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Development of bisphosphonates controlled delivery systems for bone implantation: influence of the formulation and process used on in vitro release

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Abstract The present study investigates the development of controlled drug delivery devices by association of bisphosphonates (BPs) with calcium-deficient apatite (CDA) to obtain a prolonged drug delivery. In a first part, we studied the microencapsulation of methylene bisphosphonic acid, our model of BPs, in biodegradable PLGA by the double emulsion (w/o/w) solvent evaporation/extraction process. Secondly, we associated BPs, either in a free form or microencapsulated, with calcium phosphate biomaterials. The association of free BPs with CDA was performed by isostatic compression at 80 MPa and we tested the interest of adding a binder, HPMC, in the formulation to reinforce the association. In parallel, microparticles were associated with calcium-deficient apatite, either by simple mixture or by isostatic compression. To compare the different formulations, in vitro dissolution studies were performed. All the formulations tested appear to be efficient to produce BPs loaded biomaterials able to deliver the drug slowly and at a constant rate. The slowest release rate (2.7% in 14 days) was obtained with the blend of microencapsulated BPs with CDA.

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1 Introduction

Bisphosphonates are one of the most potent inhibitors of bone resorption by preventing osteoclast activity. Thus, they constitute a class of drug that are increasingly used to treat bone diseases characterized by enhanced osteoclastic bone resorption [1, 2] and are prescribed for the treatment of patients suffering from osteoporosis, Paget's disease, or bone cancers [3].

For these treatments, bisphosphonates are administrated either by intravenous route every 3–4 weeks, or once daily by oral route. These two modes of administration are not convenient for the patients. Indeed, parenteral administration requires a patient monitoring for several hours [4] and oral route often suffers from low bioavailability due to poor gastrointestinal absorption, and may even induce side effects such as osteonecrosis of the jaws [5].

An interesting alternative to this kinds of treatments would be the local administration by mean of a drug delivery system (DDS) able to release the active compound at a constant rate during several weeks. Some recent works have already shown the great interest of the local delivery of bisphosphonate from implants. Some recent works have already shown the great interest of the local delivery of bisphosphonates from implants. Tengvall et al. [6] proved the efficiency of immobilized bisphosphonates to improve early fixation of screws used in fracture surgery, thanks to an inhibition of bone resorption; Stadelmann demonstrated that local delivery of zoledronate increased periprosthetic bone density in sheeps [7].

Concerning the treatment of osteolytic bone diseases, calcium-phosphate biomaterials, which are spontaneously resorbed and replaced by normal bone architecture in a few weeks are of particular interest for the development of bisphosphonate controlled local delivery devices. Thanks

to their biocompatibility and bioresorbability, these materials are already commonly used as bone substitutes [8]. Furthermore, bisphosphonates are stable pyrophosphate analogs with a "P–C–P" structure that have a high affinity for hydroxyapatite (calcium ions) [9] and a recent study showed that bisphosphonates could be chemisorbed on their calcium-phosphate biomaterials surface [10]. Faucheux et al. also proved the efficiency of zoledronate coated calcium deficient apatite to inhibit osteoclastic resorption in vitro [11].

The aim of this study was to develop a BPs controlled release device able to deliver the drug in situ at a constant rate over a long period.

This work was divided in two parts. In a first step, we studied the feasibility of the microencapsulation of methylene bisphosphonic acid, a working model of BPs, into Polylactic-co-glycolic acid 50/50 (PLGA) microspheres. This polymer, widely used as microencapsulation agent, is biocompatible and biodegradable. The process of double emulsion (w/o/w) solvent evaporation/extraction, optimised in a previous study, was performed [12].

In a second step, we developed implants associating BPs with calcium-deficient apatite (CDA) to produce a drug loaded biomaterial able to deliver the drug in situ. Blocks were produced by isostatic compression, a process already studied in our laboratory [13]. The effect of the addition of a binder, hydroxypropylmethycellulose, in the formulation was also evaluated.

Finally, to combine the release properties of microparticles with the interests of CDA, we associated microencapsulated methylene bisphosphonic acid and calcium-deficient apatite either by simple mixing or isostatic compression.

The different samples obtained were then compared through the evaluation of drug release by an in vitro dissolution study.

2 Materials and methods

2.1 Materials

Methylene bisphosphonic acid was kindly given by the laboratory of organic synthesis (Nantes). Poly (lactide-co-glycolide) (Medisorb® 5050 DL high I.V., $M_w = 114{,}000$) was purchased from Alkermes (Cincinnati, USA). Methylene chloride with a rectapur grade was obtained from Prolabo (Paris, France); Polyvinylalcohol (PVA, $M_w =$ 30,000–70,000) from Sigma (Saint Quentin Fallavier, France); Span®80 and Sodium chloride from Cooper (Melun, France); CDA [Ca/P = 1.53] was obtained by alkaline hydrolysis of 400 g of dicalcium phosphate dihydrate (DCPD), using 100 ml of a aqueous solution of aqueous ammonia (28%) and 4.9 l of deionized water at 100°C for 5 h. Vanadate–Molybdate reagent was obtained from VWR (Fontenay sous bois, France).

HydroxyPropylMethylCellulose (HPMC) was graciously sent by Colorcon (Bougival, France).

2.2 Methods

2.2.1 Bisphosphonates microencapsulation

A double emulsion (w/o/w)/solvent evaporation–extraction process was used. The drug dissolved in the internal aqueous phase was emulsified in the polymer organic phase, the external phase being saturated by sodium chloride salt. Three batches of microparticles were produced for each experiment.

0.1 g of methylene bisphosphonic acid was dissolved in 1.5 ml of cold (4°C) distilled water and emulsified in 30 ml of a cold solution of poly (lactide-co-glycolide) (2.25 g) in methylene chloride containing Span 80® (0.5% w/w). This first inner emulsion (w_1/o) was formed by mechanical stirring using an Ultra-Turrax® (T25, Ika, Staufen, Germany) during 1 min at 20,000 rpm. This emulsion was then poured into 1,200 ml of a cold aqueous solution of PVA (0.1% w/w) and sodium chloride (2.5% w/w) and submitted to mechanical stirring at 500 rpm (Heidolph® RZR2101, Germany) to set a double emulsion ($w_1/o/w_2$). Stirring was continued for 4 h at room temperature under air flow. This first evaporation step was followed by a solvent extraction step by rapidly transferring the w/o/w emulsion into a large volume of distilled water (1 l). The complete extraction of the solvent was obtained by diffusion through the external aqueous phase, which led to the formation of solid microparticles. They were finally collected by filtration (Büchner, Millipore, France), washed with distilled water (2 l) and dried at room temperature in darkness for 24 h.

2.2.2 Microparticle characterisation

2.2.2.1 Microparticle drug loading Methylene bisphosphonic acid chlorhydrate was detected indirectly in the external aqueous phase by UV spectrophotometry (Shimadzu UV-160 spectrophotometer, Roucaire, France) at a wavelength of 405 nm. Methylene bisphosphonic acid encapsulation efficiency, i.e. the ratio between actual and theoretical methylene bisphosphonic acid content is expressed as the mean percentage (w/w) ± SD of three formulations.

2.2.2.2 Microparticle size distribution One gram of dried microparticles was suspended in 50 ml of a 0.5% Methocel® aqueous solution (w/v) containing 1% of Tween® 80

(v/v), ultrasonicated for a few seconds and subjected to magnetic stirring for 1 h to prevent aggregation. The microparticles were then sized using a laser granulometer (Coulter LS 230, Coultronics, Villepinte, France). Three different analysis were made on each suspension, which corresponded to a single batch. Results are expressed as the mean particle diameter in volume ± SD (μm) of the three batches as a function of the occupied volume (%).

2.2.2.3 Microparticle morphology Microparticles morphology was investigated by scanning electron microscopy (SEM) (Leo-1450VP, LEO Electron Microscopy Ltd., Cambridge, Great Britain). Dried microparticles or blocks were deposited on a black adhesive tape area, vacuum coated with gold–palladium film (Emscope AEI230, Ashford, UK) for 15 min and analysed directly.

The same study was performed on samples of blocks associating CDA with microparticles.

2.2.3 Association of methylene bisphosphonic acid with CDA

Methylene bisphosphonic acid was associated with CDA either in a free form or after microencapsulation.

2.2.3.1. Association of free methylene bisphosphonic acid

a. Association A: Preparation of CDA-Methylene bisphosphonic acid blocks by isostatic compression.

Methylene bisphosphonic acid and CDA powder (7/100) were first mixed for 10 min. using a planetary mixer (Turbula T2C, WAB, Switzerland). The mixed powders were then agglomerated by wet granulation using distilled water in a mortar. The wet mass obtained was forced through the mesh of a 1.040 mm grid in an oscillated granulator (Erweka AR 400 Apparatebau GmbH, Germany). The granules obtained were then oven-dried at 40°C to a constant weight.

These granules (1 g per batch) were subjected to isostatic compression using hyperbar equipment (Alstom, Nantes, France). The granules were placed in an elastomer mold under vacuum and transferred into a high-pressure chamber containing water. They were compressed to 80 MPa for 5 min. The blocks obtained were then cut to form samples of about 100 mg.

b. Association B: Preparation of CDA-Methylene bisphosphonic acid blocks containing HPMC.

The samples were prepared as described for the process A. But, in this case HPMC was added to the formulation: 10% w/w of HPMC were first mixed with CDA before adding methylene bisphosphonic acid.

2.2.3.2. Association of microencapsulated methylene bisphosphonic acid

a. Association C: Preparation of CDA-Methylene bisphosphonic acid microencapsulated blend.

Microencapsulated methylene bisphosphonic acid (175 mg) and CDA powder (100 mg) were mixed for 10 min using a planetary mixer (Turbula T2C, WAB, Switzerland).

b. Association D: Preparation of CDA-Methylene bisphosphonic acid microencapsulated blocks.

Microencapsulated methylene bisphosphonic acid (175 mg) and CDA powder (100 mg) were first mixed for 10 min. using a planetary mixer (Turbula T2C, WAB, Switzerland) and submitted to isostatic compression (80 MPa, 5 min.).

2.2.4 Methylene bisphosphonic acid in vitro release study

In vitro methylene bisphosphonic acid release studies from functionalized materials were performed using a previously described dissolution test [14]. Samples of about 150 mg loaded with 7% w/w of methylene bisphosphonic acid were deposited in a Millicell® culture chamber (CM PICM, Millipore) equipped with a Biopore® membrane (Millipore). The chambers were immersed in six-well culture plates (AES, Combourg, France) containing 13 ml of distilled water that were subsequently placed in an oven at 37°C on a 3D rocking platform (Stuart Scientific, STR 9, UK) (5 rpm). Aqueous solutions were removed and replaced with fresh distilled water at 1, 2, 4, 8 h and 1, 2, 3, 4, 5, 6, 8, 10, 13 and 15 days. Each in vitro release study was repeated in triplicate. Results are expressed as the mean percentage of drug released as a function of time ± SD.

2.2.5 Methylene bisphosphonic acid UV spectrophotometric assay

The phosphorus content in each dissolution media sample was determined using the method described by Ames [15], using a vanadate–molybdate reagent to determine inorganic phosphates, the phosphorus concentration being obtained from the absorbance measured at 405 nm using a Hitachi U-2000 UV–visible spectrophotometer.

3 Results

3.1 Microencapsulation

As methylene bisphosphonic acid is a hydrosoluble molecule, the double emulsion/solvent evaporation–extraction

method was chosen to encapsulate methylene bisphosphonic acid. This technique induced the formation of microparticles with a high yield of 92.1% ± 0.14 and a drug encapsulation efficiency of 94.6% ± 0.18.

The mean diameter of the microparticles determined by laser granulometry was 75.8 ± 2.31 μm.

The observation of the microparticles by SEM (Fig. 1) showed round shaped particles with a smooth surface presenting no pores.

The microparticles were then associated with CDA. The morphology of the association samples obtained by isostatic compression was also studied by SEM (Fig. 2). On the photograph (a), we can observe the formation of blocks presenting a very rough surface. With a higher magnification (photograph b), we recognize round shaped forms, indicating the presence of the microparticles, covered by a layer of CDA. This indicates that the microparticles are embedded in a kind of matrix made of CDA.

3.2 In vitro release study

Four different kinds of association between methylene bisphosphonic acid and CDA were realized, two with free BPs and two with microencapsulated BPs. The different formulations were then compared through an in vitro dissolution study performed on three samples. The dissolution study was also carried out on the microparticles. The results are presented in Fig. 3 showing graphs representing the mean percentage of drug release versus time.

All these profiles show that methylene bisphosphonic acid is released very slowly from these different drug delivery systems: during the 15 days of the study the amount of drug released is comprised between 2.7 and 5.8%, the minimal value being obtained with the association C, and the maximal value with the association A.

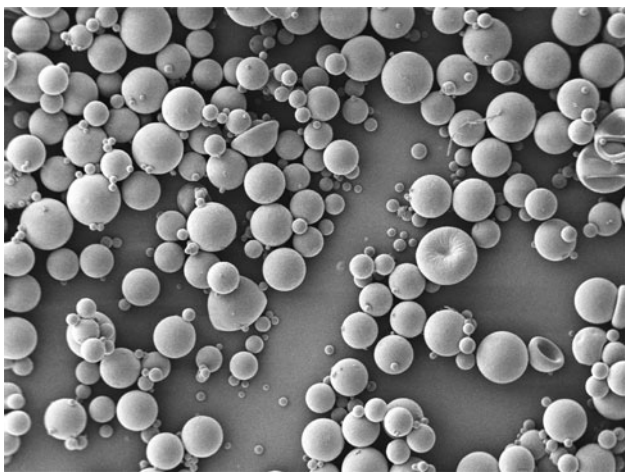

Fig. 1 SEM photograph of methylene bisphosphonic acid/PLGA microparticles (×50)

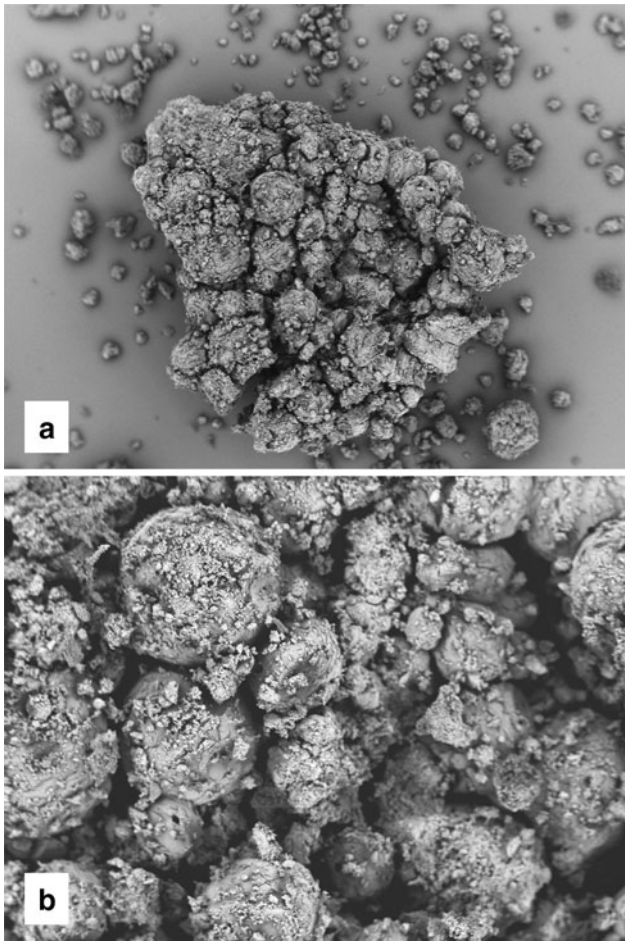

Fig. 2 SEM photograph of mixture of microparticles and CDA after compression (**a** ×50; **b** ×300)

We can also observe that the different materials tested behave in the same way and two phases can be distinguished: during the first day the curves show a quick increase of the percentage of drug released indicating a high dissolution rate; the second part of the curve (from the 24 h to the end of the study) becomes linear with a weak slope.

4 Discussion

To promote a better efficiency of bisphosphonates and enhance patient comfort, this work studied the association of the drug (either free or encapsulated) with a calcium-phosphate biomaterial, CDA. The aim of these drug loaded materials was to induce prolonged drug release while maintaining an adequate drug rate in situ.

In the first part of this work, the microencapsulation technique was tested as a mean to prolong methylene bisphosphonic acid release. The high yields obtained by the double emulsion/solvent evaporation-extraction method

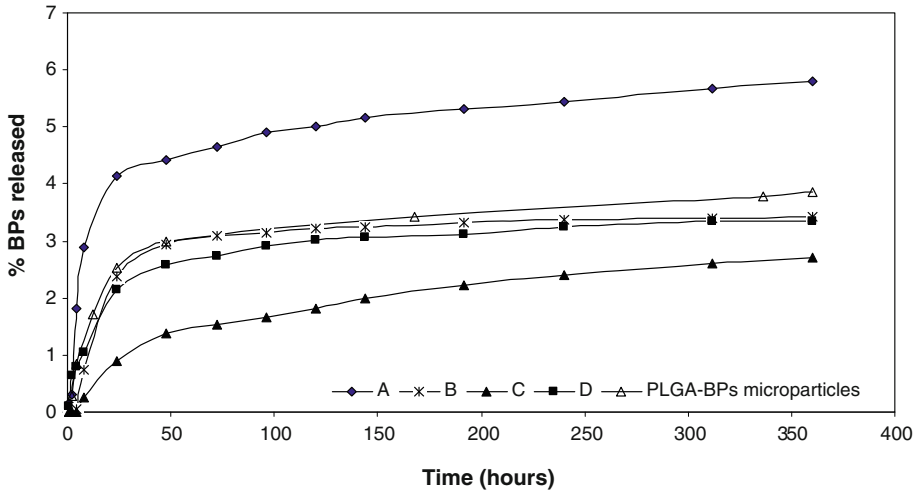

Fig. 3 Cumulative release of methylene bisphosphonic acid from microencapsulated form and formulations A, B, C and D

proved the feasibility of this process. We can also note the excellent encapsulation efficiency obtained, in spite of the strong hydrosolubility of methylene bisphosphonic acid. This good result can be attributed to the saturation of the external aqueous phase by NaCl, during the encapsulation process.

In a second step, methylene bisphosphonic acid was associated with CDA by mean of isostatic compression (association A) to form controlled drug delivery devices. This technique has already proved his efficiency to produce drug loaded biomaterials for a prolong release [13]. The influence of adding a binder in the formulation was also tested (association B). The chosen agent was HPMC, a well known binder usually used in tablet formulation. HPMC swells in contact with an aqueous medium and forms a gel around the blocks. The aim was to study if the formation of a gel network could affect the dissolution rate.

In parallel, we decided to combine the properties of microparticles and calcium phosphate devices by associating them either by simple mixing (association C) or by isostatic compression (association D).

The ability of these different formulations to prolong drug release was evaluated by an in vitro dissolution study.

From these results, it can be observed that all the samples tested lead to a dissolution profile divided in two steps. So, a general model of methylene bisphosphonic acid release profile from CDA blocks can be determined. The first part of the curve, from 0 to 24 h, corresponds to a burst release with a mean of about 1 to 4.1%. Then the dissolution rate decreases considerably with only 1 to 1.7% of methylene bisphosphonic acid released during the 14 following days. This step follows a zero order kinetic.

The first phase is due to the dissolution of the active drug present on the surface of the materials. Once the sample is put in contact with the dissolution media, the methylene bisphosphonic acid dissolves quickly. This is a common phase observed for most of prolonged drug release systems.

The second phase represents the release of the drug incorporated either in the CDA blocks or in the microparticles. The graphs show that the dissolution of the methylene bisphosphonic acid occurs with a slow and constant rate allowing the presence of the drug on the injection site over a long period.

Concerning PLGA encapsulated methylene bisphosphonic acid, the dissolution studies confirmed the great interest of this technique to modify drug release. As expected, we can observe a burst release during the first 48 h followed by a zero order release at a very slow rate.

With the two formulations containing free methylene bisphosphonic acid, the results prove that the process of isostatic compression induces the formation of hard blocks able to retain the drug. A slow diffusion through the pores of the CDA blocks occurs to permit the release of the drug gradually.

To reinforce this effect, we modify the formulation by the addition of a binder. The results obtained confirm the efficiency of HPMC to prolong drug release as shown in Fig. 3, curve B. Considering the second part of the curve, this formulation induces the slowest release. The drug is completely enclosed in the network formed by the gel. The release of the drug is then realized by a diffusion mechanism through the jellified barrier.

When studying the association of PLGA-methylene bisphosphonic acid microparticles with CDA, two behaviors could be distinguished. Indeed, the simple mixture (process C) of encapsulated methylene bisphosphonic acid and CDA, resulted in the slowest release with a mean of 2.7% in 15 days. The drug diffuses progressively and at a constant rate through the polymer barrier. Even during the first hours, the burst effect is very attenuated in comparison with the other formulations. The drug can be detected in

the dissolution medium only after 8 h of study. This corresponds to the time required for the dissolved drug to cross the membrane and reach the surface of the particle.

On the contrary, when the microparticles were associated with CDA by using the isostatic compression (process D), the results of dissolution obtained were not in accordance with our expectation. Actually, the compression of the encapsulated drug with CDA leads to compact blocks which release the methylene bisphosphonic acid at a higher rate than the simple blend (3,4% in 14 days). To analyze this result, we observed the blocks by SEM. On the photographs (Fig. 2) we could observe the fixation of the CDA on the surface of the microparticles. After the compression, the microparticles seem to still be round shaped but when observed with a higher magnification some cracks on the surface appear. This may explain the higher release rate.

5 Conclusion

This study demonstrated the feasibility to develop drug delivery systems able to deliver bisphosphonates in situ at a constant rate over several months. Both approaches tested gave interesting results. The association of CDA and bisphosphonates by isostatic compression induced a prolonged drug released, which was reinforced by the presence of HPMC. Encapsulation technique using PLGA allowed also the preparation of microparticles with high yields and releasing the drug at the slowest rate.

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