method was applied, the composition of the mobile phase is given in Table 1, the flow rate was 1 mL/min. The effluent peaks were monitored at λ = 278 nm and 230 nm for ciprofloxacin hydrochloride and vancomycin hydrochloride, respectively.

Table 1
Composition of the mobile phase used for vancomycin hydrochloride analysis by HPLC.

Time (min)	Ammonium formate (%, v/v)	Methanol (%, v/v)
0–12	90–40	10–60
12–12.5	40–0	60–100
12.5–13.5	0	100
13.5–14	0–90	100–10
14–15	90	10

Due to the symmetry of the agarose gels (Fig. 2), it is assumed that the drug concentration in the withdrawn gel sample (c_i) is constant within the zone with the same distance from the implant. This allows calculating the amount of drug present in the corresponding concentric ring (q_i), which is described by rotation of the gel sample around the drug source (Fig. 2). Summing up the drug amounts in all concentric rings allowed for the calculation of the cumulative amounts of drug released at a given time point.

2.2.4. Characterisation of the drug:HPβCD complexes

The interactions between the antibiotic drugs and the HPβCD were studied by DSC and NMR analysis. In the first case, samples were placed in non-hermetic aluminum pans and heated from −10 °C to 380 °C at 10 °C/min under nitrogen atmosphere in a DSC 1 STAR^e (Mettler Toledo, Greifensee, Switzerland). The reference was an empty aluminum pan. Temperature and enthalpy readings were calibrated using pure indium and zinc. Drug (10^{-2} mol/L) and HPβCD solutions (10^{-2} mol/L) were prepared in water and combined in a 1:1 molar ratio mixture for complex formation. The water was evaporated under vacuum at 37 °C. Drug:HPβCD physical mixtures (1:1 molar ratio) were prepared by grinding with a mortar and pestle for reasons of comparison. For NMR measurements, an Ultrashied 400 MHz apparatus (BRUKER, Wissembourg, France) was used (5 mm tubes). Drug (10^{-2} mol/L) and HPβCD solutions (10^{-2} mol/L) were prepared in D_2O. Six hundred microliters of equimolar drug:HPβCD blends were sonicated at 20 °C for 20 min and agitated for 1 h prior to analysis. The ^{1}H NMR experiment was

performed to determine the chemical shift of internal proton of cyclodextrin and proton of drug molecules. The 2D ROESY NMR experiment was also carried out with the drug:HPβCD complex to determine the geometry of the complex.

2.2.5. Statistical analysis

The drug release profiles observed under the various test conditions were compared using the analysis of variance (ANOVA). The data was processed using MS Excel.

3. Results and discussion

3.1. Characterisation of the inclusion complex

The cyclodextrin contents in the implants determined by thermogravimetric analysis were found to be around 1.7% and 1.5% (w/w) after the functionalizing process for CD-HA-M (monomer pathway) and CD-HA-P (pre-polymerized pathway) implants, respectively. Fig. 3 shows the DSC thermograms of HPβCD,

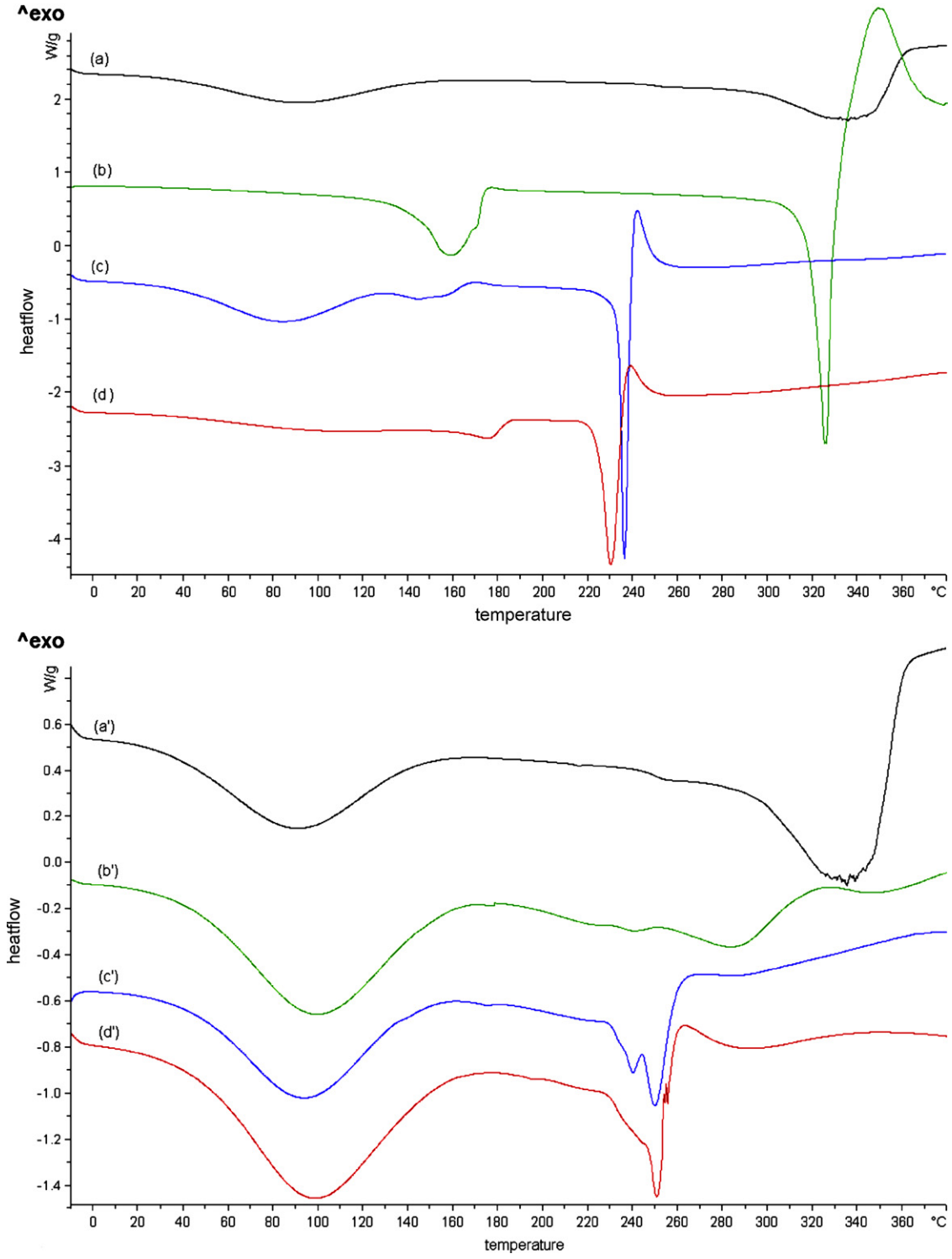

Fig. 3. DSC thermograms of pure HPβCD (a, a′); ciprofloxacin (b); vancomycin (b′); ciprofloxacin:HPβCD physical mixture (c); vancomycin:HPβCD physical mixture (c′); evaporated solution of ciprofloxacin:HPβCD (d); and evaporated solution of vancomycin:HPβCD (d′); molar ratios 1:1.

the pure drugs, drug:HPβCD (1:1 molar) physical mixtures and dried samples from drug:HPβCD (1:1 molar) solutions. HPβCD (a and a′) was characterized by a broad endothermic peak around 100 °C associated with water loss, and a second endothermic peak at 340 °C, due to thermal decomposition as observed in parallel by TGA (not shown). The thermogram of ciprofloxacin hydrochloride (b) showed a first endothermic peak in the range of 130–170 °C due to loss of moisture/sample dehydration, and a sharp endothermic peak at 329 °C corresponding to drug melting, followed by thermal decomposition at 350 °C. The physical hydrochloride:HPβCD mixture (c) and the dissolved and dried ciprofloxacin hydrochloride:HPβCD mixture (d) presented different thermograms, especially below 200 °C. Though, in both cases, the drug melting point was shifted to lower values pointing out that new compounds were formed through interactions between ciprofloxacin hydrochloride and the cyclodextrin. Considering the differences between both thermograms, it can be deduced that physical mixture between the drug and HPβCD resulted in a non-specific association between these components whilst in the case of the dissolved and dried system, inclusion complexation occurred. The melting point of the drug did not completely disappear probably because the drug was only partially combined with HPβCD.

Fig. 4a–d displays the compared DSC curves of HPβCD and polyBTCA–HPβCD combined with ciprofloxacin hydrochloride by physical mixture and by the evaporation method. If one focuses on the signal relative to the melt of ciprofloxacin, it clearly appears that the sharp signal observed at 230–235 °C in the presence of HPβCD (Fig. 4b and d) almost completely disappeared when polyBTCA–HPβCD was used (Fig. 4a and c). This means that the crystalline form of the drug was destructed in the presence of the cyclodextrin polymer and that a good dispersion of the drug is obtained in the polymer matrix.

Vancomycin hydrochloride is a glycopeptide antibiotic that presents a broad endothermic event at 105 °C (Fig. 3b′) due to its thermal decomposition. Upon mixing with HPβCD, the DSC profile pointed out new endothermic sharp peaks in the range of 230–265 °C (Fig. 3c′ and d′) indicating interactions between the drug and the cyclodextrin. As observed hereunder in the NMR study, no inclusion was observed between the antibiotic and HPβCD, due to the bulky structure of vancomycin. Therefore, the peaks that appear at 250 °C in the physical mixture and in the dissolved and dried mixture are not related to inclusion complexation, but only to an association of vancomycine with HPβCD, probably through hydrogen bonding with hydroxyls present on the outer rim of the macrocycle.

Fig. 4a′–d′ displays the DSC curves of vancomycin combined with HPβCD under its raw and polymerized forms, by the physical mixture and evaporation methods. One can observe that the sharp peaks relative to the melt of vancomycin (at 250 °C) physically mixed with HPβCD, or obtained by the evaporation method (Fig. 4b′ and d′ respectively) disappeared when the raw CD was replaced by its polymer (Fig. 4a′ and c′). This indicated that the CD polymer, despite its inability to exert inclusion complexation with vancomycin, is notwithstanding capable of interacting with this drug that results to its efficient dispersion in its matrix.

In conclusion, the thermal analysis showed the possible inclusion of ciprofloxacin into the HPβCD cavity, on the contrary of vancomycin. Though, it appeared that both antibiotics presented strong interactions with the polyBTCA–HPβCD. These interactions are probably due to hydrogen bonding involving the outer rim of HPβCD, and to acid–base interactions that may occur between the carboxylic acid groups carried by the crosslinks of polyBTCA–HPβCD (see Fig. 1a) and the amino groups present on both antibiotics (see Fig. 1b and c). These results are in good correlation with our previous study (Hornez et al., 2007) that reported that the HA–CD systems were capable of adsorbing twice and three times more vancomycin and ciprofloxacin respectively than raw microporous HA.

The ^{1}H and 2D ROESY NMR experiments were performed to obtain further supporting evidence of complex formation, as well as to characterize the potential binding mode. Fig. 5 shows the ^{1}H NMR spectrum of HPβCD that is to say variations of chemical shifts of the H_3 proton located inside the cavity of HPβCD alone or in presence of the antibiotic drugs, under the same experimental conditions. In the presence of vancomycin hydrochloride, the signals did not show significant chemical shift changes in the ^{1}H NMR spectra (data not shown). This could be explained by the bulky molecular structure of this drug, hindering the encapsulation in the CD cavity. In contrast, the addition of ciprofloxacin hydrochloride led to a shielding of the observed proton (H_3 proton triplets at 3.933 ppm, 3.909 ppm, 3.866 ppm and 3.926 ppm, 3.896 ppm, 3.873 ppm for HPβCD alone and in the presence of ciprofloxacin hydrochloride, respectively). This evidenced the existence of an interaction between the guest molecule and the interior of the host cavity via the formation of an inclusion complex. The 2D ROESY NMR experiment carried out with the equimolar ciprofloxacin hydrochloride:HPβCD solution and reported in Fig. 6 allowed establishing the geometric structure of the complex. Observed cross-peaks demonstrated the interaction between the protons inside the cavity of HPβCD and protons on the piperazine cycle of the ciprofloxacin hydrochloride. The H_3 proton located at the wider rim interacted with the H_2 and/or H_6 of ciprofloxacin and the H_5 proton at the narrow end interacted with H_3 and/or H_5 of ciprofloxacin hydrochloride. These results suggest that the complex was formed and involved the piperazine cycle of ciprofloxacin hydrochloride molecule which entered the HPβCD cavity. The proposed structure of ciprofloxacin hydrochloride:HPβCD inclusion complex is displayed in Fig. 7. Because of the lower resolution of the ^{1}H NMR spectrum of polyBTCA–HPβCD, in addition with an overlapping of the signals of ciprofloxacin hydrochloride, it was not possible to evidence by this way any possibility of inclusion complex formation between the polymerized cyclodextrin and the antibiotics.

3.2. In vitro drug release measurements

As the drug release measurements were partially performed during extended time periods, the stability of the drugs under the various conditions was studied. Only in one case, drug degradation was detected: when vancomycin hydrochloride release was measured into agarose gels. In this case, the crystalline degradation products (CDP-1) were detected, indicated by 2 novel peaks observed in the HPLC chromatogram (Fig. 8) (Marshall, 1965; White et al., 1988). Thus, the total amount of drug released was calculated as the sum of the detected vancomycin and its degradation products. As the release medium was completely exchanged at every sampling time point when using the "agitated vial method", no drug degradation products were observed. This also indicates that vancomycin hydrochloride degradation is not likely to occur *within* the implants during drug release, but only *outside*: once released into the agarose gel. *In vivo*, released drug is likely to be taken up by surrounding cells or to be transported away from the implant. Thus, the observed vancomycin hydrochloride degradation within the agarose gel does not necessarily occur in living tissue.

In addition to the knowledge of the cumulative amount of drug released as a function of time, the gel method also allowed to get insight into the distribution of the drug within the surrounding implant environment. For instance, Fig. 9 shows the concentration–distance profiles of ciprofloxacin hydrochloride upon exposure of: (a) CD-HA-M implants, (b) CD-HA-P implants, (c) non-functionalized implants, to agarose gels after 3 h, 48 h, 96 h, 216 h, 336 h. Drug concentration in microgram per gram

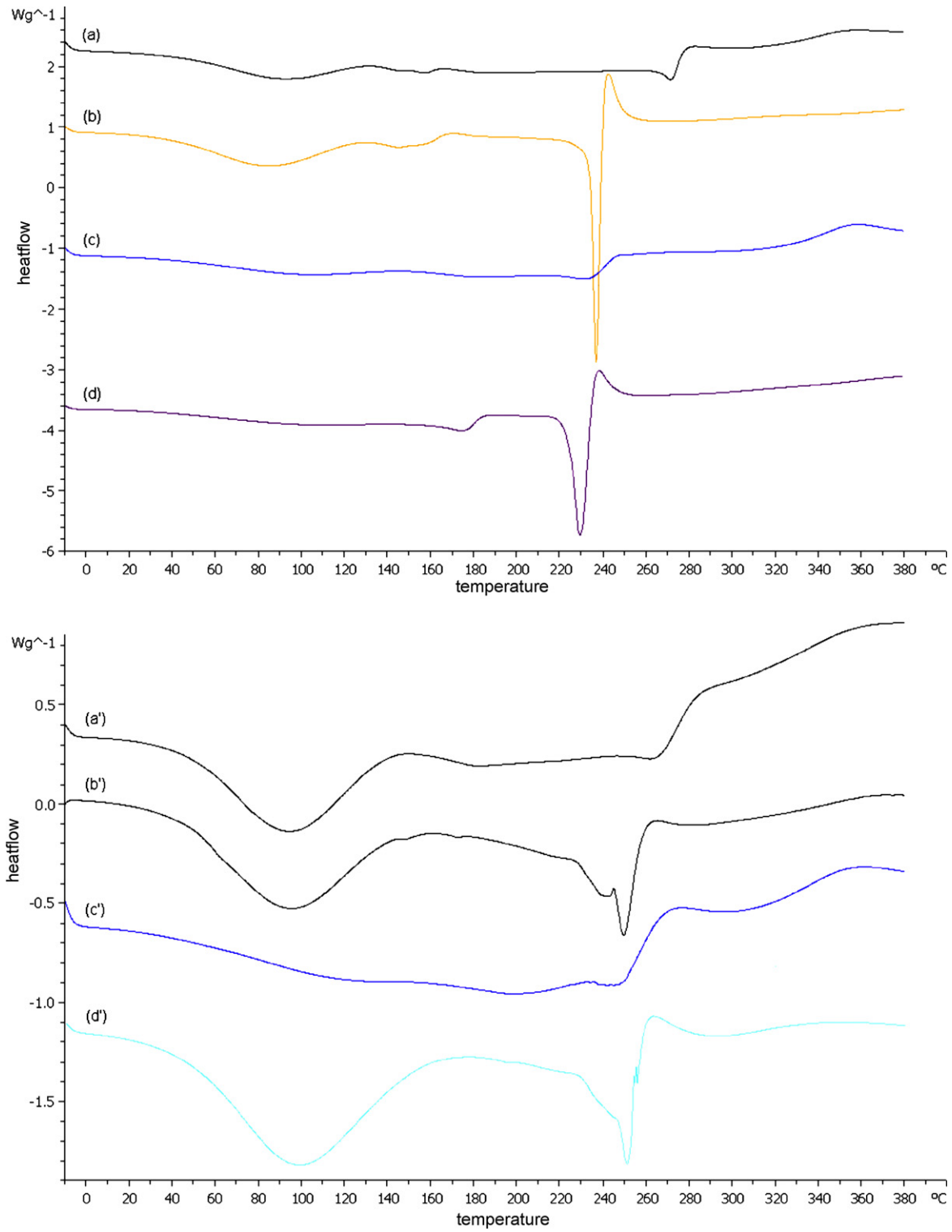

Fig. 4. DSC thermograms of physical mixtures of polyBTCA–HPβCD and ciprofloxacin (a), vancomycin (a′); physical mixtures of HPβCD and ciprofloxacin (b), vancomycin (b′); evaporated solutions of polyBTCA–HPβCD and ciprofloxacin (c), vancomycin (c′); evaporated solutions of HPβCD and ciprofloxacin (d), vancomycin (d′); molar ratios 1:1.

of agarose gel (*y*-axis) was plotted against distance from the drug source in cm (*x*-axis). The equilibrium concentrations were 4.8 μg/g, 1.3 μg/g and 5.9 μg/g for CD-HA-M implants, CD-HA-P implants and non-functionalized implants respectively. Clearly, when time progressed, the amount of drug released from the CD-HA-M implants increased, leading to further diffusion and an increase in drug concentration in the various zones (Fig. 9a). When the release rate decreased with time, e.g. at later time points in the case of CD-HA-P implants, the drug concentration in close vicinity of the implant decreased again (due to drug diffusion into the agarose gel further away from the implant) (Fig. 9b). These results indicate that drug was continuously released from the device and transported within the agarose gel by diffusion due to concentration gradients. Sink conditions were assured throughout the observation period (no saturation effects in the entire gel mass) because drug concentrations in the agarose gel were much lower

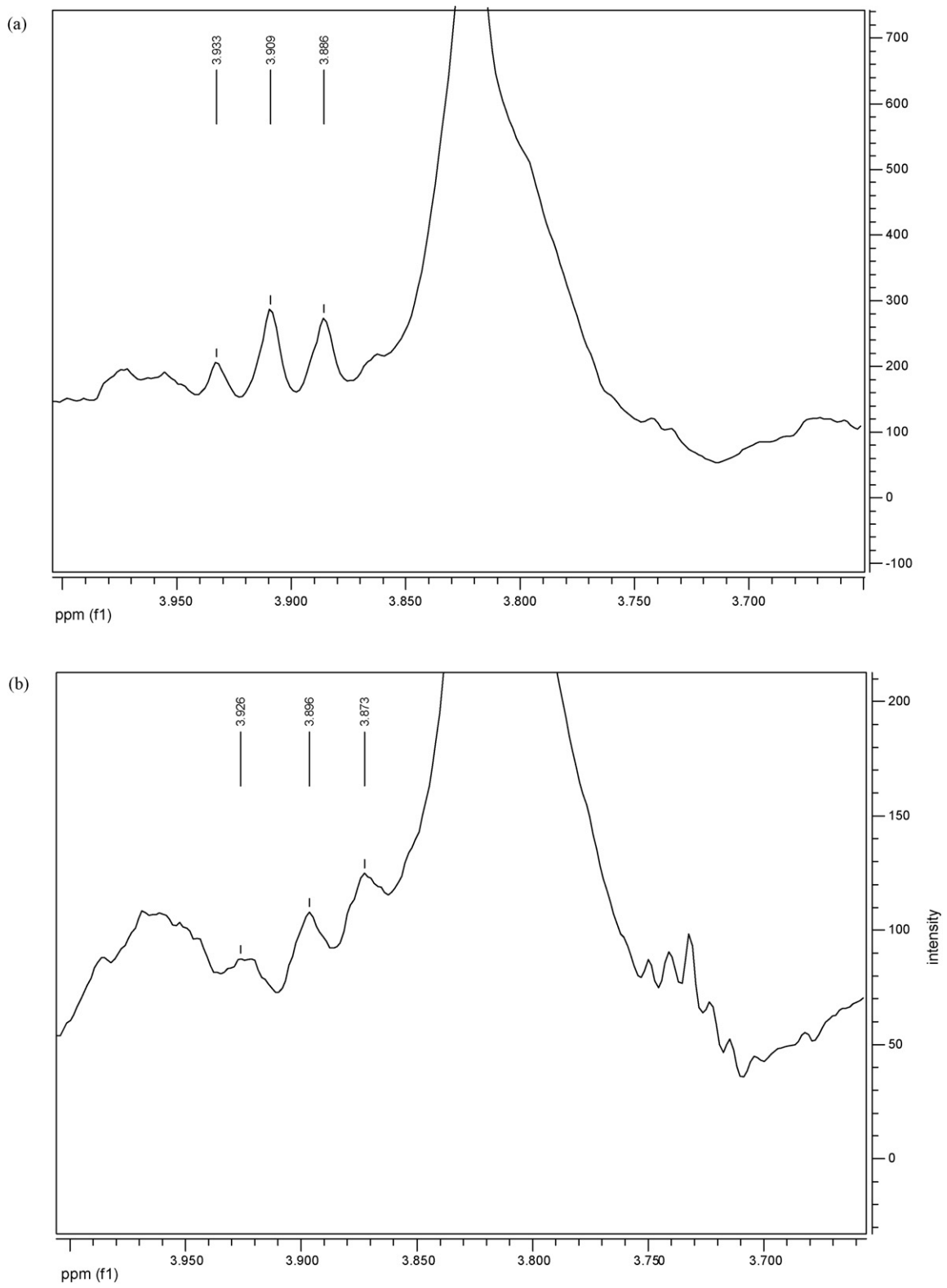

Fig. 5. ^{1}H NMR spectra of a solution of HPβCD in D_2O (a); a solution of HPβCD and ciprofloxacin hydrochloride at 1:1 molar ratio in D_2O (b).

than drug solubilities (60 mg/mL for ciprofloxacine hydrochloride and about 100 mg/mL for vancomycine hydrochloride). The presence of cyclodextrin polymers has proven to sustain the release of drug in comparison with the non-functionalized implants, with which very high concentrations of ciprofloxacin hydrochloride were observed in the zones close to the drug source at early time of points (after 24 h and 72 h) (Fig. 9c). Drug release from the non-functionalized implants is likely to be controlled by the penetration of the release medium into the system, dissolution of the drug and diffusion of dissolved drug through the water-filled "pores" of the matrix. In the functionalized implants, drug release is additionally controlled by the dissociation of the drug from the cyclodextrin

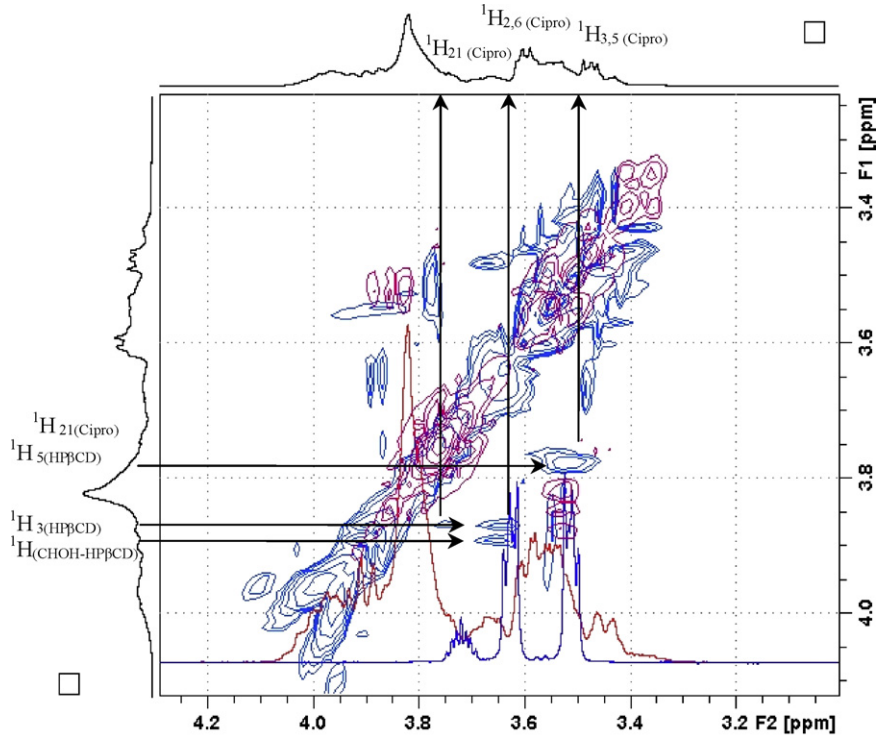

Fig. 6. 2D ROESY NMR spectra of the ciprofloxacin:HPβCD complex.

complex into the release medium, described by the equilibrium which is determined by the complexation strength:

drug : CD complex ⇄ drug + CD

Furthermore, the presence of the HPβCD polymer might hinder drug release. Interestingly, in the case of vancomycin hydrochloride, there was no major difference between the three types of implants with respect to the drug concentration–distance profiles as a function of time (Fig. 10). Thus, the absence of complex formation in the case of vancomycin hydrochloride (as discussed above) leads to similar drug release kinetics for all types of implants. This points out the importance of drug:HPβCD complex formation for the control of drug release in the case of ciprofloxacin hydrochloride. The equilibrium concentrations were 8.3 μg/g, 6.5 μg/g and 7.3 μg/g for CD-HA-M

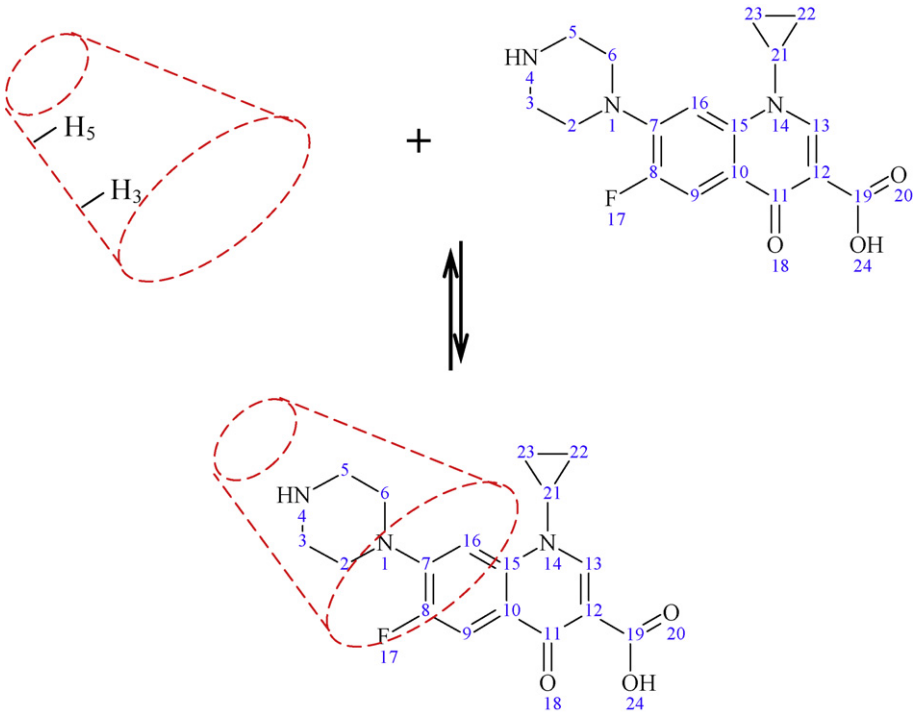

Fig. 7. Proposed structure of the ciprofloxacin:HPβCD complex.

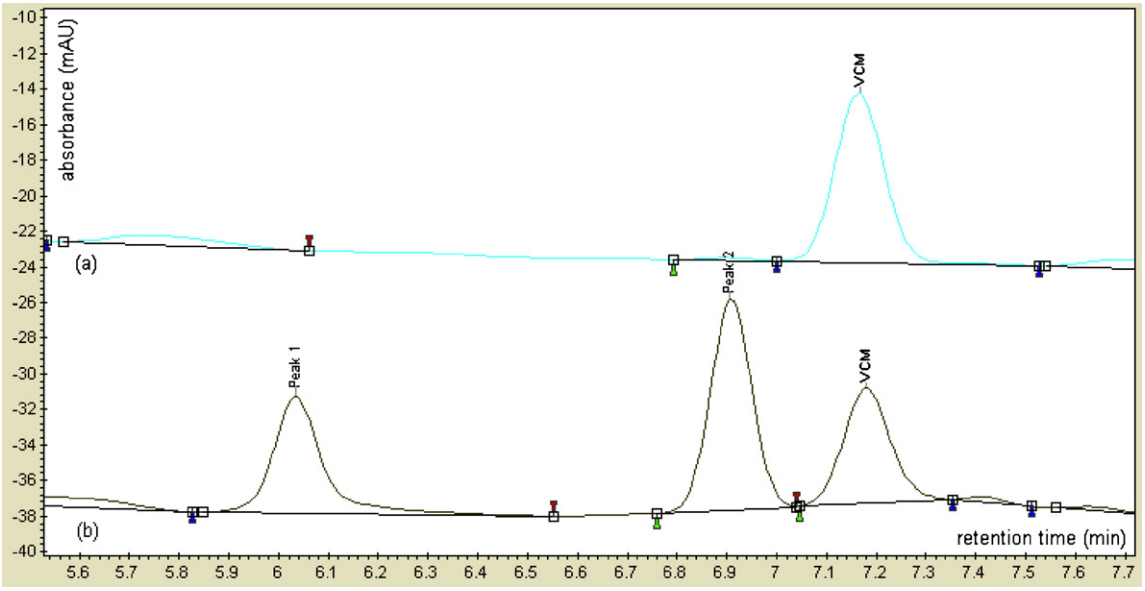

Fig. 8. HPLC chromatogram of a vancomycin hydrochloride reference solution (a); and vancomycin hydrochloride extracted from an agarose gel sample (after 96 h at 37 °C) (b). Peaks 1 and 2 correspond to the crystalline degradation products CDP-1 (VCM = vancomycin).

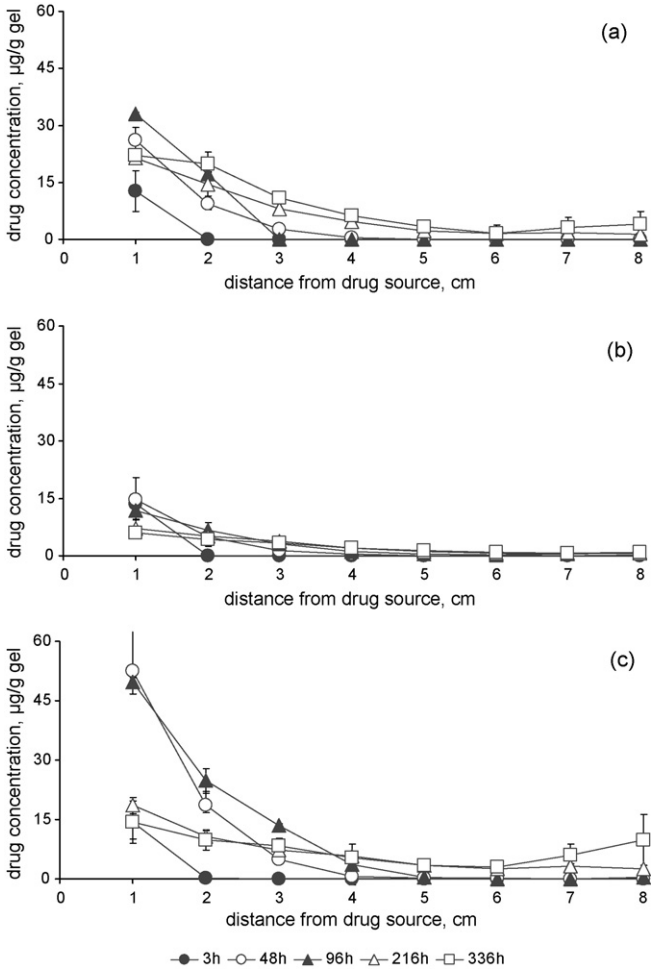

Fig. 9. Ciprofloxacin hydrochloride concentration-distance profiles within the agarose gels CD-HA-M implants (a); CD-HA-P implants (b) and non-functionalized implants (c), after different times of exposure (indicated in the diagrams).

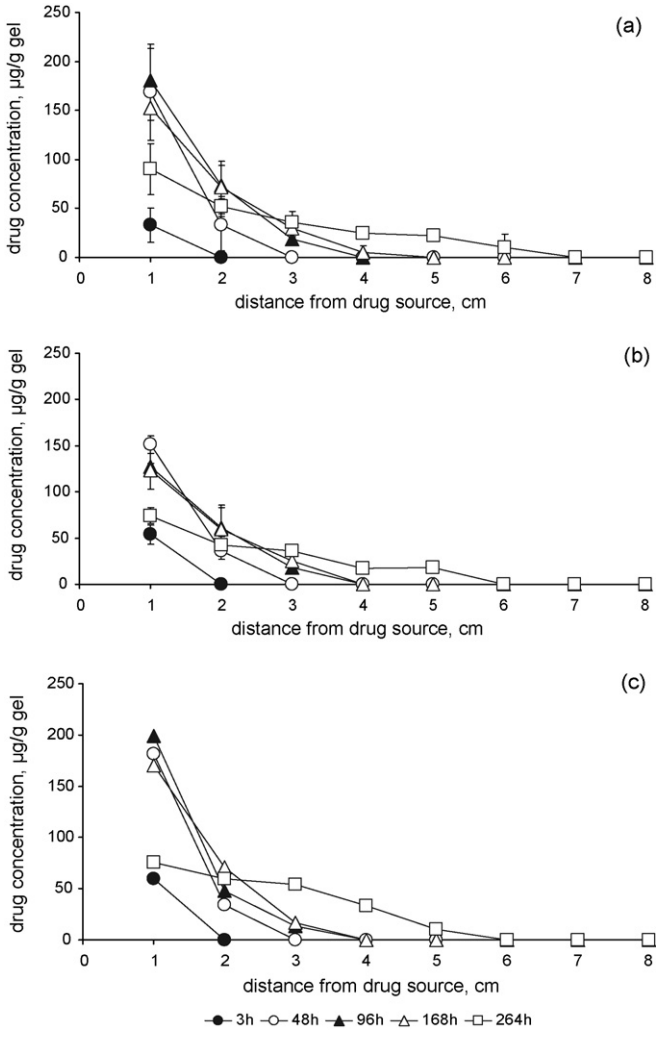

Fig. 10. Vancomycin hydrochloride concentration-distance profiles within the agarose gels from CD-HA-M implants (a); CD-HA-P implants (b) and non-functionalized implants (c) after different times of exposure (indicated in the diagrams).

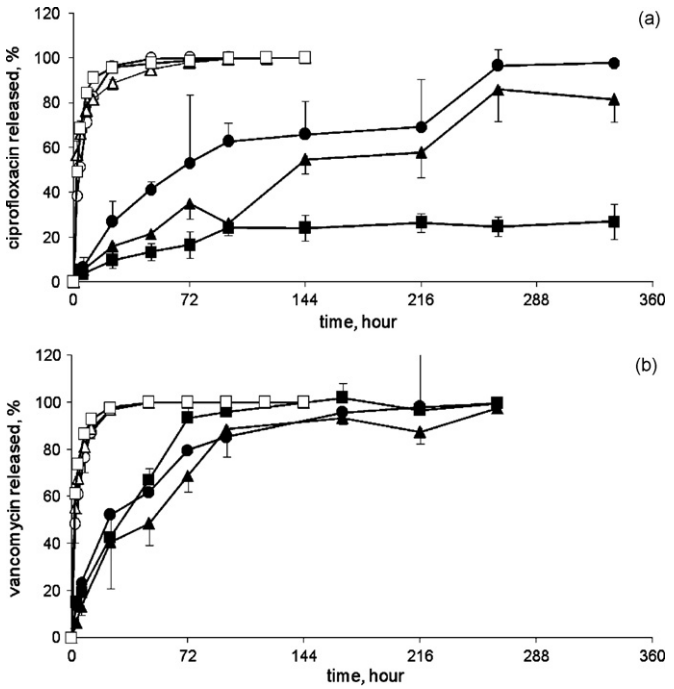

Fig. 11. Effects of type of experimental setup (●, ▲, ■ agarose gels; ○, △, □ agitated vials) and of the type of implants (●, ○ non-functionalized implants; ▲, △ CD-HA-M implants; ■, □ CD-HA-P implants) on the observed ciprofloxacin hydrochloride release kinetics (a) and vancomycin hydrochloride release kinetics (b).

implants, CD-HA-P implants and non-functionalized implants respectively.

Fig. 11 shows the release profiles of ciprofloxacin hydrochloride and vancomycin hydrochloride under the two test conditions: in agitated vials and agarose gels. Irrespective of the type of drug, the release kinetics determined with the vial method was much faster and did not show any significant difference between the three types of implants (ANOVA, $p > 0.05$). But, there was a difference between the two drugs: almost 100% of the vancomycin hydrochloride was released within 48 h, whereas it took about 96 h in the case of ciprofloxacin hydrochloride. The duration of ciprofloxacin hydrochloride release was much longer, probably because of its complexation with the cyclodextrin polymer. In agitated vials, convection rapidly homogenises the drug concentration within the release medium and, thus, maintains high drug concentration gradients at the interface "implant-bulk fluid". Agitation also promotes erosion of the matrix into smaller fragments (visual observation), resulting in an increased surface area exposed to the release medium. However, living tissues exhibit very different mass transport phenomena compared to those encountered in agitated bulk fluid conditions. Connective tissues like bone are fibrous tissues made of cells separated by an extracellular matrix. The gel used for *in vitro* release measurements consists of agarose chains linked via hydrogen bonds. The high water content and the absence of significant convective mass flow render the gel likely to better mimic the tissue at the implantation site. Under such conditions, the tested drugs were observed to be progressively released from the devices during extended periods of time. This prolonged release is fundamental for an effective antibiotic prophylaxis of osteomyelitis *in vivo* (Fig. 11). As it can be seen, the release of vancomycin hydrochloride (which is not able to form a complex with HPβCD) was rather similar for all types of implants (non-functionalized, CD-HA-M and CD-HA-P implants) (ANOVA, $p > 0.05$) (Fig. 10b). This is consistent with the above discussed drug concentration–distance profiles. In contrast, the release of ciprofloxacin hydrochloride (which is able to form a complex with HPβCD) was strongly affected by the type of implant when using the agarose gel method (ANOVA, $p < 0.05$) (Fig. 11a). Thus, *in vivo* the different compositions and structures of the implants are likely to play a major role. This does not become obvious with the agitated vial method. Ciprofloxacin release was most rapid into agarose from non-functionalized implants, followed by CD-HA-M and CD-HA-P implants. In the first case, no cyclodextrins are available for complex formation, in the second case the spatial arrangement of the cyclodextrin polymer inside the pores of the system seems to offer less hindrance for drug release in the case of CD-HA-M compared to CD-HA-P implants.

4. Conclusion

This paper displayed through the DSC study that the raw and polymerized HPβCD systems did not interfere in the same manner with a drug. It was shown that if a drug was not included inside the CD cavity, complementary interactions occurred especially with the carboxylic acid functions carried by the crosslinks of the polymer. Therefore, even vancomycin that presents a bulky structure that prevents its inclusion inside the CD cavity could exert interactions with the polymer. This confirms the interest of functionalizing medical implants (Tabary et al., 2007; Blanchemain et al., 2008) and HA (Lepretre et al., 2009) with cyclodextrin crosslinked with a polycarboxylic acid for prolonged drug delivery purposes.

Concerning the kinetic study, the type of experimental setup can significantly affect the observed drug release kinetics from bone implants. Thus, great care must be taken when defining the conditions for such *in vitro* measurement. While the vial method is simple to use, its predictive value for *in vivo* release may be limited, because the mass transport processes in the surrounding tissue are very different. The use of the agarose gel method is rather cumbersome, but offers more biorelevant conditions and a higher discriminatory power.

The functionalized HA implants allowed sustaining antibiotic release during about 300 h and 150 h for ciprofloxacin hydrochloride and vancomycin hydrochloride, respectively. The method of functionalization (monomer or pre-polymer pathways) is of great importance with regard to the accessibility of the drugs to the CD polymer complexing sites. Consequently, this also influences the rates of release of the drugs from these systems.

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