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Research paper

Nanoshells prepared by atomic layer deposition – Long acting depots of indomethacin

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ABSTRACT

There is a trend in pharmaceutical research and development to develop depot formulations with dosing once weekly, once monthly, or even less frequently. A novel approach to achieve long acting injectable suspensions is to produce dense inorganic nanoshells with atomic layer deposition (ALD) on active pharmaceutical ingredients. Such particles can be suspended in an aqueous vehicle and administered subcutaneously. The purpose of this work was to study the release of a model drug, indomethacin, coated with aluminium oxide nanoshells.

Indomethacin was ball-milled to a median particle size of 6 μm. The nanoshells were produced with a proprietary ALD process that is trademarked as PharmaShell® by Nanexa AB. The drug load was determined with HPLC-UV to 82 wt%. The test materials were administered subcutaneously in rats (1, 10, and 100 mg/kg) from which blood samples were collected during 12 weeks. Plasma was generated and analyzed with regards to indomethacin using UPLC-MS/MS.

The release rate was dramatically slower for the nanoshell coated indomethacin compared with uncoated indomethacin. Drug was released *in vivo* during more than 12 weeks for the 10 and 100 mg/kg doses, and during 10 weeks for the 1 mg/kg dose, while uncoated indomethacin was eliminated with a half-life of 15 h, as calculated from the release data by fitting a one phase decay function. The exposure levels were similar as earlier reported for therapeutic indomethacin doses, but significantly sustained in the present study using coated drug particles in rats.

In conclusion, this is the first long-term *in vivo* evaluation of nanoshell depot formulations. The stable plasma concentrations for more than 12 weeks demonstrate that nanoshells can enable long-term depot injections with high drug load.

1. Introduction

The therapeutic effect of many drugs may be enhanced by using sustained release formulations. Constant drug levels can improve the effect, but also reduce negative side effects. Plasma level time-profiles of repeated dosing using high clearance drugs have high maximum drug levels, where toxic effects are more pronounced, and deep valleys where the drug levels may be subtherapeutic. However, it is possible to avoid high toxic levels and low subtherapeutic levels by formulating such drugs with sustained-release formulations, achieving simply high enough drug levels within the therapeutic concentration range [1,2].

Cancer treatment is one therapeutic field where sustained release formulations probably will increase in importance. Many anti-cancer drugs exercise their effect at a certain phase in the cell-cycle and therefore require therapeutic drug levels over long time periods. However, rapid plasma clearance leads to short tumor exposure times unless the drug is formulated in long-acting sustained release depots [3]. Further, less frequent administration by the aid of, for instance, long acting injections is more convenient for the patient and will therefore increase the medication adherence [1]. In general, adherence is poor for chronic therapies. One example where the treatment success is assured by strict adherence, by correctly following medicinal advices, is HIV antiretroviral therapy. However, as many as two thirds of the patients taking reverse transcriptase inhibitors may end their drug therapy or intentionally alter prescribed doses [4], which may lead to poor therapeutic effect [5] and development of drug resistance [6].

There are several long acting injectable or implantable formulation strategies, including oily solutions and suspensions, aqueous suspensions, microspheres, lipid systems, *in situ* forming systems, and implants [1,2]. PharmaShell® is a patent pending [7] drug delivery system that

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enables sustained release over long time following the formation of nanoshells on the surface of the particles. The nanoshells are produced directly on particles of an active pharmaceutical ingredient (API) with Atomic Layer Deposition (ALD), a chemical vapor deposition technique with its main application within thin film coatings in semiconductor processing [8]. One challenge when coating drug particles with wet chemical techniques is that the drugs dissolve in the solvent. ALD is a dry process, thus avoiding the aforementioned problem.

In ALD, thin film deposition is achieved through self-limiting gas-solid reactions, typically under low pressure and elevated temperature in a reaction chamber. The gas-solid reactions occur in a predetermined sequence, which usually consists of four steps that are repeated. The four steps are individually called pulses and together they are referred to as an ALD-cycle. In the case when aluminium oxide is grown directly on the surface of the particles the ALD cycle consists of the following four pulses:

1. Trimethyl aluminium (TMA) is evaporated and carried into the reaction chamber by an inert gas, typically nitrogen. TMA adsorbs to the surface of the particles, typically to a hydroxyl, as a monolayer and in doing so losing two of its methyl ligands.
2. The chamber is purged with nitrogen to remove excess of TMA and by-products. Only the monolayer of TMA that is adsorbed to the surface of the particles remains.
3. Water is evaporated and carried into the reaction chamber by nitrogen. Water reacts with the monolayer of TMA to form a monolayer of aluminium oxide.
4. The chamber is purged with nitrogen to remove excess of water and by-products, leaving one monolayer of newly formed aluminium oxide on the surface of the particles.

The ALD cycle is repeated until the desired thickness of aluminium oxide coating on the drug particles is achieved. The thickness produced by one cycle is dependent on the process but is in the range of 0.1–0.3 nm per ALD-cycle. The thickness of the nanoshells is a few nanometers, which results in high drug loads.

There are only a few earlier scientific reports where ALD on pharmaceutical relevant systems have been studied, all published last year. We [9] found that the ALD process did not lead to recrystallization of amorphous spray-dried lactose in the lower micron size-range and that the produced nanoshells were pin-hole free since they prevented the highly hygroscopic material from absorbing moisture. Zhang et al. [10] demonstrated how dissolution rate, dispersibility, and heat transfer could be modified for individual lactose monohydrate and budesonide particles in the lower micron to higher nano size range by ALD coatings. Käärinäinen et al. [11] showed that it was possible to deposit ALD coatings on individual acetaminophen particles in the size-range 10–200 μm without changing the polymorph of the drug and observed modified *in vitro* drug release. Hautala et al. [12] deposited ALD films on minitablets to achieve taste masking, but did unfortunately not find the taste masking capacity sufficient.

This work presents the first pharmacokinetic study of ALD coated nanoshells as a drug delivery system for sustained release. The purpose of this work was to study the drug release of a model drug, micronized indomethacin, coated with aluminum oxide nanoshells. Indomethacin is a non-steroidal anti-inflammatory agent with analgesic and antipyretic properties [13]. The drug is low-toxic, poorly soluble in water, with an intrinsic solubility of 8.8 μg/mL and a pK_a of 4.13 [14]. It has melting point at 151 °C and the mean half-life of neat indomethacin suspensions administered subcutaneously in adult humans was estimated to 4.5 h [13]. *In vitro* testing was performed and the purpose with the *in vitro* testing was to investigate if a delayed drug release *in vivo* was to be expected. The *in vitro* method and results are included as Supplementary Material.

2. Materials

Indomethacin (Hangzhou APIChem Technology Co., Ltd., China), indomethacin CRS batch 3.0 used for analytical standards (EDQM, France), indomethacin-d4 (Toronto research chemicals, Canada) used for analytical standards, polysorbate 80 (Sigma-Aldrich/Merck KGaA, Germany), trimethyl aluminium (98%, ABCR GmBH, Germany), water (Ph. Eur, Sigma-Aldrich/Merck KGaA, Germany), nitrogen (99.999%, AGA, Sweden), polyethyleneglycol 4000 (PEG4000) (BioUltra Sigma-Aldrich/Merck KGaA, Germany), polyvinylpyrrolidone K30 (PVPK30) (Sigma-Aldrich/Merck KGaA, Germany), polysorbate 20 (Sigma-Aldrich/Merck KGaA, Germany), phosphoric acid 85% (AcroOrganics, Belgium), acetonitrile (HPLC/gradient grade, Rathburn, UK), phosphate buffered saline (PBS) (0.2 μm filtered, Sigma-Aldrich/Merck KGaA, Germany), acetic acid glacial (Fischer Scientific, USA), formic acid (ACS reagent, Ph Eur, > 98%, Sigma-Aldrich/Merck KGaA, Germany), dimethyl sulphoxide (DMSO) (Reagent Plus > 99.5%, Sigma-Aldrich/Merck KGaA Germany), Phosphate buffer (PB) (APHA, pH 7.2, 25 mM, Sigma-Aldrich/Merck KGaA Germany).

3. Methods

3.1. Indomethacin micronization

Micronization of indomethacin was performed by milling using a Fritsch Pulverisette 7 premium line using 80-mL zirconium oxide milling vessels, with 5 mm zirconium beads and 0.5 wt% polysorbate 80 solution at 100 rpm for 1050 min. The milled material was rinsed with water after the ended milling. The suspensions were centrifuged for 15 min at 10,000*g* and the supernatant was decanted. The sediment was re-suspended in water and centrifuged again. This process was repeated twice to remove polysorbate 80 from the milled particles. The sediment was then dried in a OV-11 vacuum oven (Jeio Tech, Korea) equipped with an Edwards 8 E2M8 two stage vacuum pump (Edwards, UK) at 50 °C over night before being manually pressed through a 100 μm test sieve (Retsch, Germany).

3.2. Nanoshell depositions

The nanoshell depositions, to produce coated indomethacin particles, were made in a Picosun SUNALE™ R-series ALD-reactor (Picosun Oy, Finland) fitted with an Picosun POCA powder cartridge (Picosun Oy, Finland) to contain particles. The POCA cartridge consisted of two glass cups with bottoms of sintered glass filters, one fitting inside the other one. The uncoated micronized indomethacin particles were placed in the larger cup and the smaller cup was inserted on top as a lid. The precursors used were trimethyl aluminium (TMA) and water. Nitrogen was used as a carrier gas and for purging.

ALD was performed using the following parameters and setting:

Reaction chamber temperature: 50 °C. *Reactor chamber pressure*: 13 hPa. *Pulses*: 0.2 s of H_2O exposure at 8 hPa partial pressure, 5 s of purging with N_2 at 8 hPa partial pressure. All this repeated 21 times and then finished with 400 s further purging with N_2 at 8 hPa partial pressure. 0.2 s of TMA exposure at 8 hPa partial pressure, 5 s of purging with N_2 at 8 hPa partial pressure. All this repeated 21 times and then finished with 400 s further purging with N_2 at 8 hPa partial pressure.

To achieve a nanoshell thickness of 30 nm, 115 ALD-cycles were used. The cycles were divided into five sets and particles were deaggregated by manual agitation between the ALD sets to move the particles in relation to each other, which is a necessity to achieve pinhole free nanoshells.

3.3. Particle size analysis

The particle sizes of the uncoated and the nanoshell coated indomethacin particles were measured using a laser light scattering

particle size analyzer (SALD–7500 nano, Shimadzu, Japan). About 15 mg of sample was suspended in 3 mL 0.5% polysorbate 80 solution using ultrasonication at 60 W (Bandelin Sonopuls 3200 equipped with a BB 6 cuphorn, Bandelin, Germany) for 1 min. Approximately 100 μL of the suspensions were added to the 8-mL measuring cuvette filled with water.

3.4. Solid-state characterization

Powder X-ray diffraction (XRD) was measured using a Bruker D8 Advance coupled 2θ-θ diffractometer (Bruker AXS, Inc., USA) with a position sensitive detector, LynxEye XE-T (Bruker AXS, Inc., USA). Specimens for XRD were placed in Ø 25 mm holders and compacted to produce an even surface. The samples were irradiated with X-rays generated by a CuKα1 tube operated at 40 kV and 40 mA with a wavelength of 1.5406 Å. A motorized primary divergence slit with 0.3° opening was used. A step size of 0.023° with an integration time of 0.68 s was used from 10 to 40° 2θ.

3.5. Morphology analysis

Particle morphology of nanoshell coated indomethacin was investigated by scanning electron microscopy (SEM, Zeiss Merlin, Germany). Powder samples were applied to electrically conductive carbon tabs. No further sample preparation was necessary prior to the analysis. Micrographs were obtained and analyzed visually.

3.6. Nanoshell thickness analysis

The thickness of the applied nanoshells was investigated by means of Transmission Electron Microscopy (TEM) using a Tecnai G2 TF20 UT (FEI, USA) with a field emission gun with a point resolution of 0.19 nm. The TEM specimen was made by embedding the powder sample into copper grid with glue and then ion milling to electron transparency.

3.7. Injectability studies

One milliliter of nanoshell coated indomethacin (400 and 520 mg/mL) suspended in 3% PEG4000, 0.3% polysorbate 20, 0.25% PVPK40 in PBS (440 and 520 mg/mL) was drawn into a 1-mL Plastipak syringe (BD, USA) with 4.7 mm inner diameter equipped with a needle. BD PrecisionGlide Needles (BD, USA) (25 mm length) ranging from 19 to 26 gauge (G) was used. The injection was performed manually at 4–6 mm/s and the force was determined using a FG-6005SD force gauge (Lutron Electronic Enterprices, Taiwan). Ten measurements per needle and suspension concentration were performed.

3.8. Drug load analysis

The indomethacin content of the nanoshell coated particles was determined by first dissolving the nanoshell in 1 M phosphoric acid followed by dilution with acetonitrile:PB (1:1) before filtration (0.2 μm RC, Lab Logistics Group, Germany) into HPLC vials 2 mL (Teknolab, Sweden) and further analyzed with HPLC (n = 2). The method was validated by treating samples of pure indomethacin this way and the recovery was > 98%. The treatment of pure indomethacin with phosphoric acid did not result in any new peeks in the chromatograms, which would have been the case if indomethacin was degraded in the process. Samples were analyzed by the assay for impurities of indomethacin in the monograph for indomethacin in the European Pharmacopeia (see section below).

The reversed-phase gradient HPLC method was based on the method for related substances of indomethacin (*Ph.Eur.* 9.0, monograph 01/2017:0092). The analysis was performed on a Prominence-*i* (Shimadzu, Japan) equipped with a diod array detector (Shimadzu, Japan) set at 320 nm. A 4.6 × 150 mm, 3 μm particles, phenyl-hexyl column (Accucore, Thermo Scientific, USA) was used with a column temperature at 40 °C. The mobile phases were 10 g/L acetic acid and acetonitrile, using the gradient program described in the monograph on *Ph.Eur.* 01/2017:0092. The peak area was used to calculate the concentration, using a six-point calibration curve (0.50–100 μg/mL). Neat uncoated indomethacin was used as standard.

As the method was set-up according to the monograph in the European Pharmacopeia, the method is already validated. A method verification was performed according to the guideline in the United States Pharmacopeia ⟨1226⟩ Verification of compendial procedures. The extended range of the method is validated in each analytical run by the analysis of a 6-point calibration curve (acceptance criteria $r^2 > 0.995$). Calibration curve samples and control samples were dissolved in and diluted with acetonitrile:water (HPLC-grade (1:1)).

3.9. Pre-clinical study

A 12-week single-dose pre-clinical study of uncoated and nanoshell coated indomethacin was performed at Charles River Laboratories, UK. The study consisted of 24 male Sprague Dawley rats with an initial body weight between 250 and 295 g divided into four groups with six rats in each group.

The rats were housed in a controlled environment in accordance with current guidelines regarding care and housing of laboratory animals. Water and food was given *ad libitum*. The samples (uncoated/neat and nanoshell coated indomethacin) were dispersed in 1% Na-CMC and 0.1% polysorbate 20 in PBS by vortexing (Vortex genie 2, Scientific Industries Inc., USA) during 1 min. Drug concentrations of 0.67, 6.7, and 67 mg/mL were administered subcutaneously in the dorsal region in the rats. The 100-fold difference in concentration of the suspensions was selected to achieve constant administration volumes (1.5 mL/kg). The injected doses were 1, 10 and 100 mg/kg.

Blood plasma samples (approx. 0.2 mL) were collected into tubes containing K_2EDTA as an anticoagulant from the jugular vein predose and at the following times post dose administration 0.5, 1, 3, 6, 12, 24, 48, and 72 h, and 1, 2, 4, 6, 8, 10, and 12 weeks (the group with unmodified indomethacin was terminated after 1 week). As soon as practically possible following blood sampling, plasma was separated by centrifugation (1500*g* for 10 min at 4 °C). The blood plasma samples were stored frozen after plasma generation by centrifugation at 1500*g* (Heraeus Megafuge, Thermo Scientific, USA) directly after sampling.

3.10. Blood plasma analysis

The indomethacin content in rat plasma samples was analyzed with UPLC-MS/MS using a Xevo TQS-micro (Waters, USA) equipped with positive electrospray ionization and multiple reaction monitoring. A sample of 20 μL of rat plasma and 40 μL of acetonitrile with internal standard (100 ng/mL indomethacin-d_4 CRS) was vortexed gently and then centrifuged in an Eppendorf centrifuge 5810 (Eppendorf, Germany) for 10 min at 3220*g* to precipitate proteins. Then, 30 μL of the supernatant was diluted with 100 μL water followed by 10 μL injection of this solution was onto the UPLC-MS/MS system. Separation was performed on an Acquity UPLC system (Waters, USA) using a Acquity UPLC-BEH column, 2.1 × 50 mm, 1.7 μm particles (Waters, USA) and a pre-column Acquity UPLC-BEH column, 2.1 × 5 mm, 1.7 μm particles (Waters, USA) maintained at a temperature of 50 °C. The mobile phases were 0.1% formic acid in water and 0.1% formic acid in acetonitrile using a gradient program. A nine-point calibration curve (5.00–50,000 ng/mL) using indomethacin dissolved in DMSO (1 mg/mL) diluted with blank rat plasma was used for quantification. The internal standard was indomethacin-d_4 dissolved in DMSO (1 mg/mL) and diluted to 100 ng/mL in acetonitrile. The indomethacin CRS standard from EDQM was used for calibration samples.

The bioanalytical method was verified when set-up, and the reliability of the generated data is assured by running two sets of calibration

standards in each analytical run. Range and linearity are therefore assessed each time analyzes were performed. The use of an labelled internal standard also enhance the reliability of the results.

3.11. Pharmacokinetic data analysis

Pharmacokinetic data analysis was performed using GraphPad Prism version 7.0d for MacOS (GraphPad Software, USA).

4. Result and discussion

4.1. Particle size

A narrow particle size distribution with a median below 10 μm is suitable for subcutaneously administered suspensions. Larger particles may lead to clogging of the injection needle [15,16]. A maximum size of one third of the injection needle inner diameter is recommended to prevent clogging of the needle during injection, which corresponds to a maximum paticle size below 100 μm when using a 25 Gauge (G) needle [15]. The median particle size of both the micronized uncoated and the nanoshell coated indomethacin particles was 6 μm in diameter. Thus surface modification of the particles did not result in any detectable increase in particle size (Fig. 1). Further, no particles of indomethacin were above 100 μm in diameter.

4.2. Crystallinity characterization

XRD diffractograms of uncoated and nanoshell coated indomethacin demonstrated no significant difference regarding crystal form or crystallinity. The γ form [17] was conserved after the ALD process (Fig. 2), i.e. the ALD process did not induce any noticeable changes in the solid-state structure, which is in line with earlier results on ALD coated pharmaceutical relevant materials [9,10,12]. The applied nanoshells consist of amorphous Al_2O_3, thus not detectable by XRD.

4.3. Morphology

A SEM micrograph of a typical nanoshell coated indomethacin sample is shown in Fig. 3a and a micrograph of uncoated indomathacin is shown in Fig. 3b. The nanoshells produced by the process were essentially crack and pin-hole free and covered the particles completely, which is essential for depot formulations. Nanoshells should not be expected to have any visible effects on morphology of the particles and when comparing with micrographs of uncoated material this is confirmed. The present results confirmed that the ALD process was gentle and did not induce changes of the primary particles of the drug. The micrographs confirmed the particle size distributions observed in Fig. 1 as well as the high crystallinity shown in Fig. 2.

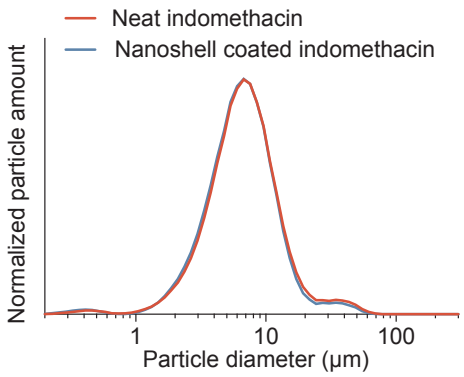

Fig. 1. Size distribution analysis of neat indomethacin and nanoshell coated indomethacin as measured laser light scattering.

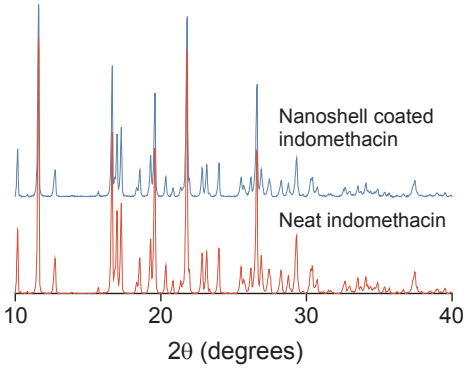

Fig. 2. X-ray diffractogram of nanoshell coated indomethacin and neat indomethacin.

4.4. Nanoshell thickness

The TEM micrographs show a uniform thickness of the nanoshells surrounding the indomethacin particles (a typical and representative TEM image is depicted in Fig. 4 in two magnifications). A homogeneous coverage is necessary to achieve a sustained release profile of indomethacin from the prepared depot formulation. In the higher magnification of the nanoshell, it can be confirmed that the nanoshell thickness was 30–35 nm, as was expected from the selected ALD approach (i.e. 0.25–0.30 nm increase in thickness for each applied ALD cycle). The thickness of the shell is evaluated from several TEM images.

4.5. Injectablity

A formulation that is aimed to be administered subcutaneously needs to be injectable. Injectability is a measure of how easily a drug/formulation is injected into a desired site of action. In the present study, the injectability was estimated by glide force measurements. The required force for a particular formulation depends on properties such as viscosity, needle size, syringe inner diameter, and in case of a suspension, particle size and morphology, as well as concentration of the suspension. The anatomic location (and species) will influence the choice of needle diameter. A typical needle size for subcutaneous injections is 21–27 Gauge (G) [18,19].

As expected, the required glide force increased as a function of increased needle gauge (Fig. 5). Further, the 520 mg/mL suspension required higher glide force than the less concentrated 400 mg/mL suspension (> 22 G). All force glide measurements except for the 520 mg/mL suspension using the 26 G needle was below a threshold for practically perform the injection (the upper limit is usually considered to be 15 N) [20]. Thus, from a technical point of view, it is possible to prepare highly concentrated injectable suspensions, i.e. at least up to 520 mg/mL, of micronized nanoshell coated indomethacin particles when using needles of Gauge size lower than 26.

4.6. Drug load

The drug load of the nanoshell coated indomethacin was 82.4 ± 1.4 w% as determined with HPLC. In general, long acting injectable drug delivery systems, such as liposomes and microspheres, have a drug load < 25 w%. In many cases just 5–10 w%. For instance, Vinarov et al. demonstrated a drug delivery system based on phospholipids and surfactants with, what was considered as high drug load, 21 w% albendazole [21]. Miller et al. confirmed a 19 w% drug load of dexamethasone into polymeric micells [22], while Costa Lima et al. loaded 10 w% bisnaphthalimidopropyldiaaminooctane into PLGA nanoparticles [23].

Preferably, for patient convenience, the entire dose should fit into one single unit or dose, which limits the use to high potency drugs when

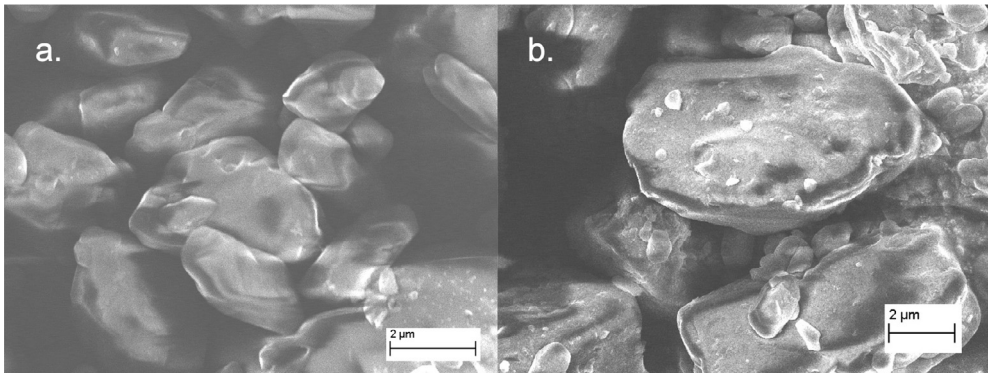

Fig. 3. Micrograph from SEM depicting a. nanoshell coated indomethacin particles and b. uncoated indomethacin particles.

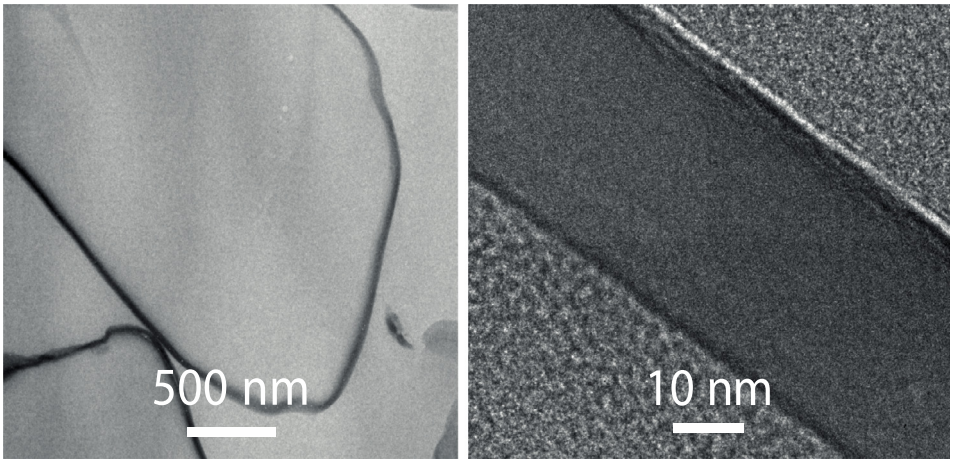

Fig. 4. TEM images of a nanoshell coated indomethacin particle in two magnifications where the left image show the nanoshell distribution surrounding the particle and the right image show the shell in higher magnification.

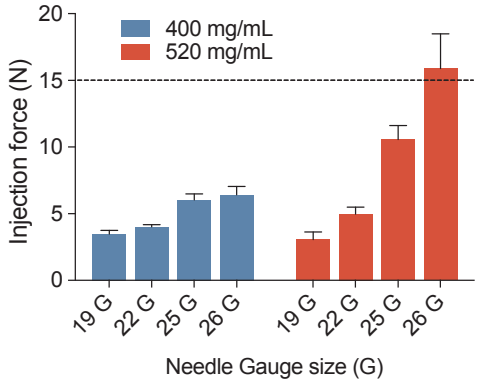

Fig. 5. Figure showing the injectablility of two different formulations of nanoshell coated indomethacin (400 mg/mL and 520 mg/mL) using needles with different gauge sizes (n = 10). Error bars indicate standard error of the mean.

developing long acting injections. The high drug load capacity for nanoshell depot formulations further support the potential for using this technology for controlled drug realease, with longer exposure possibilities for more potent compounds.

In addition to the high drug load, the remaining content of the formulation was measured. No unknown impurities above 0.05% was detected, and the content of the two impurities 4-chloro benzoic acid and 5-methoxy-2-methyl-indol acetic acid were present at 0.1% each. The nanoshell accounted for approximately 17.4 w% of the sample weight, determined by HPLC.

4.7. Indomethacin pharmacokinetics

No animals were terminated prematurely or displayed any negative symptoms in general condition during the study, which demonstrates that the nanoshell coated indomethacin was safe and well tolerated for doses up to 100 mg/kg. As a reference, the LD50 of indomethacin is 13 mg/kg for rats [24]. Thus, the use of nanoshells as drug delivery system in long acting depots enables administration of high doses of an API that is toxic in its neat form, a feature that can be enabling on drugs that otherwise would not be possible to use.

No local inflammation was observed at the site of administration 48 h after dosing indicating that the nanoshells did not produce an inflammatory response in rats at the present administration site.

The plasma concentration-time curves for indomethacin following subcutaneous administration are displayed in Fig. 6 and the pharmacokinetic (PK) parameters (maximum indomethacin plasma concentration (C_{max}), area under the curve for 0–72 h ($AUC_{0\text{-}72h}$), area under the curve for 0–12 weeks ($AUC_{0\text{-}12\ weeks}$), and steady-state indomethacin plasma concentration ($C_{steady\text{-}state}$)) are shown in Table 1. The two highest doses of nanoshell coated indomethacin (10 and 100 mg/kg) kept releasing indomethacin throughout the whole 12 week study, while the lower dose (1 mg/kg) went below the lower limit of quantification (LLOQ), that is, 0.5 ng/mL, in plasma levels after 10 weeks. As expected, the plasma levels of indomethacin in the rats administered with neat indomethacin decreased much faster. It should also be noted that a neat indomethacin suspension has a half-time of 4.5 h when administered subcutaneously [13], which means that the drug also is protected from degradation by the nanoshells.

For the nanoshell coated indomethacin, a steady state drug release was reached after approximately one week (Table 1). The achieved

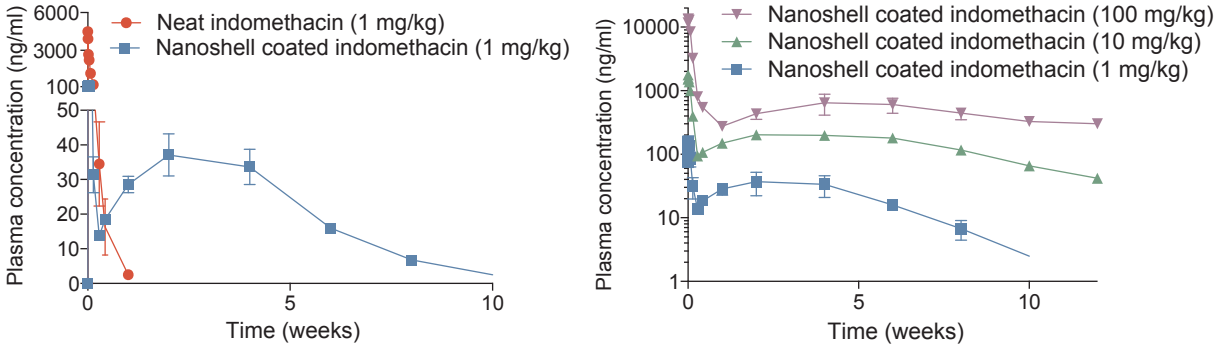

Fig. 6. Plasma concentration-time curves for indomethacin following subcutaneous administration, as measured by HPLC-MS/MS on blood plasma from test animals at different times. Error bars indicate standard error of mean, n = 6.

Table 1
Pharmacokinetic (PK) parameters for indomethacin following subcutaneous injection.

PK parameters	Neat indomethacin (1 mg/kg)	Nanoshell coated indomethacin (1 mg/kg)	Nanoshell coated indomethacin (10 mg/kg)	Nanoshell coated indomethacin (100 mg/kg)
C_{max} (ng/mL)	4515 ± 474	157 ± 40	1780 ± 190	14,200 ± 2500
$AUC_{0\text{-}72h}$ (h ng/mL)	40,400 ± 4352	3060 ± 245	32,700 ± 2390	276,000 ± 24,000
$AUC_{0\text{-}12\ weeks}$ (weeks ng/mL)	245 ± 26	219 ± 25	1810 ± 150	7010 ± 1080
$C_{steady\text{-}state}$ (ng/mL)	N.A.	28.8 ± 12.6	169 ± 63	433 ± 304

plasma levels were well in range for many drug substances, including indomethacin where 150–600 ng/mL has been reported as effective drug levels [25,26]. The indomethacin plasma levels started to decrease after approximately four weeks (1 mg/kg) and eight weeks (10 mg/kg), respectively, while no obvious decrease in plasma levels was observed for the highest dose (100 mg/kg). The duration of the depot was 10 weeks for the 1 mg/kg dose. At the end of the study, the 10 and the 100 mg/kg doses had not stopped releasing indomethacin. By assuming a steady-state release for the full length of the continuation of the depot for the 10 and 100 mg/kg, the total length of these doses can be estimated to 16 and 52 weeks, respectively.

The release of API from the nanoshells are regulated by dissolution of the aluminium oxide nanoshells. The reason why there is a difference in depot length and not a linear correlation between administered dose and steady state plasma levels, is likely a buildup of a local saturation of aluminium ions at the site of administration due to a higher concentration of nanoshell coated indomethacin for the higher doses. Furthermore, for the lower dose, it is reasonable to expect that the particles spread out more in the tissue than the higher doses did, which leads to a larger surface available for dissolution of the nanoshells.

In summary, the plasma concentration-time profile of nanoshell coated indomethacin demonstrated sustained release over 12 weeks when administered subcutaneously at 10 or 100 mg/kg. This is the first study to demonstrate the concept of nanoshells as formulation strategy for long-term sustained release.

5. Conclusions

This study was the first to demonstrate that inorganic nanoshells can be used to achieve sustained release of a model drug for more than 12 weeks, which should be compared with the fast elimination of neat indomethacin. The studied formulation consisted essentially of primary particles of micronized indomethacin coated with pin-hole free aluminium oxide nanoshells. The drug load was higher than 80%. Thus, it can be concluded that the drug delivery system based on nanoshell coatings, using atomic layer deposition, on micronized API particles show potential for long acting depot treatments.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2019.04.019.

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