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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

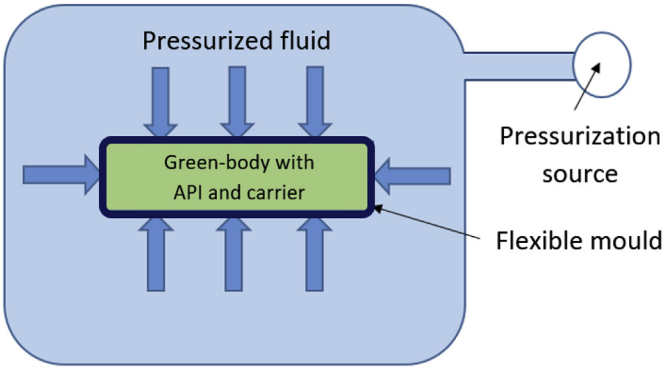

Fig. 1. Schematic illustration of a Cold Isostatic Press (CIP) (e.g. Quintus CIP42260, USA) loaded with a green-body of calcium sulfate and active substance encased in a non-permeable mould (e.g. a latex membrane) and exposed to pressurized fluid (e.g. water). The addition of purified water corresponding to 1.5 crystal water is performed directly before the compression, causing the recrystallisation to proceed simultaneously to the cold isostatic compression (hydration under pressure). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

were varied to find a suitably low porosity. The compression process in the CIP press essentially involves the application of a non-permeable membrane covering the green-body as protection against the pressurization medium (e.g. water) encased in a high-pressure chamber, as shown schematically in Fig. 1.

The compressed material was size reduced with a centrifugal mill (Retsch model ZM200, Germany). The milled material was passed through two sieves of mesh sizes 125 and 300 µm. The milling process leaves approximately 5 wt% of the size reduced material undersized and approximately 25 wt% oversized, depending on milling apparatus settings. These granules outside the range were discarded.

For evaluation of process parameters such as of amounts of added crystallisation water, residual porosity (measured as density) and pressurizing time, compressed material was also manufactured from drug-free calcium sulfate hemi-hydrate.

2.6. The NanoZolid® formulation

The novel NanoZolid® controlled-release parenteral powder formulation containing 2-HOF is composed of one third by weight of each of the three powder fractions described above: i) The milled calcium sulfate hemihydrate; ii) the calcium sulfate 2-HOF mixture; iii) and the compressed granules with 2-HOF (Liproca® Depot [30]). This powder composition was weighed into specially designed mixer syringes (with an internal mixer propeller) with 4.0 g in each syringe. The mixer syringes were individually packed in aluminium pouches and sterilized with a dose of 25–50 kGy of γ-radiation.

To produce an injectable paste, the sterilized powder was mixed with a 0.25 wt% carboxymethyl cellulose aqueous diluent in proportions 3.3 ml diluent to 4.0 g of powder. The hence formed viscous suspension (paste) solidifies within approximately 15 min after adding the diluent to the powder loaded syringe.

2.7. Evaluation methods

The particle size distributions of milled and un-milled calcium sulfate and compressed granules were characterised with laser light diffraction using a Malvern Mastersizer 2000 (Malvern Instruments Ltd, UK). Crystal structure of calcium sulfate was evaluated with X-ray powder diffraction (XRPD) (X:Pert Powder, PANalytical, The Netherlands). Specific surface area was evaluated with Brunauer-Emmet-Teller (BET) (Micrometrics Gemini 2370, USA). Powder morphology formulation microstructure were characterised with scanning electron microscopy (SEM) (Zeiss 1530, Germany).

Powder flowability as an indication of the possibility to fill the syringes with a uniform content was characterised as the ratio of the powder bulk density and the tapped density (compressibility), presented as Carr's index and as static angle of repose [8,25]. The tapped density measurements were not performed according to the European pharmacopoeia but with only 4.0 g of powder in a plastic syringe with scale.

The concentration of 2-HOF, potential drug loss and 2-HOF degradation after the complete production process was analyzed with Waters High Performance Liquid Chromatograph (HPLC). The system consisted of a Separations module (Waters 2695) and a Photodiode Array Detector (Waters 2996). Two mobile phases were prepared based on acetonitrile (ACN), water and trifluoroacetic acid (TFA). Mobile phase A was water:ACN in proportions 50:50 by volume with 0.1% TFA; mobile phase B was pure ACN. The mobile phases were mixed stepwise as 80, 45, 10, 10, 80 and 80% by volume of Mobile phase A in mobile phase B, starting at times: 0, 15, 20, 21, 21.1 and 25 min, respectively.

The analytical column was a reverse-phase HPLC C18 column; Hypersil GOLD (150 × 4.6 mm id, particle size 5 µm), and a Guard column (Phenomenex C18 4 mm, i. d. = 3 mm). The injection volume was 10 µl, flow rate 1.0 ml/min, and 2-HOF was UV-detected at 237 nm. The calibration curve of 2-HOF was linear over the range of 0.17–660 µg/ml with a correlation coefficient $R^2 > 1.000$. For this method, the retention time for 2-HOF is 13.5 min. The area under the curve was used for the quantification of the drug concentration.

The release rate of 2-HOF from the formulations was determined with an adopted *in vitro* method. Solidified 300 mg lumps of the formulations were individually immersed in 300 ml of 0.9 wt% sodium chloride solution in a flat bottomed 500 ml clear glass flask with lid, stored at 37 ± 0.5 °C. Liquid samples of 20.0 ml per time point (each week) were withdrawn and compensated for by adding 20.0 ml of fresh medium. The concentration of 2-HOF in the dissolution medium was quantified with HPLC-UV. The dissolution profile comparison was performed using the similarity factor f_2 defined as:

$$f_2 = 50 \cdot log\left\{\left(1 + (1/n)\sum_{t=1}^{n}(R_t - T_t)^2\right)^{-0.5} \cdot 100\right\} \quad (2)$$

where the variables are: n is the total number of drug release test points, R_t and T_t are the average percentages of drug released from reference formulation and from test formulation at test t, respectively [20].

3. Results

3.1. Powder properties and characteristics

The XRPD analysis was used to identify crystalline phases of calcium sulfate anhydrite or dihydrate in the hemihydrate powder after heating of the dihydrate. The starting powder from Carl Roth showed the typical XRPD spectrum of a calcium sulfate dihydrate powder with the main peaks at 2Θ: 11.7°; 20.8° and 29.2° [23]. After the heat treatment at 200 °C and the mild wet milling process at ambient temperature, the XRPD spectrum changed to that of the expected calcium sulfate hemihydrate, i.e. with main diffraction peaks at 2Θ: 14.7°; 25.7° and 29.8°, as shown in Fig. 2. If the heat treatment is not gentle enough (excessive time or temperature), formation of the calcium sulfate anhydrite (complete dehydration) phase is risked. This crystal phase does not rehydrate to dihydrate and is therefore not useable in this context neither as a solidifying phase of the formulation nor for the densification in the isostatic pressing process. The presence of the anhydrite phase would have been identified in the XRPD spectrum.

Considering the heat treatment conditions in the dehydration process, the powder is expected to be of the β-form [9]. The α-form of calcium sulfate hemihydrate requires a more complicated fabrication

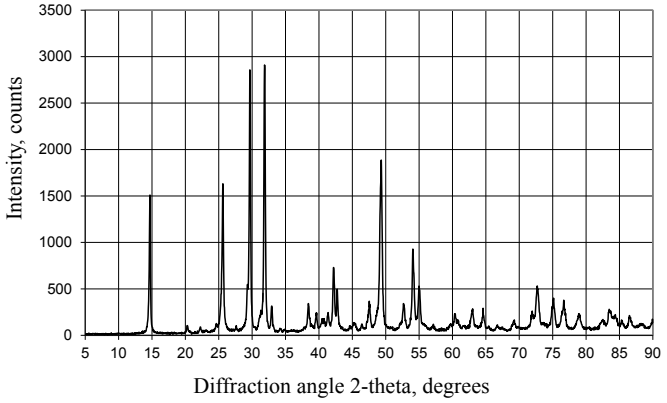

Fig. 2. X-ray powder diffraction (XRPD) spectrum of calcium sulfate hemihydrate powder after heat treatment at 200 °C for 4 h including milling, with the main diffraction peaks at 2Θ: 14.7°; 25.7° and 29.8°.

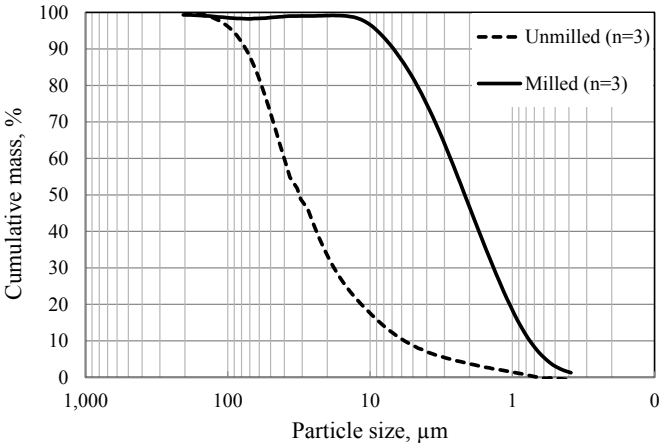

Fig. 3. Particle size distributions of calcium sulfate hemihydrate before and after milling.

process involving a controlled humidity and over-pressure (autoclavation). The purpose of the powder milling was to facilitate the formation of a fine-structured, non-porous and homogenous structure in the following compression step. After heat treatment, the hemihydrate powder particle size was specified to d_{50} = 31 µm. The milling resulted in a particle size reduction to d_{50} = 2.2 µm, as seen in Table 1. The effect of the milling process on the particle size distribution, which is important for the homogeneous wetting prior to compression, is shown in Fig. 3.

The active agent 2-HOF was freely soluble in 2-propanol. After evaporation of the 2-propanol the concentration of 2-HOF in the dry powder mixture was 42.3 wt%. There was thus no measured loss of drug substance in this process step and no detectable molecular degradation. The 2-HOF calcium sulfate mixture showed a homogeneously distributed yellowish colour. As seen in Table 1, the flowability of the hemihydrate as well as for the powder mix with 2-HOF was ambiguous and characterised as very poor as regards compressibility (Carr's index) and as good or excellent as regards static angle of repose.

3.2. Compression by hydration under pressure

The pre-compression procedures of the dry powder mix generated porous and fragile green-bodies needing gentle handling. The porous structures, however, facilitated a quick (10–20 s), homogenous and complete wetting of the structure, as the added hydration water was sucked into the structure by capillary action. The reason for the addition of water is to facilitate the creation of a dense structure free from micro-pores after the following cold isostatic compression procedure, as the water initiates a recrystallisation from the hemihydate to the dihydrate phase.

Powder compression tests with varied amounts of hydration water, isostatic pressure and amounts of powder were performed with hemihydrate powder free from active substance, see Table 2. It was confirmed that totally pore-free bodies can be achieved for at least 300 g of power with a pressure of 400 MPa and a pressure time of 60 min. The lower evaluated pressures, 200 and 300 MPa, reduced the obtained density. A 100% ratio between achieved density and optimal theoretical density (2.32 g/cm^3) was achieved with a pressure of 400 MPa and amounts of water near or slightly below the stoichiometric water amount (0.186 g of water per g of calcium sulfate hemihydrate). An addition of more than about 10% above the stoichiometric amount of water, decreased the achieved density noticeably. Slightly under-stoichiometric amounts of water did not reduce the achieved density,

Table 2
Densities of bodies after isostatic compaction for 60 min.

Water to powder ratio (−)	Isostatic pressure (MPa)	Density vs. max. density (%)	Powder weight (g)	Comments (Stoichiometric water to powder ratio is 0.19)
0.50	0	70.0	1.0	Solidified hemihydrate-water paste, without pressure
0	200	79.2	1.0	Powder compacted without water
0.19	200	86.9	10.0	Stoichiometric amount of water
0.09	200	94.0	10.0	½·stoichiometric amount of water
0.15	300	97.8	150	Under-stoichiometric on water
0.18	300	95.7	150	Under-stoichiometric
0.20	300	94.0	150	Near stoichiometric
0.22	300	91.0	150	Over-stoichiometric
0.25	300	86.0	150	Over-stoichiometric
0.17	400	100	300	Under-stoichiometric on water
0.20	400	100	300	Near-stoichiometric
0.20	400	100	300	Near-stoichiometric
0.22	400	98.7	300	Over-stoichiometric
0.23	400	96.9	300	Over-stoichiometric

Table 1
Powder data for intermediates and final drug-loaded powders. Data are mean values of n measurements. Not analyzed = n.a.

Powder	Particle size, d_{10}, d_{50}, d_{90} (µm) (n = 2)	Powder density (g/cm^3) (n = 3)	Angle of Repose (°) (n = 3)	Compressibility Carr's index (−) (n = 3)	Specific surface area (m^2/g) (n = 1)	Assay2-HOF (mg/g) (n = 3)
Unmilled hemihydrate	5.7, 31, 75	n.a.	n.a.	n.a.	n.a.	–
Milled hemihydrate	0.75, 2.2, 6.8	0.58	31-35 (good)	37 (very poor)	5.4	–
Powder mix with 2-HOF	n.a.	0.67	25-30 (excellent)	34 (very poor)	n.a.	428.6
Compressed granules	98, 206, 330	0.74	n.a.	n.a.	n.a.	321.4
Final powder	n.a.	0.73	31-35 (good)	40 (very poor)	n.a.	250.0

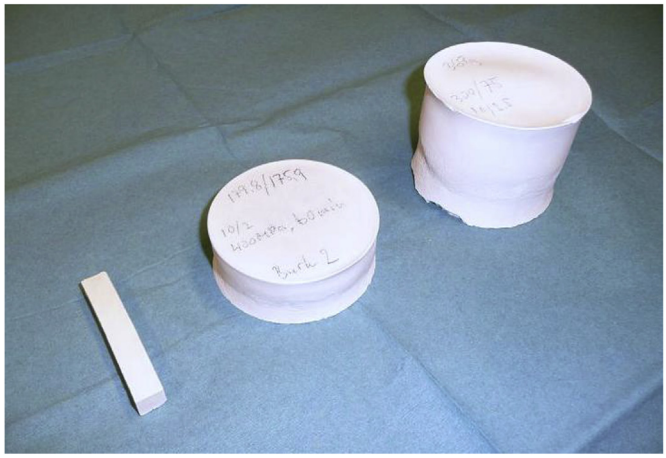

Fig. 4. Three examples of compacts made by hydration under pressure of 10, 150 and 300 g of calcium sulfate hemihydrate and stoichiometric amounts of water (19 wt%).

instead for the lower pressures the density actually increased when using under-stoichiometric amounts of water.

It was confirmed by calorimetric measurements that the hydration reaction was completed well before 60 min. Therefore 60 min of compaction was routinely used. However, in a test series with 150 g of powder and varied water amounts, the densities were similar also after 30 min of hydration. In summary, 60 min of isostatic compression at 400 MPa of pre-compressed bodies with near- or slightly under-stoichiometric amounts of water gave rise to highly dense (minimal amounts of macro-pores) and homogenous bodies of calcium sulfate dihydrate. The compressed bodies showed typical signs of a volume shrinkage compared to the pre-compressed forms, corresponding to an increase in density, see some examples of compressed bodies in Fig. 4.

Compression of 2-HOF impregnated hemihydrate powder was performed in the same way as described above. However, the API had a powder bonding effect and facilitated the powder pre-compression. The API also slightly prolonged the time necessary for total wetting of the pre-compressed powder.

After pre-crushing, milling and sieving, the yield of material with a grain size between 125 and 300 μm was approximately 70 wt%, after discarding the material outside the 125–300 μm range. The average particle size was measured to d_{50} 206 μm, see Table 1. The concentration of 2-HOF in the compressed granules was 32.1 wt%, i.e. lower than in the starting powder (42.3 wt%).

3.3. Pharmaceutical powder formulation

The pharmaceutical powder formulation was obtained by mixing the three individual precursor powders in equal amounts by weight, see Fig. 5. The reason for using one third of each powder fraction was the estimated equal importance of achieving a cohesive and strong depot (pure hemihydrate), an initially faster release (hemihydrate with API without isostatic densification) and the long-term slow release (from the isostatically densified granules). By doing the three-fraction powder mix, all these characteristics were achieved in combination. The combined formulation powder showed a Carr's index indicating poor flow and an angle of repose indicating good flow, see Table 1.

The combined powder was shown to have a drug load of 25.0 wt%, corresponding to the mean of the drug concentrations of the individual powders. The powder was easily mixed with the carboxymethylcellulose diluent forming a paste that was injectable through a 17-gauge needle.

The drug release was evaluated *in vitro* from 300 mg moulded and solidified depots. The solidified formulations gave a long-term and controlled drug release with excellent repeatability for five months for

Fig. 5. The three powder components of the NanoZolid powder formulation: milled hemihydrate (white); 2-HOF hemihydrate mix (yellowish); and compressed and milled granules (coarse particles with intense yellow-brownish colour). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

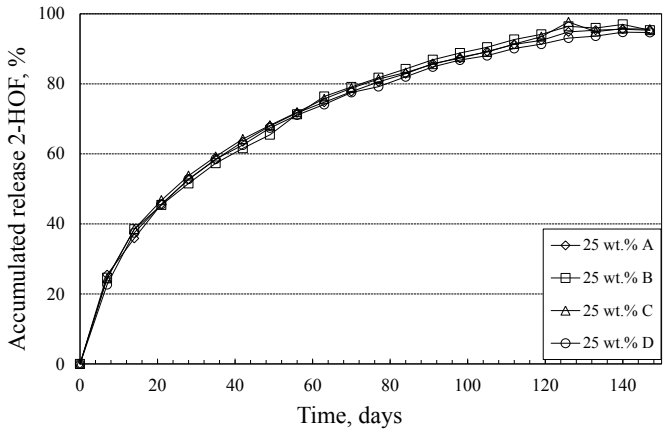

Fig. 6. Accumulated *in vitro* drug release of 25 wt% 2-HOF over time from 300 mg solidified depots of NanoZolid® formulations in 300 ml of 0.9% saline water [28].

the 25 wt% formulation, see Fig. 6.

The drug release profiles showed an initial faster phase for the first 3–4 weeks, followed by a slower release for about five months. An additional formulation containing 33 wt% of 2-HOF in the powder was also evaluated during this first release phase (3 weeks) –and showed an essentially similar *in vitro* release compared to the 25 wt% version. For statistical aspects, such as average accumulated release, relative standard deviation and similarity factor (f_2), see Table 3. This NanoZolid® formulation was manufactured with the processing steps, but differed from the 25 wt% version as follows: the total of 4 g of powder was composed of 1.10 g calcium sulfate hemihydrate, 1.45 g powder mix and 1.45 g compressed granules – where the powder mix contained 50 wt% 2-HOF. The microstructural design was intended to produce a faster release (a few weeks) from the porous matrix and a slower release (for up to 5 months or more) from the isostatically compressed granules. The solidified depots release drug as a result of both dissolution and erosion. From SEM of a cross-section of moulded and solidified depots, the isostatically compressed granules are distinguishable in the more porous matrix (consisting of both drug-loaded and pure calcium sulfate), see Fig. 7. With EDX-mapping the precipitations of the drug, i.e. in this case 2-HOF, can be seen both in the porous matrix as well as

Table 3
Three weeks *in vitro* drug release for 25 and 33 wt% 2-HOF formulations.

	Test no.	Accumulated release (%) after 7 days		Accumulated release (%) after 14 days		Accumulated release (%) after 21 days	
		25 wt% 2-HOF	33 wt% 2-HOF	25 wt% 2-HOF	33 wt% 2-HOF	25 wt % 2-HOF	33 wt% 2-HOF
	A	25.47	29.69	35.86	43.12	45.65	54.19
	B	24.61	32.41	38.47	42.41	45.40	50.84
	C	24.43	28.04	38.48	42.15	46.77	51.95
	D	22.66	27.33	37.46	38.78	45.57	49.86
	E	–	28.78	–	39.40	–	47.58
	F	–	28.30	–	42.29	–	52.57
Average		24.29	29.09	37.57	41.36	45.85	51.17
SD		1.179	1.807	1.235	1.801	0.624	2.298
RSD (%)		4.85	6.21	3.29	4.36	1.36	4.49
Similarity factor, f_2 (%)							66.0

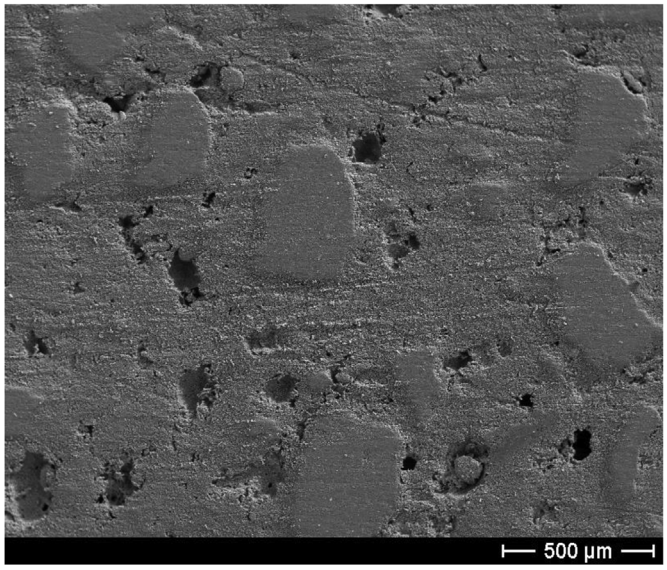

Fig. 7. Scanning electron microscopy image of a cross-section cut through a 25 wt% 2-HOF solidified depot, showing the microstructure consisting of compressed granules in a porous matrix.

in the compressed granules, see Fig. 8.

4. Discussion

Products combining injectable and long-term drug release formulas for local treatment of diseases, directed to specific organs or tumor sites, may be advantageous both for precise and individually adjusted doses as well as for lower risk for systemic exposure and hence decreased adverse effects. The present paper describes a novel procedure for the preparation of such products; the NanoZolid® technology. The new technology was used for encapsulation of drugs or pharmaceutical compounds in a densified, non-porous, matrix of bioresorbable material. This was carried out utilizing the principles of cold (room temperature) isostatic pressing in combination with the ability of hydrating ceramics to recrystallise when exposed to water. The combination of the nano-recrystallisation process and a high external pressure can be compared to a low temperature sintering as described previously [10]. Instead of achieving atomic mobility by increasing the temperature, as in standard powder sintering, the local material diffusion in the new process is caused by the recrystallisation coupled with the uptake of additional crystallisation water (hydration). The nano-recrystallisation

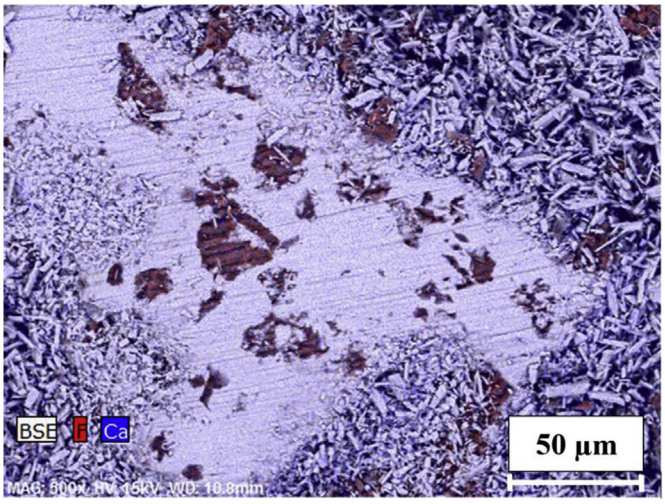

Fig. 8. Scanning electron microscopy with energy dispersive X-ray mapping image of a cross-section of a 25 wt% 2-HOF solidified depot, showing the microstructure with precipitates of 2-HOF (red) and calcium (bluish) in the porous matrix as well as in the compressed granules (white-grey). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

in combination with an external pressure causes the starting powder to compress, under ideal conditions to a fully non-porous microstructure (matrix) in which active agents may be encapsulated. Potentially, with large and dense implants the release may proceed for periods longer than the five months achieved in the present investigation. Also, cold isostatic pressing is an unusual technique in the pharma powder industry, which when applied innovatively to e.g. hydrating ceramics may open up for new depot formulations for parenteral use.

All crystal forms of calcium sulfate are water-soluble. The dihydrate dissolves maximally to 2.67 g/l at 20 °C. In many organic solvents such as ethanol or iso-propanol, the calcium sulfate salts are practically insoluble [7]. The drug substance is therefore ideally dissolved in an organic solvent when combined with calcium sulfate which enables a drug to distribute evenly over the powder grains as a result of the evaporation process of the solvent. The organic solvent may preferably be evaporated in a closed system, such as e.g. a rotational evaporator, for collection and recycling.

This study shows that a fairly normal small-drug molecule withstands the exposure to a pressure of 400 MPa (4000 bar) for 60 min. Utilizing the same NanoZolid® technology we were successfully able to produce and evaluate several other molecules (unpublished results). A comparably gentle processing to the active agent, while still producing completely dense microstructures, is not possible to achieve with conventional tablet compression or granulation without significant, at least temporary, heat generation. A relative loss of active substance, however, appeared in the milling and sieving processes used for producing the size-fractionized compressed granules. This is probably explained by a higher degree of active pharmaceutical ingredient in the discarded fine-particle fraction compared to in the remaining coarser size fractions.

Both the precursor powders and the final drug pharmaceutical powder show ambiguous flowability characteristics. This may be due to that the tapping was not performed according to the pharmacopoeia based method, but may also be due to the high complexity in powder flow, where particle tumbling and interparticulate friction may play an important role [14,31]. Taking both used powder flow tests into account, the flowability may be described as acceptable, as the powder product may directly be filled into primary packages (such as syringes or vials) without being further processed. However, due to the distinctively different grain sizes of the mixed powder fractions, a low

vibration powder filling process is preferable in order to avoid segregation. Preliminary evaluations indicate that a pneumatic powder filler functions satisfactory.

In the present investigation we have shown that a precise amount of crystallisation water and a sufficient pressure during the entire hydration time should be maintained to obtain the required dense body, which in a subsequent step can be milled and used as one part in a powder formulation that creates a sustained drug release of up to at least five months. Our results show that shorter pressure times (30 instead of 60 min) have also produced satisfactory densified bodies. Presumably, the pressurization should proceed for most of the time required for full hydration. A pressure of 400 MPa was necessary to produced totally dense structures of pure calcium sulfate. However, a pressure of 300 MPa also resulted in very low residual porosities (2%). It is believed that such low porosity is manifested mainly as closed pores, which in turn may affect the drug release. Water contents above the stoichiometric amount produce increased porosity, presumably due to remaining pockets of free water. However, the stoichiometric amount of water or slightly lower amounts seem to be ideal. A non-ideal hydration process requiring slightly less water compared to the theoretical value might explain this phenomenon. However, when wetting the calcium sulfate-active substance powder mixtures prior to applying the isostatic pressure, more water may be needed to compensate for any water absorbed by the active substance.

The solidified 2-HOF formulation produced a two-phase drug release feature. The initial higher release rate of 2-HOF during the first weeks can be explained by the faster dissolving of the non-compressed material in the powder mix. The two phases are not distinctly separated as the release from the compressed granules also starts early and continues throughout the whole release test time. The long-term drug release of five months or even more with a completely bioresorbable formulation indicates that this type of formulation, including densified and slowly resorbable bioceramics, may compete successfully with existing slow-release parental depots. This is therefore a promising formulation for potential use of wide range of unmet medical needs and therapies. The other NanoZolid® formulation containing 33 wt% was followed only during three weeks as the main purpose was to confirm that a drug load of up to 33 wt% is possible to achieve keeping the similar initial release pattern.

The drug product containing 2-HOF described in this work has been evaluated clinically on patients with prostate cancer. The reconstitution process for a NanoZolid powder and diluent (a 0.25 wt% sodium carboxymethyl cellulose), as well as the injectability of the hence formed viscous suspension through 17 gauge needles, has been confirmed in clinical trials presented in Ref. [30]. Furthermore, it has been shown that the described NanoZolid® technology is applicable also to other drugs e.g. docetaxel [11].

5. Conclusions

Highly dense and slowly bioresorbable drug formulations for long-term release of the drug substance 2-HOF can be achieved with the NanoZolid® technology by exploring isostatic pressing at room temperature together with hydrating calcium sulfate that recrystallises during the compression.

To achieve an optimally compressed drug carrier microstructure, the isostatic pressure, the pressure time and the amount of added crystallisation water are essential parameters that need to be optimised for each specific requirement profile and for each active substance. In the present study, 400 MPa, 60 min pressure time and near stoichiometric and slightly under-stoichiometric amounts of water, respectively, resulted in the most compressed structures.

The compressed drug carrier containing the active substance, as produced using the NanoZolid® technology, may be explored in several forms including pre-manufactured depots that are surgically implanted. However, in the present study, we made an injectable formulation containing granules from the compressed material, mixed with non-compressed calcium sulfate hemihydrate powder. By mixing such a formulation with an aqueous solution, an injectable suspension is formed that solidifies *in vivo* forming a long-term drug release depot, resembling a local drug eluting implant. By combining non-compressed and compressed calcium sulfate powder fractions in a NanoZolid® formulation, an initial boost dose followed by a slower release over up to five months was achieved.

Conflicts of interest

SG and NA are both consultants for and have stock ownership in LIDDS AB. SG and NA are also both registered inventors for patent applications owned by LIDDS.

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