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# Cold isostatic pressing of hydrating calcium sulfate as a means to produce parenteral slow-release drug formulations

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ABSTRACT

In the present study, cold isostatic pressing of hydrating biocompatible inorganic materials was used to encapsulate active substance in a highly dense microstructure of bioresorable/biodegradable material. This forms the basis in the NanoZolid® technology. It was shown that such microstructures can be used to produce slow-release parenteral formulations for long-term drug release.

The described novel principle to produce slow-release depot powder formulations was explored to manufacture a water-solvable calcium sulfate formulation encapsulating the anti-androgen substance 2-hydroxyflutamide (2-HOF) in a microstructurally designed calcium sulfate matrix. The microstructure of the solidified depot consisted of a composite of porous and dense material providing a combination of faster and slower release features. By mixing the drug loaded powder, consisting of densified and non-densified granular components, with an aqueous sodium carboxymethyl cellulose solution, an injectable suspension was formulated, which is injectable and which solidifies *in vivo* as a result of the ability of calcium sulfate to solidify by hydration.

The NanoZolid powder was characterised regarding pharmaceutical process parameters and the microstructure of the solidified formulation was evaluated with scanning electron microscopy and elemental mapping. The *in vitro* drug release was evaluated with a specially designed dissolution method with convection only at sampling occasions.

## 1. Introduction

Biodegradable parenteral slow-release formulations offer several potential advantages over traditional methods of administration. These advantages include: A controlled drug-release pattern during a defined period of time after each injection; an enhanced local concentration and effect; enhanced patient compliance; avoidance of first pass metabolism (improved bioavailability); decreased dosing frequency; lower incidence of systemic adverse effects and reduced medical care cost [12,16]. Several principles for drug release over many months or years have been reported [35]. Examples include surgically inserted drug loaded implants, e.g. drug eluting stents and drug filled reservoirs [24,27], as well as needle or catheter administered suspensions or emulsions based on polymeric drug-eluting microspheres (beads) or lipid-based formulations [3,4,18].

Biodegradable implants, e.g. some polymer based drug delivery systems, are in clinical use for parenteral controlled-release of drugs ranging from both hydrophilic and hydrophobic small molecules to small water-soluble peptides [13,26,34]. Issues that often need to be considered for polymers in parenteral controlled-release formulations include: (a) protein instability [2], (b) difficulties associated with use of organic solvents [32], and (c) large needle sizes [22]. Also, the future clinical use of new modalities (e.g. biologics) will rely on the development of novel parenteral drug delivery systems.

The present study describes the formulation and small-scale manufacturing of a novel controlled-release drug delivery system based on hydrating inorganic biomaterials, specifically calcium sulfate with designed powder characteristics, using the NanoZolid® technology. Calcium sulfate is an established biomaterial for bone void fillers in orthopedic and dental applications [17], and also a well-known pharmaceutical excipient for oral use [33]. There are also some approved pharmaceutical products based on calcium sulfate, such as implantable beads containing antibiotics [1].

The solid-state properties and the crystal structure of calcium sulfate are related to the amount of crystal water bonded in the lattice. The calcium sulfate dihydrate, with two units of crystal water per unit of calcium sulfate, salt contains the highest amount of crystal water that can be accommodated by calcium sulfate. When a crystalline powder of

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calcium sulfate dihydrate is heated under controlled conditions to above 100 °C, it may totally transform to a semi-stable hemihydrate, with only half a unit of crystal water per calcium sulfate. This solid-state transformation is reversible if a sufficient amount of water is added (again) to the hemihydrate powder, thus returning to the di-hydrate state [9], according to (1):

$$CaSO_4{\cdot}0.5H_2O\ (s)\ +\ 1.5H_2O\ (l) \leftrightarrow CaSO_4{\cdot}2H_2O\ (s) \quad (1)$$

It is well-established knowledge that the rehydration of a powder-water suspension to dihydrate is also associated with a solidification of the suspension to a cohesive structure. The water-free calcium sulfate is not or only slowly transformable to the dihydrate form. There are also identified over- and under-stoichiometric versions of both the hemi-hydrate and the dihydrate [9]. Thus, if a fine-grained powder of drug loaded calcium sulfate hemihydrate is mixed with water, an injectable suspension or paste is formed, which may solidify *in vivo* forming a local slow-release depot, as demonstrated previously with the cytostatic drug docetaxel [11]. However, such a depot releases its drug relatively fast, (typically within a few weeks depending on amount of formulation), as the solidified microstructure is characterised by a relatively high (approximately 30%) open porosity [21].

In the present study, the porosity of solidified calcium sulfate is reduced by allowing the hydration to proceed under a high external isostatic pressure without heat (a cold isostatic pressure, CIP). To accomplish this, a powder mixture of calcium sulfate hemihydrate and the active agent is wetted with a fixed amount of water and an external pressure is applied. As the nano-recrystallisation associated with the hydration proceeds while applying the pressure, a dense structure free from macro-pores can be obtained. This reduction in porosity is possible due to the increased molecular mobility that is created by the nano-recrystallisation associated with the rehydration process from the hemi-hydrate to di-hydrate of calcium sulfate. This pore-reducing process is comparable to a sintering at room temperature. In traditional sintering of powder materials that are not able to recrystallise by hydration, the pore-reducing process generally needs high heat to create the required molecular or atomic mobility during the compression phase, as is the case in hot isostatic pressing (HIP) [5], or as with isostatic ultrahigh pressing (IUHP) to modify starch or colloidal triglyceride dispersions with only a slight heating up to approximately 40–50 °C [6,29]. By applying the above mentioned CIP process, a totally dense calcium sulfate matrix with finely dispersed and encapsulated drug precipitates could be obtained without applying heat that could induce degradation of sensitive drugs. The encapsulated drug would then slowly be released as the matrix is dissolved and eroded by dissolution in a water-based environment such as tissue, similarly as described elsewhere [19].

The main objective of this report is to describe the key features and the pharmaceutical relevance of cold isostatic pressing as a means of producing non-porous drug carriers of calcium sulfate for use as parenteral administration forms, here referred to as the NanoZolid® technology.

## 2. Materials and methods

### 2.1. Starting materials

For the manufacture of a controlled-release formulation in a calcium sulfate carrier, a Ph. Eur. grade calcium sulfate dihydrate from Carl Roth GmbH, (Karlsruhe, Germany, art. No. 0256) was used as starting material. The particle size of the as-purchased powder was specified to $d_{50}$ = 40 µm. Chemoswed AB (Malmö, Sweden) manufactured the active substance 2-hydroxyflutamide (2-HOF) (described in section 2.2. below). Also 2-propanol (AnalaR Normapur®) from VWR International GmbH (Darmstadt, Germany) was used in the manufacturing process.

A diluent for reconstitution of powder to a suspension was provided by APL AB (Umeå, Sweden), consisting of purified water with 0.25 wt% carboxymethyl cellulose, grade 9M31XF (Ashland, the Netherlands). For drug release evaluation purposes a 0.9 wt% sodium chloride aqueous solution (Volusol®) was obtained from VWR.

### 2.2. Active pharmaceutical ingredient – 2-hydroxyflutamide (2-HOF)

2-Hydroxyflutamide (2-HOF) is an anti-androgen receptor antagonist and the active metabolite of flutamide with a molecular weight of 292.22 g/mol 2-HOF is a crystalline, yellowish to brown powder and has no basic pH-properties and no physiologically relevant proteolytic properties, as the predicted $pK_a$ for the acidic hydroxyl group is 10.0. 2-HOF is unionized throughout the physiological pH range. The estimated log P (partition coefficient) value is 2.1 [28]. The solubility of 2-hydroxyflutamide in saline was found to be about 49 µg/ml at 20 °C and 110 µg/ml at 37 °C. None of the biological or biochemical processes for 2-HOF is pH dependent.

### 2.3. Preparation of calcium sulfate hemihydrate

A micronized calcium sulfate hemihydrate was produced by a two-step process starting with a thermal treatment of the calcium sulfate dihydrate (Carl Roth, Germany) in flat and broad glass crystallisation dishes (200 mm diameter) in a heating cabinet (Termaks, Norway) at 200 °C for 4 h. The obtained hemihydrate calcium sulfate was thereafter size reduced by wet milling in a tumbling mixer (Turbula, Switzerland) with 2-propanol.

### 2.4. Preparation of a calcium sulfate and 2-HOF powder mixture

To produce a fine-grained and homogenous mixture of the active agent 2-HOF and the milled calcium sulfate hemihydrate, 75 g of 2-HOF powder was dissolved in 1200 g of 2-propanol in a flat-bottomed crystallisation dish. Once the dissolution of 2-HOF was complete, 100 g of the calcium sulfate powder was added to the solution. The hence achieved mixture was left to dry in a fume hood on a 35 °C heating plate with mild magnet stirring. As above, this dried powder (residual 2-propanol content not more than 0.5 wt%) was de-agglomerated by being passed through a 450 µm mesh.

### 2.5. Preparation of dense calcium sulfate granules with 2-HOF

To produce dense granules of calcium sulfate with encapsulated 2-HOF, the powder mixture of calcium sulfate hemihydrate and 2-HOF as described above was wetted with controlled amounts of water and compressed to a dense structure in a two-step compression procedure. The amount of water was selected to satisfy the uptake of crystallisation water during hydration. To transform 1.0 g of calcium sulfate hemihydrate to calcium sulfate dihydrate, the uptake of crystal water is 0.186 g. (Molecular weights of $CaSO_4{\cdot}½H_2O$ and $CaSO_4{\cdot}2H_2O$ are 145.14 and 172.16 g/mol respectively; (172.16–145.14)/145.14 = 0.186).

As a first step in the encapsulation process, the powder mixture was pre-compressed (forming an intermediate green-body) for approximately 2 min at a pressure of 35–100 MPa depending on size in a dedicated rubber mould in a cold isostatic press (CIP) (Quintus CIP42260, USA). This procedure leads to manageable but porous and brittle bodies [15]. As a second process step, purified water was added and soaked into the porous pre-compressed body in amounts corresponding to about 18.6 wt%. The sealed rubber moulds were then exposed to 400 MPa of pressure lasting for approximately 1 h in the same CIP press. After 1 h the hydration reaction is expected to be completed. An efficient cooling system allows the process to be performed at room temperature. No temperature rise of the densified material was observed, as the samples were removed from the pressure chamber less than 1 min after completion of the pressure cycle. The process parameters of isostatic pressure, amount of added water, and pressure time