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Jet milling—a new technique for microparticle preparation

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Abstract

An innovative technique for solvent free preparation of microparticles is described. Microparticles were prepared by a melt grinding technique which consists of three consecutive steps of melting in case of placebo microparticles or co-melting of polymer and drug in case of drug loaded microparticles, respectively, and pregrinding. In a final jet milling step the reduction of the particle size and smoothening of the microparticle surface occurred. Different polymers of PLA and PLGA type were utilised. The influence of the preparation parameters during the process were investigated according to microparticle properties like particle size distribution, habitus or surface morphology by executing a $2^{(5-2)}$ factorial design. The minimum mean particle size distribution (x_{50} value) reached 4–6 μm. Scanning electron microscopy revealed that non-porous microparticles with a smooth surface were prepared. The release pattern of estrioltriacetate loaded microparticles of Resomer® R 202H nearly followed a zero order release kinetic over a period of 21 days without an initial burst effect. The preparation process can be carried out in a reproducible manner. The results demonstrate that microparticle preparation is possible by the following unique melt grinding technique without using organic solvents.

Keywords: Microparticles; PLA; PLGA; Zero order release kinetic; Initial burst; Solvent free preparation; Factorial design; Estrioltriacetate

1. Introduction

During the past two decades, interest in developing microparticles with the ability of a continuous drug release over a long period of time as modern parenteral drug delivery systems has obviously grown. Considerable interest has been focused on the use of biodegradable polymers for specialised applications such as controlled release of drug formulations. Meanwhile, microsphere drug-delivery systems using various kinds of biodegradable polymers have been extensively studied (Franssen and Hennink, 1998). Polylactide (PLA) and poly (lactide-co-glycolide) (PLGA) polymers have been proven to be excellent drug carriers for microparticulate systems due to their advantages, e.g. biocompatibility and regulatory approval. Applicable formulations for subcutaneous and intramuscular application are widely known so far (Del Pozo et al., 1987). So far a great variety of processes to prepare biodegradable polymeric microparticles is well

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known. The most popular techniques are the double emulsion methods, solvent evaporation, organic phase separation (coacervation), spray drying or also the ASES process (Müller and Fischer, 1989; Lin and Yu, 2001). Of course, all of them have their specific advantages and disadvantages. But all techniques suffer one common drawback: The need of organic solvents in at least one of the production steps (Ghaderi et al., 1999; Johansen et al., 1999; Kim and Park, 1999; Major et al., 1999; Sendil et al., 1999). This fact leads to toxicity problems with the residual organic solvents, such as methylene chloride, chloroform, acetonitrile and tetrahydrofuran or methanol as co-solvent after the preparation (Bodmeier and Mc Ginity, 1987; Birnbaum et al., 2000). The residual content in the microspheres after preparation has to be removed in time consuming drying steps. Very often drying processes have to be realised at low temperatures due to low glass transition temperatures of the applied polymers. So drying times of up to several weeks appear. Furthermore the capacity of active agents in microparticles is strictly limited due to the fact that drugs have to be deposited in or on the microparticles out of a solution. A new process to prepare microparticles without the use of organic solvents including a higher portion of drug loading had to be developed in order to circumvent all mentioned drawbacks of other techniques. The aim of this study was to investigate the suitability of a new melt grinding technique to prepare microspheres. Most impressive advantages are the prevention from toxicity due to the absence of organic solvents during the process and the short preparation time with no drying step necessary (Wichert and Rohdewald, 1990). The solvent free melt grinding technique has been shown to be a suitable preparation method for microspheres without a drying step and a very high limit of loading with active agents (Carstensen and Müller, 2000). In this paper the new preparation process shall be presented.

2. Experimental section

2.1. Materials

2.1.1. Polymers

The used polymers Resomer® RG 502H (PLGA, hydrophilised), Resomer® R 202H (PLA, hydrophilised) or Resomer® R 202 (PLA) (see Table 1) were obtained from Boehringer Ingelheim (Ingelheim, Germany).

2.1.2. Drugs

A laboratory batch of micronised estrioltriacetate was used as model drug in case of drug loaded microparticles. The raw substance was purchased from Jenapharm (Jena, Germany, Lot-No. 3005K1).

2.1.3. Release medium

The composition of the release medium for investigation of the release pattern of estrioltriacetate loaded microparticles was Pluronic® F 68 0,05g (ICI, Cleveland, GB), sodiumazide 0.04g (Merck KG, Darmstadt, Germany), HP-β-cyclodextrine 5 g (Encapsin®, Fa. Janssen, Olen, Belgium) and Sörensen buffer solution pH 7.4 ad 100 ml.

The prepared buffer was sterilised at 121 °C, 2 bar for 15 min.

Table 1
Physicochemical properties of polymers used (Resomer®, Boehringer Ingelheim, Ingelheim, Germany)

Name	Type	M_W	M_n	i.v. (dl/g)	T_G (°C)
Resomer® R 202H	Poly-D,L-lactide (hydrophilised)	15 000	10 000	0.2	41.6
Resomer® RG 502H	Poly-D,L-lactide:glycolide 50:50	13 000	7000	0.2	33.4
Resomer® R 202	Poly-D,L-lactide	17 000	8000	0.2	40.6

2.2. Methods

2.2.1. Microparticle preparation

Placebo and drug loaded microspheres were prepared by using a unique melt grinding technique. This technique is a combination of three consecutive steps, namely: melting of the polymer (if desired drug dispersing or solvation)—step 1, rotor speed milling of the congealed melt—step2, and jet milling for microparticle comminution and surface smoothening—step 3.

2.2.1.1. Step 1—melting. Melting of polymer and dissolving or dispersing of drug substance in case of drug loaded microparticles was done in a beaker on a heat plate. The required temperature was dependent on the physicochemical behaviour of polymer and drug used. It was adjusted slightly higher than the melting point or the glass transition temperature of the chosen polymer. However, a low viscous solution or suspension had to be obtained. The melt was transferred into another tumbler to congeal. Several melting cycles were necessary to obtain a homogenous solution or suspension.

2.2.1.2. Step2—pregrinding. After congealing of the melt a pregrinding step using a rotor speed mill was done in a FRITSCH Pulverisette 14 (Fritsch, Idar Oberstein, Germany) at 18 000 rpm. In a second step a breaker plate (500 μm) took place around the rotor.

2.2.1.3. Step 3—jet milling. The microparticle forming step was done in a Jetmill Jet-O-Mizer 000 (Fluid Energy Aljet, Plumsteadville, US; see Fig. 1). The nitrogen for milling purpose was of quality 5.0. Jet milling conditions were investigated in a factorial design. Exact conditions of jet milling for microparticle production will be defined in Table 2.

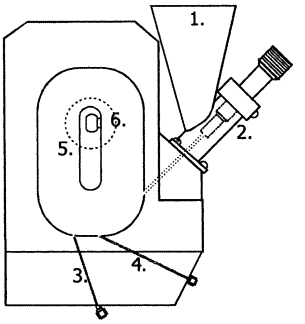
The microparticles were characterised according to particle size distribution, surface morphology, drug release and thermal properties.

2.2.2. Step c—jet milling: investigation of production parameters by a $2^{(5-2)}$ factorial design

A $2^{(5-2)}$ factorial design was performed to investigate important influence factors during preparation (see Table 2) (Sansdrap and Moes, 1993). Placebo microspheres of Resomer® RG 502H were prepared. This kind of statistical design was chosen because of its advantage in getting first impressions of many influence factors in a technique by running only few trials. The reduced factorial design was the first choice because influence strength of nearly all adjustable parameters were completely unknown before. The investigated production parameters were fixed and varied within determined ranges:

(a) number of grinding cycles in the rotor speed mill from one to four times, number of grinding cycles in the jet mill from one to three times,
(b) pressure at the pushing inlet (see Fig. 1) from 4 to 9 bar,
(c) pressure at the tangential grinding inlet A (see Fig. 1) from 3 to 8 bar,
(d) pressure at the tangential grinding inlet B (see Fig. 1) from 3 to 8 bar, and
(e) milling cycles in the rotor speed mill, one or two times.

Generally the pressure at the pushing inlet was adjusted to a higher level to avoid grist leaving the milling chamber through the feed hopper.



1. feed hopper
2. pushing inlet
3. grinding inlet B
4. grinding inlet A
5. milling chamber
6. separator

Fig. 1. Drawing of the jetmill Jet-O-Mizer 000 (Fluid Energy Aljet, Plumsteadville, USA).

2.2.3. Particle size

The Particle size distribution was determined by laser diffraction in a HELOS laser diffractometer equipped with the RODOS Module, a dry measurement module, (Sympatec, Clausthal-Zellerfeld, Germany). A Fourier lens with a focus of 50 mm was installed.

2.2.4. Scanning electron microscopy

Microparticle shape and surface morphology were analysed by scanning electron microscopy (SEM) using a Philips XL 20 scanning electron microscope (Philips, Kassel, Germany). The samples were fixed on a carbon fibre film and sputtered with gold in an argon atmosphere at a sputter current of 50 mA for 180 s using a SCD 005 Sputter coater (BalTec, Balzers, Liechtenstein). High tension in the SEM was set at 10–15 kV, to avoid softening of microparticles.

2.2.5. Drug release

Drug release behaviour of estrioltriacetate loaded microspheres (5% [w/w]) was investigated using a flow through cell apparatus (Ph. Eur. Nr X, SOTAX Dissotest CE 70, SOTAX, Allschwil, Switzerland) combined with reversed phase HPLC analysis. 50 mg of particles were transferred into implantate cells and fixed with a glass fibre filter (Whatman, Maidstone, GB). The release medium was pumped by a calibrated tube pump type Watson Marlow 205 C (Watson Marlow Bredel, Rommerskirchen, Germany). The temperature of the release medium was adjusted to 37 °C with a flow rate of 100 ml per 24 h. 0.5 ml of released buffer was ejected at each sampling occasion. This volume was replaced by fresh medium. Released drug content was investigated by reversed phase HPLC with a HPLC System consisting of a HPLC Detector 430, a HPLC Autosampler 465, a column Thermostat Jetstream 2 plus (all components by Kontron Instruments, Watford, UK), and an Applied Biosystems Solvent Delivery System 400 (Applied Biosystems, Foster City, USA). As stationary phase a Merck LiChroCart 4.8 × 125 mm column, filled with LiChrospher 100 RP18, 5 μm (both Merck KG, Darmstadt, Germany) was used. The mobile phase was composed of 20% acetate buffer (consisting of sodiumacetate, 8.11 g, 1N HCL Titrisol®, 93 ml, aqua. bidest. Ad 1000 ml) and 80% methanol (pH 3 (HOAc)). All substances were obtained from Merck KG, Darmstadt, Germany. The flow rate was fixed at 1.5 ml, detection wavelength was set to 215 nm.

2.2.6. Thermal analysis

Thermal analysis was done by differential scanning calorimetry (DSC 7, Perkin–Elmer, Norwalk, US) and thermogravimetry (TGA 7, Perkin–Elmer, Norwalk, US). Differential scanning calorimetry was done by sealing 2 mg of microparticles or raw polymer into open pans. The samples were measured with a heating rate of 10 K/min followed by a fast cooling ramp at 40 K/min and a second heating ramp at 10 K/min related to an empty reference pan.

3. Results and discussion

The $2^{(5-2)}$ factorial design yielded the following results: The reduced design revealed that the num-

Table 2
Composition of the $2^{(5-2)}$ factorial design plan (10 batches to prepare, 10 runs resp.)

Run	Pressure pushing nozzle (bar)	Pressure grinding nozzle A (bar)	Pressure grinding nozzle B (bar)	Milling cycles (jetmill)	Milling cycles rotor speed mill
1	9	8	8	3	4
2	9	8	2	3	1
3	9	2	8	2	4
4	9	2	2	2	1
5	4	8	8	2	1
6	4	8	2	2	4
7	4	2	8	3	1
8	4	2	2	3	4
9 (C)	6	5	5	2	2
10 (C)	6	5	5	2	2

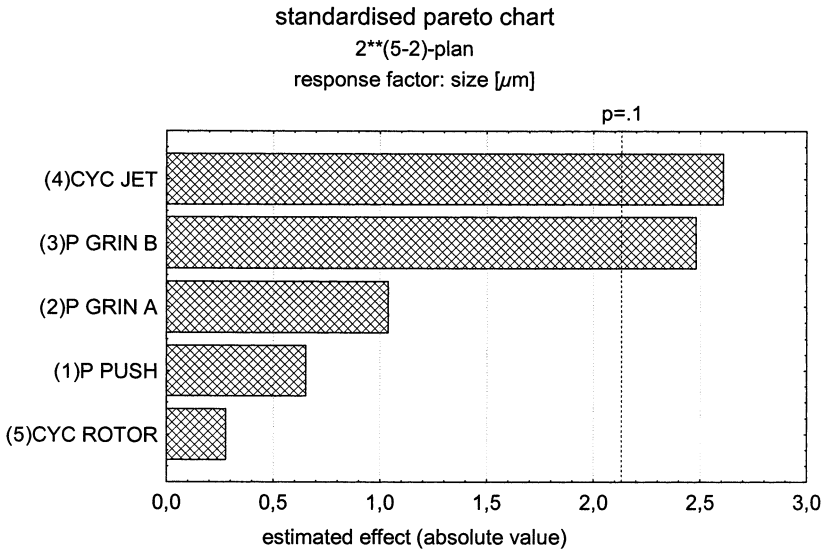


Fig. 2. Pareto chart for the response factor size (cyc jm = milling cycles jetmill; p grind B = pressure at grinding nozzle B (bar); p grind A = pressure at grinding nozzle A (bar); p push = pressure at the pushing nozzle (bar); cyc rsm = milling cycles rotor speed mill).

ber of milling cycles in the final production step (jet milling) and the nitrogen pressure at the tangential grinding inlet B strongest influence particle size distribution, mean particle size ($P < 0.1$) and surface morphology. Other factors investigated within the $2^{(5-2)}$ factorial design, namely the pressure at the pushing inlet, at the tangential grinding inlet A and pre-grinding in the rotor speed mill, did not show strong influence ($P > 0.1$) on mean particle size, particle size distribution (see Fig. 2) and surface morphology.

Fig. 3 shows the influence of the number of grinding cycles versus nitrogen pressure at the tangential grinding inlet B on particle size. Three grinding cycles in combination with a nitrogen pressure of 5–7 bar at the tangential inlets showed the best results according to particle size distribution ($x_{90} < 13.78$ μm, see Fig. 4), whereas the smoothest surface resulted after three milling cycles in the jet mill at higher pressures of 9 bar at the delivery inlet and 8 bar at both tangential grinding inlets (see Fig. 5). These results are due to pressure at the pushing nozzle serves the purpose that grist cannot leave the milling chamber through the feed hopper. Rotor speed milling fulfils a preliminary function for the final microparticle building step, namely jet milling, by providing particles which are small enough to pass the feed hopper into the milling chamber of the jet mill. Both parameters nearly do not touch the particle comminution and surface smoothing process itself. Whereas the pressure at the grinding inlet B and the milling cycles in the jetmill

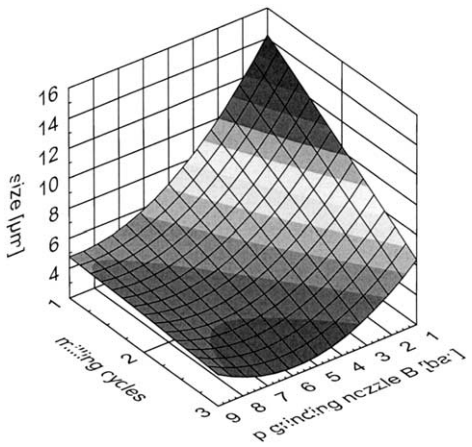


Fig. 3. Dependence of number of grinding cycles in the jetmill and the pressure of milling gas at the tangential inlet B on microparticle size.

Fig. 4. SEM of placebo microparticles made of Resomer® RG 502H after preparation as described in Table 2 run 7.

interfere with the grinding and smoothening process. A larger number of grinding cycles gives more opportunity to the microparticles to collide with each other or with the surface of the milling chamber and to abrase the surface, which is a requirement for a small particle size, a narrow particle size distribution and a smooth surface. The small influence of the pressure at the grinding nozzle A is due to the acute tangential angle of the inlet in relation to the walls of the milling chamber. Its nitrogen flow provides a high speed of airflow only, but it does not make the particles collide with

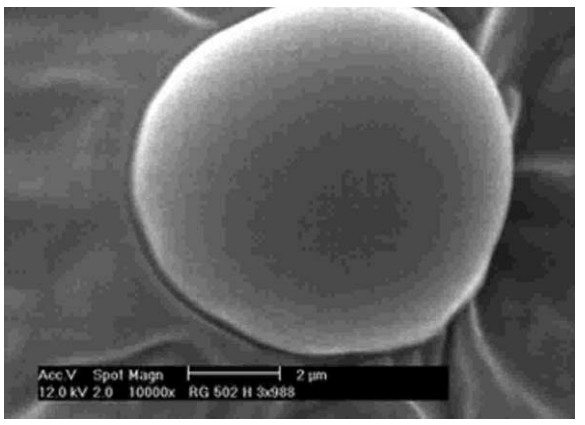


Fig. 5. SEM of a placebo microparticle made of Resomer® RG 502H after preparation as described in Table 2 run 1.

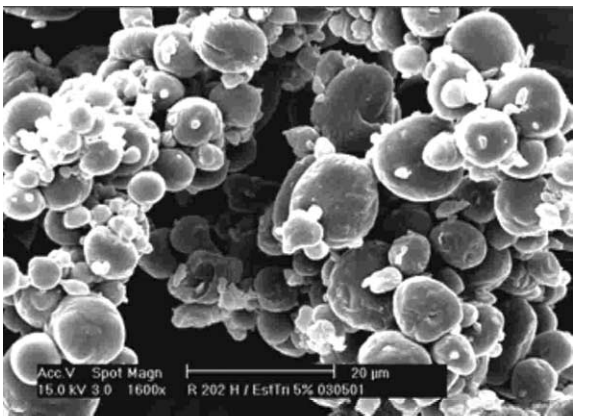


Fig. 6. SEM of estrioltriacetate loaded microparticles made of Resomer® R 202H (5% w/w).

each other. Whereas grinding inlet B comes into the chamber in a more obtuse angle which provides a high speed of the nitrogen but also forces particles to collide with each other by redirecting the particles. As the function principle of this type of mill is grinding by cannon forces, the result is quite easy to explain (Rumpf, 1959, 1960a,b).

For further investigations, Resomer® R 202H microspheres loaded with 5% [w/w] estrioltriacetate were prepared as described above. The microspheres showed a small particle size distribution ($x_{90} < 13.36$ µm) and a smooth surface (see Fig. 6). The release pattern of the drug nearly followed a zero order kinetic behaviour for a period of 21 days (see Fig. 7) without an initial burst effect.

This fact could be due to changes in thermal properties of superficial polymer of the microparticles. To investigate this phenomenon the thermal properties of Resomer® R 202 during the jet milling process were studied. A batch of Resomer® R 202 was melted, congealed and preground. Then jet milling with nitrogen pressures of 9 bar at the pushing nozzle and 8 bar at both grinding inlets was repeated five times. After each milling step a sample was taken and analysed according to particle size distribution and thermal properties by differential scanning calorimetry. Thermogravimetrics was done to assure that effects, which are seen in DSC curves are not due to evaporating residual water in the microparticles after the preparation procedure.

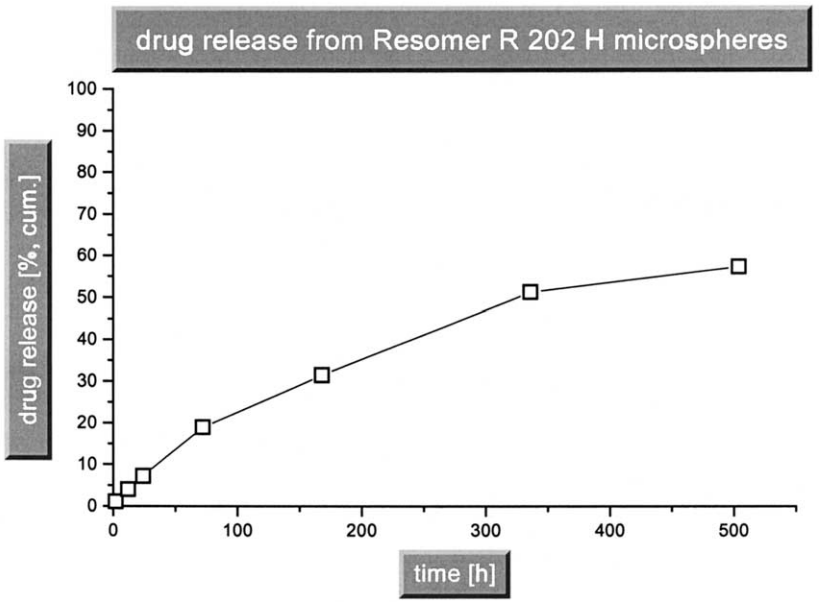


Fig. 7. Estrioltriacetate release from Resomer R 202H microspheres (drug loading 5% [w/w]).

Fig. 8 shows the influence of the number of grinding cycles on enthalpy relaxation. It was demonstrated that the heat capacity (Delta H) decreases to about 50% of the origin value in dependence of the number of jet milling cycles carried out. This behaviour is supposed to be

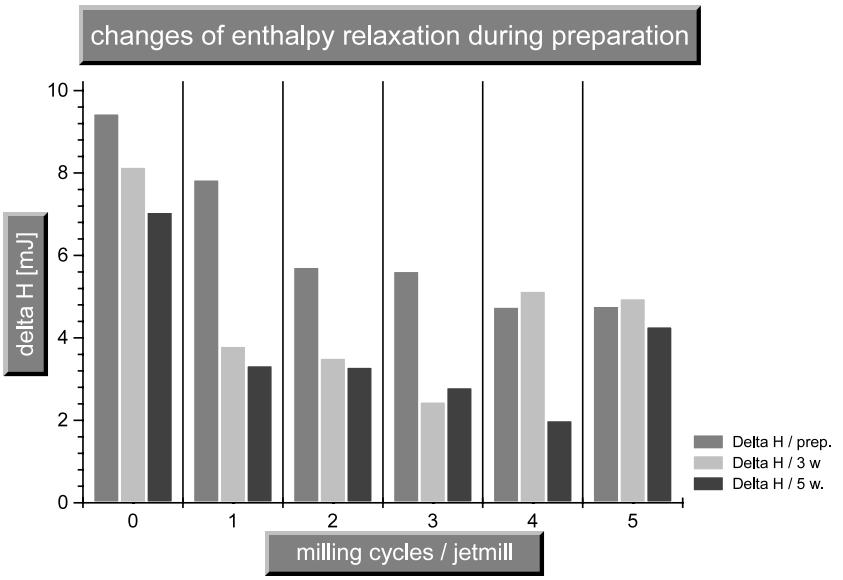


Fig. 8. Changes of enthalpy relaxation [delta H] (first heat curve, 10 K/min) in dependence of milling cycles and storage time. (Delta H/prep = enthalpy relaxation directly after preparation; Delta H/3 w = enthalpy relaxation after 3 weeks of storage; Delta H/5 w = enthalpy relaxation after 5 weeks of storage).

related to the release pattern. Mechanical stress during jet milling could provide an opportunity to polymers to arrange in a more stable conformation mainly in superficial areas of microspheres. This could be the reason for no burst release at all. Maybe sintering of superficial areas occurred.

4. Conclusion

The results of the experiments revealed that the new melt grinding technique is a valid method for preparation of microspheres. The most important advantages are the absence of organic solvents as well as time and energy consuming drying steps. The method also provides the possibility of a very high drug load by solving it molecular disperse or simply dispersing in the polymer melt. The release pattern of the microparticles showed a zero order release kinetic behaviour. Properties of the resulting microparticles regarding particle size, surface morphology and release pattern will be controllable over a wide range by careful choice of polymer and co-grinding with modifiers. Thermal analysis showed that enthalpy relaxation is influenced by mechanical stress during jet milling. It can be presumed that this behaviour relates to the release pattern of manufactured microspheres loaded with estrioltriacetate by superficial sintering of the polymer and stabilisation of polymer chain conformation.

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