J. DRUG DEL. SCI. TECH., 18 (6) 438-444 2008

Validation of a manufacturing process of pellets for bone filling and drug delivery

E. Chevalier[1], M. Viana[1]*, S. Cazalbou[2], D. Chulia[1]

[1]Université de Limoges, CNRS SPCTS UMR 6638, Faculté de Pharmacie, Laboratoire de Pharmacie galénique,
2, rue du Docteur-Marcland, 87025 Limoges Cedex, France
[2]CNRS CIRIMAT UMR 5085, Faculté des Sciences pharmaceutiques, Laboratoire de Pharmacie galénique,
35, chemin des Maraîchers, 31062 Toulouse Cedex 09, France
*Correspondence: marylene.viana@unilim.fr

Wet high shear granulation followed by heat treatment was used to manufacture calcium phosphate porous spherical pellets. Experimental conditions were determined in order to obtain pellets presenting required specifications, i.e. size of about 800 µm, maximum sphericity and high microporosity. These pellets were intended for bone defect filling and in situ drug delivery and the final step therefore consisted in ibuprofen loading. In a context of quality assurance, the aim of the present work was to validate each step of the manufacturing process (i.e. granulation, calcination and drug loading). Indeed, reproducibility of the pellet characteristics, especially the drug content and release kinetics from the ceramic to be implanted, has to be ensured through the manufacture quality control.

Key words: High shear granulation – Heat treatment – Drug loading – Reproducibility – Process validation – Porous pellets – Drug delivery – Bioceramics.

Calcium phosphate ceramics are widely used as bone-graft materials [1-3] due to their chemical composition and crystalline structure similar to the mineral bone phase. These biomaterials are biocompatible, bioactive and osteoconductive [4-6]. To be effective for bone ingrowth, calcium phosphate bioceramics have to comply with certain specifications. In particular, macropore diameters must be at least 100 μm to host the cellular and extracellular components of bone and blood vessels and greater than 200 μm in diameter to allow osteoconduction [7]. Compared to the various physical presentations that are developed, calcium phosphate pellets present several advantages: they enable complex shaped bone defects to be filled while maintaining regular intergranular porosity due to their spherical shape.

High shear wet granulation may produce large granules, up to 1,000 μm, from primary particles [8] and improve their flow properties [9]. This granulation consists in several steps: dry mixing in order to homogenize the initial powder mix, then wetting in order to induce nucleation and particle growth. The resulting agglomerates are finally dried. If spheronization, i.e. high speed rotation of the impeller without liquid addition, follows the agglomeration step, it is possible to manufacture spherical pellets.

In the context of a bone substitute development, the creation of intrapellet micropores is sought to promote chemical exchanges with the biological fluids and enhance bioactivity. As wet granulation binders are organic components, further heat treatment can remove them, thus creating intrapellet porosity. This additional microporosity can also be used to load pellets with therapeutic agents. The objective is to control the in situ release of the active pharmaceutical ingredient.

In as far as the implantation of biomaterials might be responsible for local inflammation [10-12], a novel therapeutic approach consists in the use of anti-inflammatory local delivery systems improving the bioavailability [10]. Among the anti-inflammatory agents suitable for drug delivery systems, ibuprofen was chosen as a model molecule since it is well documented and widely used [13].

One manufacturing process of porous spherical pellets involving high shear wet granulation followed by heat treatment was previously developed [14]. In the present paper, the aim is to validate the process in order to control, from one step to another, the properties of the final pellets. In fact, whatever the scale and field, reproducibility requirements are of major importance to ensure constant end-use properties of the final product, i.e. for biomaterial application, morphology, textural properties, drug content of the pellets as well as release kinetics of the included drug substance.

This work describes each step of the manufacturing process of porous spherical phosphocalcic pellets loaded with ibuprofen. Four granulations are performed with the same process parameters and the corresponding batches are calcined and divided into three parts to be loaded. All along the process, the properties of four pellet batches are determined and compared in order to validate the manufacturing process and to evaluate its ability to produce pellets with the expected specifications concerning shape, size and porosity, as well as release characteristics.

I. MATERIALS AND METHODS

1. Materials

A calcium phosphate derivative (CaP, batch number G8138/3, Cooper, France), consisting of two phases (monetite and hydroxyapatite) with a global Ca/P molar ratio equal to 1.5, was used as a pellet skeleton [14]. Pregelatinized starch (Sepistab ST 200, batch number 80551, Seppic, France) was used both as a binder for granulation and as a pore forming agent after elimination by heating. Ibuprofen (Ibuprofen 50, batch number IB1M738, BASF, Ludwigshafen, Germany) was used as drug substance.

2. Methods

2.1. Pellet manufacturing process

Wet granulation process (*Figure 1*) was performed on 200 g of powder mix containing 10% of ST 200 in a Mi-Pro high shear granulator (Pro-C-epT, Zelzate, Belgium) equipped with a 1,900-mL capacity glass bowl, a three-blade impeller and a chopper.

In order to obtain the expected pellet characteristics (good sphericity, mean diameter around 800 μm for macropore size of about 150 μm and high microporosity) the following experimental conditions were applied [14-15]:

- mixing of the CaP and ST 200 powders at impeller and chopper speeds of 500 rpm for 180 s;
- granulation of the powder mix by addition of water at a distri-

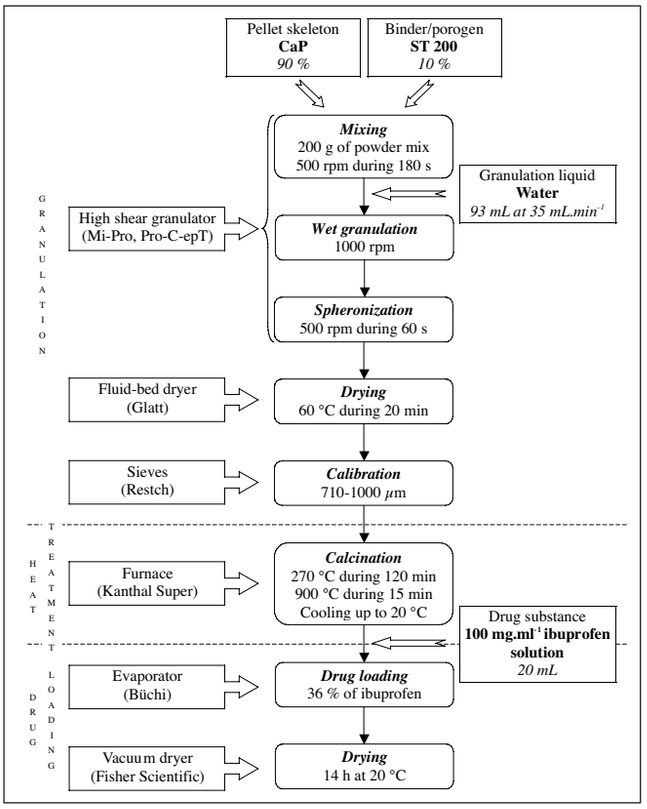

Figure 1 - Pellet elaboration chart.

bution rate of 35 mL.min^{-1}, with impeller and chopper speeds of 1,000 rpm;
- spheronization of the granules at impeller and chopper speeds of 500 rpm for 60 s.

Pellets thus obtained were dried in a fluidized bed (Glatt, Haltingen-Binzen/Baden, Germany) at 60°C for 20 min and finally sieved in order to retain the 710-1,000 μm fraction.

These granules were then submitted to a heat treatment, i.e. calcination (Kanthal Super, Rapid High Temperature Furnace, Bulten-Kanthal, Sweden) in order to remove the binder, thus acting as a pore former, and create the porosity. Pellets were first heated at 270°C with a heating rate of 2°C.min^{-1} and maintained at this temperature for 2 h in order to burn out the pregelatinized starch. Then, in a second step, they were heated at the same heating rate, up to 900°C in order to obtain a monophasic component, β-TCP. This temperature was kept constant for 15 min before being cooling down at the rate of 10°C.min^{-1} [14].

In order to validate the manufacturing process, four pellet batches were produced. Calcined pellets were then loaded with ibuprofen. In order to validate this step, 3.5 g of calcined pellets were sampled three times in each batch and loaded according to the following process: 20 mL of an ibuprofen ethanolic solution (100 mg.mL^{-1}) were introduced in a 250 mL flask containing the pellets and were evaporated in a Rotavapor (Büchi, Switzerland). Then, pellets were vacuum-dried at 20°C in an oven (model 45001, Fisher Bioblock Scientific, Illkirch, France) for 14 h. The theoretical ibuprofen ratio in the loaded pellets was equal to 36%.

2.2. Raw material and unloaded pellet physicochemical characterizations

2.2.1. Particle size

The raw material size distribution was determined in triplicate with a laser diffraction analyzer (Mastersizer 2000, Malvern Instruments Ltd, Worcestershire, UK) under dry conditions. The median diameter ($d_{0.5}$, μm) was determined from the distribution curve.

2.2.2. Pycnometric density

Pycnometric density (d_{pycno}, g.cm^{-3}) was determined using a helium pycnometer AccuPyc 1330 (Micromeritics Instruments Inc., Norcross, GA, USA). Prior to measurements, samples were degassed at a pressure lower than 50 mTorr vacuum (VacPrep 061, Micromeritics Instruments Inc., Norcross, GA, USA). Measures were repeated until the value stabilized [16].

2.2.3. Specific surface area

Specific surface area (S_{spe}, m^2.g^{-1}) was measured by nitrogen adsorption using a Gemini 2360 Analyzer (Micromeritics Instruments Inc., Norcross, GA, USA). Measurements were repeated until the value stabilized. Prior to evaluation, samples were degassed under the same conditions as for pycnometric density measurements. The specific surface areas were calculated from the BET multipoint equation [17] in the relative range of pressure of 0.05-0.30. In all cases, sample quantities ensured a total measured surface of at least 1 m^2.

2.2.4. Morphology

Raw material morphology and texture were observed using scanning electron microscopy (SEM, Stereoscan S260, Leica, Cambridge, UK).

Granule morphology was observed by optical microscopy (MZ 16, Leica, Cambridge, UK). The pellet sphericity was determined by calculating the circularity coefficient on 20 pellets per batch with the Image J free software [18]. The circularity formula is the following:

$$\text{Circularity} = 2\sqrt{\pi \times \text{area}} \Big/ \text{perimeter} \qquad \textbf{Eq. 1}$$

2.2.5. Porosity

Porosity measurements were carried out with a mercury intrusion porosimeter (Autopore IV 9500, Micromeritics Instruments Inc., Norcross, GA, USA) with a 5-cm^3 powder penetrometer. Cumulative and differential intrusion curves were recorded. The intrusion volume V_{intra} (mL.g^{-1}) corresponding to the pellet porosity was deduced and the corresponding pore size diameters were noted.

The pellet porosity (%) was calculated according to *Equation 2*:

$$\text{Pellet porosity (\%)} = [V_{intra}/(V_{solid} + V_{intra})] \times 100 \qquad \textbf{Eq. 2}$$

where V_{solid}, the volume occupied by the solid fraction of the granules, was determined by pycnometric measurements.

The inter-pellet porous volume V_{inter} (mL.g^{-1}) was calculated according to:

$$V_{inter}\ (\text{mL.g}^{-1}) = V_{packed} - V_{pellets} = V_{packed} - (V_{intra} + V_{solid}) \qquad \textbf{Eq. 3}$$

where V_{packed} (mL.g^{-1}) was the apparent volume obtained by close packing of pellets without any agglomerate deformation [19] and $V_{pellets}$ (mL.g^{-1}) was the volume of the pellets.

The pellet bed which would result from the organization of unloaded pellets inside the bone defects could be characterized by the inter pellet porosity, i.e. macroporosity of the bone filling material according to:

$$\text{Macroporosity (\%)} = (V_{inter}/V_{packed}) \times 100 \qquad \textbf{Eq. 4}$$

The total porosity of the biomaterial such elaborated, composed of both macroporosity and microporosity, therefore equalled:

$$\text{Total porosity (\%)} = [(V_{inter} + V_{intra})/V_{packed}] \times 100 \qquad \textbf{Eq. 5}$$

2.3. Dissolution kinetics

In vitro release studies were performed on about 550 mg of loaded pellets in 500 mL of phosphate buffer solution (pH 7.48) at 37°C using