European Journal of Pharmaceutics and Biopharmaceutics 204 (2024) 114515

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Control of encapsulation efficiency and morphology of poly (lactide-co-glycolide) microparticles with a diafiltration-driven solvent extraction process

Florian Kias, Roland Bodmeier *

College of Pharmacy, Freie Universität Berlin, Kelchstr. 31, 12169 Berlin, Germany

ARTICLE INFO

Keywords:
Biodegradable drug delivery systems
Microparticles
Poly(lactide-co-glycolide)
Solvent extraction/evaporation method
Solvent removal
Diafiltration
Encapsulation efficiency

ABSTRACT

The removal of organic solvents during the preparation of biodegradable poly(D,L-lactide-co-glycolide) (PLGA) microparticles by an O/W- solvent extraction/evaporation process was investigated and controlled by diafiltration. Emulsification and steady replacement of the aqueous phase were performed in parallel in a single-vessel setup. During the process, the solidification of the dispersed phase (drug:PLGA:solvent droplets) into microparticles was monitored with video-microscopy and focused beam reflectance measurement (FBRM) and the residual solvent content was analyzed with headspace gas chromatography (organic solvent) and coulometric Karl-Fischer titration (water). Microparticles containing dexamethasone or risperidone were characterized with regard to particle size, morphology, encapsulation efficiency and in-vitro release. Diafiltration-accelerated solvent extraction shortened the process time by accelerating solidification of dispersed phase but reduced the residual dichloromethane content only in combination with increased temperature. Increasing the diafiltration rate increased particle size, porosity, and the encapsulation efficiency of risperidone. The latter effect was particularly evident with increasing lipophilicity of PLGA. A slower and more uniform solidification of end-capped and increased lactide content PLGA grade was identified as the reason for an increased drug leaching. Accelerated solvent extraction by diafiltration did not affect the in-vitro release of risperidone from different PLGA grades. The initial burst release of dexamethasone was increased by diafiltration when encapsulated in concentrations above the percolation threshold. Both porosity and burst release could be reduced by increasing the process temperature during diafiltration. Residual water content was established as an indicator for porosity and correlated with the burst release of dexamethasone.

1. Introduction

Biodegradable PLGA microparticles are prepared by manufacturing processes such as solvent extraction/evaporation, organic phase separation or spray drying [1,2]. In these methods, organic solvents (e.g., dichloromethane (DCM), chloroform, or ethyl acetate) are used to dissolve the PLGA polymer. These solvents must be removed during the manufacturing process to a concentration in the final product that ensures that the permitted daily exposure for the patient recommended by the ICH is not reached (e.g., 6 mg/d DCM) [3–5]. The solvent removal rate can be decisive for the morphology of the solidified microparticles and thus the release of encapsulated drug [6–8]. In the conventional solvent extraction/evaporation process, after emulsification, a fast diffusion-driven extraction is caused by the organic solvent concentration gradient between the inner drug:PLGA:solvent phase (O) and the external aqueous phase (W) [9,10]. The proportion of organic solvent extracted depends on both its solubility in the aqueous phase and the O/W phase ratio [6,11,12]. Thus, different organic PLGA solvents will show different extraction rates, for example, the water solubility of ethyl acetate is approximately four times higher than the solubility of dichloromethane. This effect can be compensated by pre-saturating the aqueous phase with organic solvent [13,14]. Once the aqueous phase is saturated, solvent evaporation and not diffusion determines the extraction rate [10,14]. This process depends primarily on the volatility of the organic solvent, temperature, ambient pressure, volume to surface ratio of the aqueous phase, and removal of the evaporated organic solvent [9,11,15,16].

To avoid the control of extraction rate by evaporation, the aqueous

* Corresponding author.
E-mail address: bodmeier@zedat.fu-berlin.de (R. Bodmeier).

https://doi.org/10.1016/j.ejpb.2024.114515
Received 10 May 2024; Received in revised form 18 September 2024; Accepted 23 September 2024
Available online 24 September 2024

phase can be diluted gradually or continuously [6,17,18]. Slow extraction of organic solvent facilitates the formation of small microparticles, which tend to have pores and a low encapsulation efficiency due to water influx [6–8,19]. A rapid extraction, by increasing the aqueous phase volume initially or by fast dilution, facilitates fast solidification of the polymer. This can increase encapsulation efficiency [20], but also particle size, pore size, burst release and residual solvent content [21,22]. Moderate continuous extraction may allow particle shrinkage, thereby reducing porosity and burst release of drugs [7,8].

The use of membrane separation techniques, in particular tangential flow filtration, enables controlled and sometimes selective removal of organic solvents, stabilizers and unencapsulated drugs [23–25]. Diafiltration, i.e. the simultaneous removal of aqueous phase and addition of the same amount of volume of fresh extractant, makes it possible to keep the vessel volume small and reduce the total extraction volume used [26,27]. Regardless of whether components or the complete aqueous phase are removed, the dispersed phase must be retained by membranes, filters, or sieves. To reduce the risk of blockage, diafiltration is usually performed in a separate vessel after the emulsion has formed and parts of dichloromethane have already been extracted.

In this study diafiltration-assisted microparticle preparation in a single-vessel design was investigated. Solvent removal, particle solidification, the effect on encapsulation and in-vitro release of two different drugs with different solubility in the organic phase (risperidone and dexamethasone) were examined.

2. Materials and methods

2.1. Materials

Micronized dexamethasone (Caesar & Loretz GmbH, Hilden, Germany); risperidone (RPG Life Sciences limited, Navi Mumbai, India); poly(lactide-co-glycolide) (PLGA) (Resomer® RG 503H; Resomer® RG 753S, Evonik Industries AG, Darmstadt, Germany); acetonitrile (HPLC grade) (VWR International GmbH, Darmstadt, Germany); dichloromethane (DCM) (HPLC grade), dimethyl sulfoxide (DMSO) (headspace grade) (Carl Roth GmbH + Co. KG, Karlsruhe, Germany); polyvinyl alcohol 4–88 (PVA) (Merck KGaA, Darmstadt, Germany).

2.2. Methods

2.2.1. Preparation microparticles

Microparticles were prepared by a modified solvent extraction/evaporation method in two different batch sizes. The organic phase was prepared by dispersing or dissolving 0–40 % (w/w) dexamethasone or risperidone (based on polymer weight) in a 10 % (w/w) PLGA 503H (dexamethasone and risperidone) or PLGA 753S (risperidone) in dichloromethane solution (VF2, IKA-Werke GmbH & Co. KG, Staufen im Breisgau, Germany). 2.5 or 10.0 mL of the organic phase was emulsified into 50 or 200 ml 0.25 % (w/v) PVA solution using a propeller stirrer (d = 3.5 cm, 800 rpm or d = 5.0 cm, 500 rpm). The 50 mL batches were prepared in a 50 mL stirred cell (Millipore Corporation, Bedford, USA) equipped with a 10 μm stainless-steel sieve at the bottom to retain the dispersed phase. The 200 mL batches were prepared in a filter funnel with an integrated sintered glass filter with 10–16 μm pore size (Vitra-POR Filter-Funnel 250 ml Por. 4, ROBU Glasfilter-Geräte GmbH, Hattert, Germany), equipped with two probes for in-process video microscopy (PVM) and focused beam reflectance measurement (FBRM) (PVM V819 and Lasentec FBRM D600T, Mettler Toledo AutoChem Inc., Redmond, USA) (Fig. 1). To control solvent extraction by diafiltration, the aqueous phase was continuously exchanged for 60 min, by removing it through a bottom outlet and replacing the same amount of volume with fresh PVA solution from above using a double-head peristaltic pump (323S, Watson-Marlow Limited, Falmouth, England). If a one-step dilution was carried out instead of continuous diafiltration, the entire batch was transferred into a ten times larger volume of aqueous phase 15 min after the start of emulsification and stirred with a magnetic stirrer. The microparticles were separated 24 h after emulsification by vacuum filtration using a 10 μm stainless-steel sieve, washed three times with 250 mL deionized water and dried under vacuum at 35 °C for 72 h. Dried microparticles were stored in a desiccator at 7 °C.

2.2.2. Solvent content

The water content of microparticles was determined according to the method for coulometric Karl-Fischer determination (Ph. Eur. 2.5.32). 10–20 mg microparticles were accurately weighed and dissolved in 1.0 mL acetonitrile. About 250 mg of this solution, accurately weighed, were analyzed in triplicate for the water content with a coulometric Karl Fisher titrator (831 KF Coulometer, Metrohm AG, Herisau, Switzerland) using HYDRANDAL Coulomat AD (Honeywell Specialty Chemicals Seelze GmbH, Seelze, Germany). The water content based on total weight was calculated after correction for the water content of a blank.

Dichloromethane content of microparticles or aqueous phase was quantified with headspace gas chromatography (GC-2014, Shimadzu Corp., Kyoto, Japan) with a method adapted from USP monograph for residual solvents using a capillary column equivalent to USP G43 phase (Rtx-1301, Restek Corp., Bellefonte, USA). 5.0 mL aqueous phase or 10.0–50.0 Mg microparticles, dissolved in 5.0 mL dimethyl sulfoxide, were sealed in a 20 mL headspace GC vial with an aluminum screw cap with PTFE septum. Samples were equilibrated automatically under shaking by an autosampler (AOC-6000, Shimadzu Corp., Kyoto, Japan) for 60 min at 80 °C (water) or 45 min at 105 °C (DMSO). 1.0 mL of the gas phase was sampled automatically, with a needle temperature 5 °C above previous equilibration temperature and injected at 140 °C with a

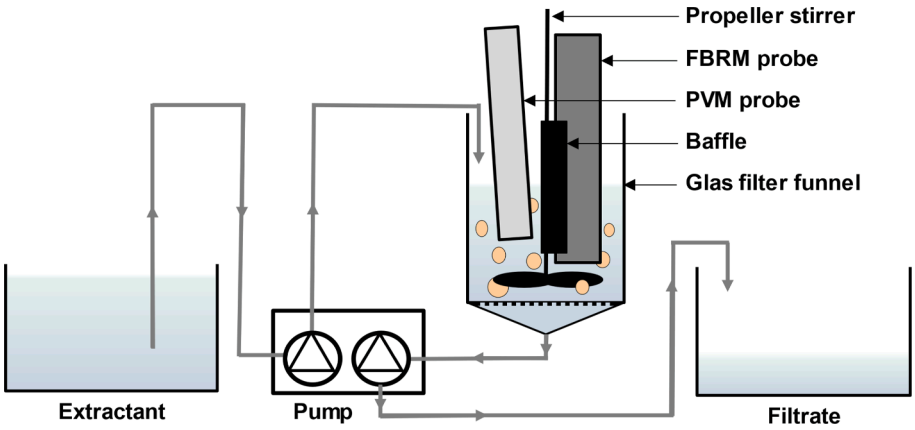

Fig. 1. Schematic setup of diafiltration-controlled extraction for microparticle preparation, equipped with probes for video microscopy (PVM) and focused beam reflectance measurement (FBRM).

subsequent split ratio of 5. The column oven temperature was maintained at 80 °C (water) or 40 °C (DMSO) and increased after 7 min to 160 °C (water) or 120 °C (DMSO) with a heating rate of 30 K/min. The carrier gas was nitrogen. Samples were detected with a flame ionization detector (FID) set to 250.0 °C. Evaluation of the spectra was performed with LabSolutions 5.98 (Shimadzu Corp., Kyoto, Japan). The dichloromethane content in the samples was calculated from peak area using a linear calibration curve obtained by dilution series. For freshly separated microparticles, the water content was deducted from the sample weight to calculate their dichloromethane content

The stirred emulsification vessel was positioned on a balance to determine the distribution of dichloromethane in an evaporation-controlled microparticle preparation process. The mass of evaporated dichloromethane was determined by the mass loss of the total batch minus the evaporation rate of water (obtained from the mass loss of a stirred pure aqueous phase). Saturation of the aqueous phase with dichloromethane was assumed to occur within seconds [10] and to be constant until solidification of the droplets. This was monitored by sampling and subsequent GC analysis of the aqueous phase. Based on this, the dichloromethane removal rate from the dispersed phase was equated with the calculated dichloromethane evaporation rate for the duration of saturation of the aqueous phase.

2.2.3. *In-process monitoring using focused beam reflectance measurement and video microscopy*

The probes for focused beam reflectance measurement (FBRM) and video microscopy (PVM) (Lasentec FBRM D600T and PVM V819, Mettler Toledo AutoChem Inc., Redmond, USA) were positioned in the emulsification vessel (Fig. 1) with the FBRM fixed beaker stand and additional clamps for the PVM. The FBRM online measurements were performed every 6 s, with a scan speed of 2 m/s and the coarse electronic discrimination setting. The data were processed using the iC FBRM 4.0 and iC PVM 7.0 software (Mettler Toledo AutoChem Inc., Redmond, USA). The resulting chord lengths from the FBRM were expressed as square weighted median to obtain the best estimate of particle size [28]

2.2.4. *Particle size analysis*

The particle size and size distribution of microparticles were measured using laser diffraction (HELOS BF and CUVETTE, Sympatec GmbH, Clausthal-Zellerfeld, Germany) after redispersing the microparticles in deionized water. Particle size distributions were analyzed using Sympatec WINDOX 5.4.1.0 software with the LD evaluation mode and expressed as volumetric density distribution (q3lg).

2.2.5. *Drug loading and encapsulation efficiency*

The actual drug loading was determined by dissolving 10.0–20.0 mg microparticles in acetonitrile and diluting 1:1 (V/V) with deionized water. The absorbance of the solution was then measured by UV–Vis spectroscopy (UV-1900i, Shimadzu Corp., Kyoto, Japan) at 242 nm (dexamethasone) or 276 nm (risperidone). Concentrations were calculated with previously established standard curves considering the effect of dissolved PLGA. The encapsulation efficiency (EE) in percent was calculated as the ratio of actual drug loading (AL) to the theoretical drug loading (TL).

2.2.6. *Morphology*

Scanning electron microscopy (SEM) (SU8030, Hitachi High-Technologies Europe GmbH, Krefeld, Germany) was used to image the surface and internal morphology of the microparticles. To investigate the inner structure, the microparticles were dispersed in a solvent-free glue (UHU GmbH & Co. KG, Baden, Germany) and the hardened matrix was cut with a razor blade. Samples were sputtered under an argon atmosphere with gold (CCU-010 HV, Safematic GmbH, Zizers, Switzerland) and then observed.

2.2.7. *In-vitro release*

Approximately 10 mg of microparticles, accurately weighed, were immersed in 100 mL of phosphate-buffered saline pH 7.4 (PBS) for risperidone or in 100 mL of 0.9 % sodium chloride solution adjusted to pH 7.4 for dexamethasone and stored in an incubation shaker (37 °C and 80 rpm). At designated time points, 10.0 mL of release medium was removed and replaced. Sink conditions were maintained throughout. The concentration of the drug in each sample was determined using the spectroscopic method described above. In-vitro release was performed in triplicate and results were depicted only as the mean value when the standard deviation was below 5 %.

3. Results and discussion

3.1. *Solvent removal in a classical evaporation-controlled process*

The amount of organic solvent extracted at the beginning of emulsification depended on its solubility in the aqueous phase and the phase ratio. At a phase ratio of 1:20 and a temperature-dependent solubility of dichloromethane of 10–20 mg/mL, about 15–30 % were rapidly extracted into the aqueous phase until saturation. As long as saturation persisted, the evaporation rate from the aqueous phase determined the further extraction [9], which was about 70 mg/min in a 50 mL Batch. In the first 45 min, more than 98 % of the dichloromethane was removed from the dispersed phase by rapid initial extraction followed by slow evaporation (Fig. 2). The viscosity of the droplets increased with time and the diffusivity of the solvent slowly decreased due to an increase in PLGA concentration. When the glass transition temperature of the PLGA: solvent mixture in the dispersed phase exceeded ambient temperature, the droplets solidified into microparticles, resulting in a sharp decline in the extraction rate of residual dichloromethane.

3.2. *Solvent removal in a diafiltration-controlled process*

Through diafiltration with fresh aqueous phase at a relative rate of 20 %/min (20 % of the aqueous phase volume were exchanged within one minute), dichloromethane was continuously removed from the system independently of evaporation. A solvent saturation of the aqueous phase is avoided and thus not limiting to solvent extraction, and dichloromethane was removed faster from the dispersed phase. Faster solidification of the microparticles enabled earlier subsequent

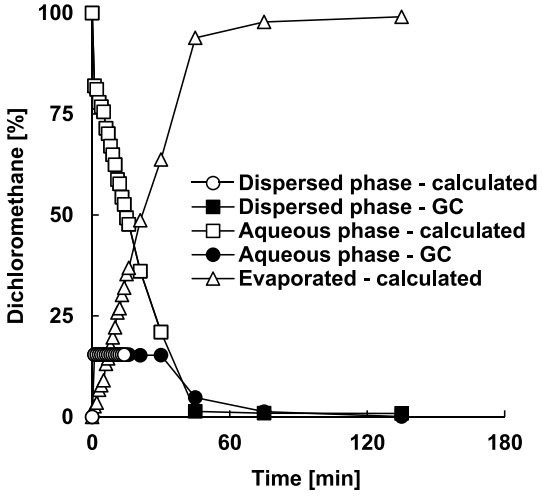

Fig. 2. Distribution of dichloromethane during solvent evaporation controlled preparation of blank PLGA 503H microparticles with an initial phase ratio of 1:20 (50 mL batch size). Filled symbols represent values determined by gas chromatography, open symbols are calculated based on the mass loss of the total batch.

processing steps, e.g. collection of the microparticles without sticking or aggregation. Once the glass transition temperature of the dispersed PLGA phase exceeded ambient temperature, further diafiltration had little effect on the residual solvent content because diffusion was limited by the smaller solvent diffusion coefficient and not by the concentration gradient. This behavior was independent of the type of drug encapsulated (Fig. 3).

When diafiltration was combined with a slightly elevated temperature (35 °C), lower residual solvent contents were achieved quickly (Fig. 4). At 35 °C, the process temperature remained longer above the glass transition temperature, thus the dispersed phase remained longer in a liquid or viscous state which favored solvent removal. The dispersion stability was not affected by the elevated temperature.

3.3. Effect of diafiltration on microparticle solidification and morphology

Focus Beam Reflectance Measurement (FBRM) can be used to monitor online the preparation of microparticles by the solvent evaporation process [29,30]. The data obtained, using FBRM, not only depends on size and geometry, but also on the optical properties of the dispersed phase [28]. A decrease in chord length may initially be noticeable due to emulsification and droplet shrinkage. Another transitionally increase in the FBRM signal can be caused by occurrence of subsurface scattering in addition to specular reflection, due the onset of phase separation during PLGA solidification. When solidification progresses, a smooth surface forms and the opacity increases, which means that specular reflection is almost exclusively present, which again results in a decreased FBRM signal. The following stagnation of the FBRM signal indicates that the particles are solidified, at least near the surface [28].

Encapsulation of drugs affected the FBRM signal in different ways. In particular, the initial subsurface scattering was enhanced by the presence of dexamethasone crystals in the dispersed phase (Fig. 5). In contrast, dissolved risperidone did not affect the initial chord length. It caused a delayed transient increase of chord length as it was completely dissolved and only precipitated in the dispersed phase after most of the dichloromethane was extracted. The media chord length increased slightly further between 60 and 65 min, if 30 % risperidone was encapsulated without diafiltration. Splitting the FBRM signal into size classes resulted in an increase in the number of particles counted, which occurred primarily in the particle size fraction < 25 µm, consisting mainly of risperidone crystals (data not shown). The formation of needle-shaped crystals was also confirmed by in-situ video microscopy (Fig. 6). Risperidone was extracted with dichloromethane from the dispersed PLGA phase and precipitated in the continuous aqueous phase when the extraction rate became slower than the evaporation rate. The risperidone solubility then decreased with decreasing dichloromethane

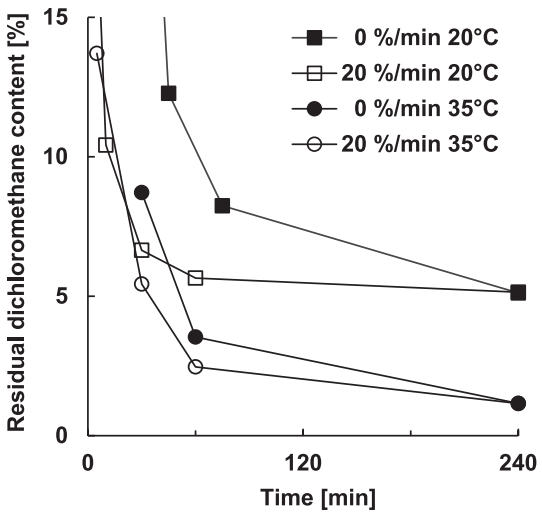

Fig. 4. Effect of diafiltration and temperature on the residual dichloromethane in blank PLGA 503H microparticles (50 mL batch size).

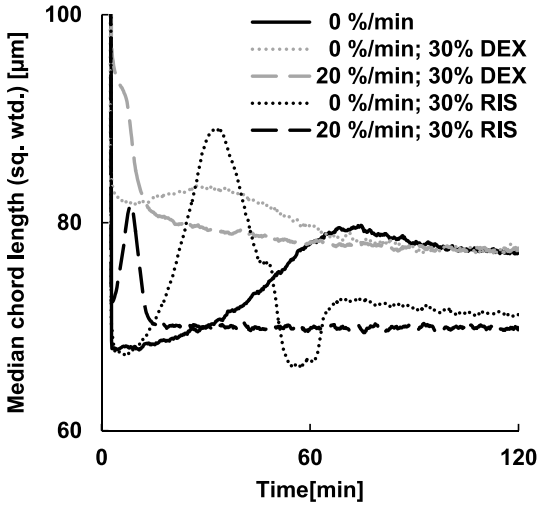

Fig. 5. Effect of relative diafiltration rate and drug loading on the square weighted median chord length of PLGA 503H microparticles (200 mL batch size).

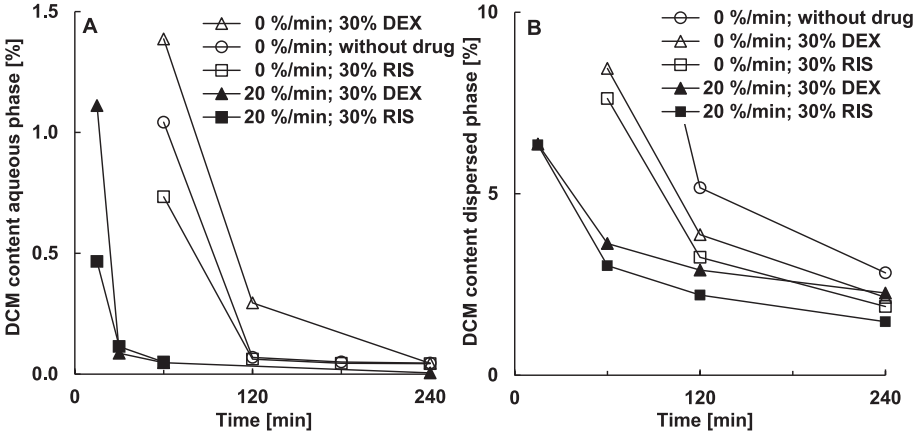

Fig. 3. Effect of relative diafiltration rate and drug loading on the dichloromethane content in (A) the aqueous phase and (B) the dispersed PLGA 503H phase (200 mL batch size).

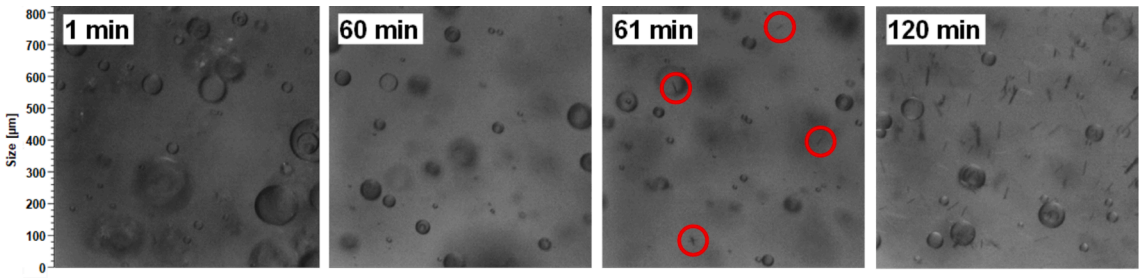

Fig. 6. Microscopic appearance (in-situ) during the encapsulation of risperidone in PLGA 503H without diafiltration (Circles mark first appearance of needle-shaped risperidone crystals).

content in the aqueous phase and the drug precipitated.

If dichloromethane extraction was accelerated by diafiltration, an earlier increase, decrease and stagnation of chord length was observed, indicating a faster solidification of the microparticles. The lack of increase in chord length after solidification also showed that unencapsulated risperidone did not crystallized (Fig. 5). Risperidone in the dissolved state was removed by diafiltration in the same way as dichloromethane with the continuous aqueous phase, so that no crystals precipitated in the production vessel even if the risperidone solubility decreased.

With increasing diafiltration rate, the average particle size of microparticles loaded with 30 % drug increased (Fig. 7). Due to the faster extraction, a skin of solidified PLGA may formed on the droplet surface. This could have prevented uniform shrinkage during further extraction of dichloromethane, resulting in larger, more porous particles [8]. The increase in particle size was greater with dexamethasone. Dexamethasone was encapsulated in the form of dispersed drug crystals, this increased the viscosity of the dispersed PLGA phase and further impeded uniform shrinkage.

Moderate diafiltration rates ($\leq$ 20 %/min) did not change the homogeneous dense morphology of blank PLGA microparticles. At high relative diafiltration rate (100 %/min), numerous slightly deformed microparticles, with a large hollow core were formed (Fig. 8).

The formation of a robust, solidified polymer skin probably almost completely prevented the shrinkage in this case. As the extraction progresses, PLGA completely precipitated under the existing skin. Water, which may have entered by rapid extraction, coalesced inside and filled a large cavity. The formation of such large hollow cores was previously observed due to rapid solvent extraction by gradually increasing temperature [18,21] or entrapment of water by $W_1/O/W_2$ double emulsion method with low O/W_2 phase ratio [7]. The encapsulation of 30 % dexamethasone generally led to a more inhomogeneous and porous morphology (Fig. 8). Dexamethasone crystals in the dispersed phase hindered shrinkage during extraction. In comparison, microparticles loaded with risperidone had a dimpled surface and the inner pores had a smaller, more regular size. The hollow core structure, formed at a high diafiltration rate (100 %/min), was less pronounced with risperidone, partially, drug crystals had grown in the cavity.

3.4. *Effect of diafiltration on drug encapsulation*

At a high diafiltration rate, diffusion of drug from the dispersed to the continuous phase was prevented earlier because of a faster droplet solidification. This was particularly the case for risperidone again, because of its greater solubility in the PLGA phase and the aqueous phase compared to dexamethasone. Increasing the relative diafiltration rate from 0 to 100 %/min increased the encapsulation efficiency from 62 to 78 % or 69 to 80 % at a theoretical drug loading of 10 % or 30 % (Fig. 9). The encapsulation efficiency has been increased more at the lower 10 % theoretical drug loading because the amount of drug extracted up to the solubility limit in the continuous phase corresponded to a larger percentage of the theoretical drug loading.

The positive effect of accelerated solvent removal by diafiltration on encapsulation efficiency was particularly evident with the more hydrophobic PLGA grade 753S. In general, it is assumed that the solubility of hydrophobic drugs within a PLGA matrix increases with increasing hydrophobicity of PLGA at the same molecular weight [31]. The encapsulation efficiency of risperidone in 753S, which has a similar molecular weight to 503H but an increased lactide content and end-capping, was only 27 %. The FBRM signal of 753S microparticles stagnated later with and without risperidone compared to 503H (Fig. 10). The signal without drug increased only slowly, without subsequently decreasing, indicating a more uniform solidification without rapid phase separation in the dispersed phase. The glass transition temperature of

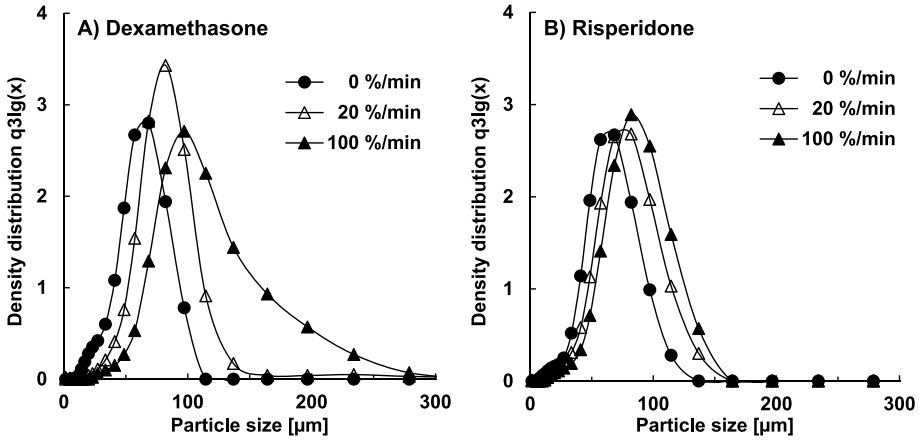

Fig. 7. Effect of relative diafiltration rate and drug type on the particle size distribution of PLGA 503H microparticles loaded with (A) 30 % dexamethasone and (B) 30 % risperidone.

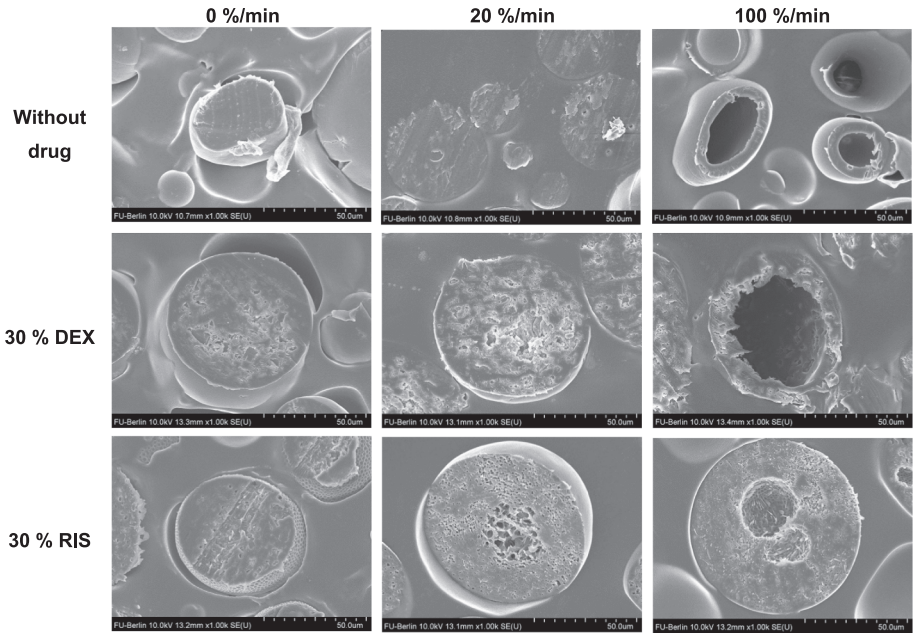

Fig. 8. Effect of relative diafiltration rate and theoretical drug loading on the morphology of PLGA 503H microparticle cross-sections.

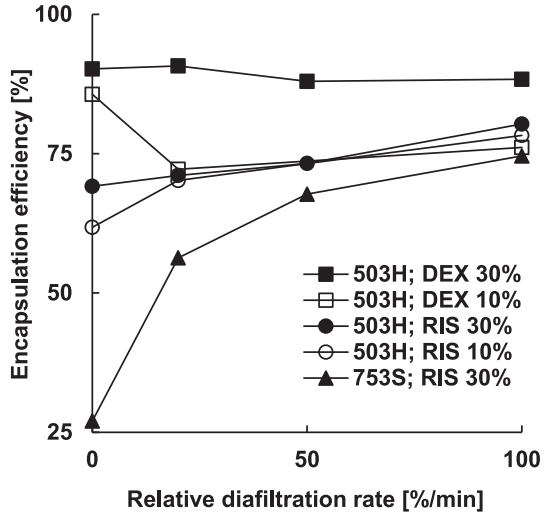

Fig. 9. Effect of relative diafiltration rate, drug loading and PLGA grade on the encapsulation efficiency.

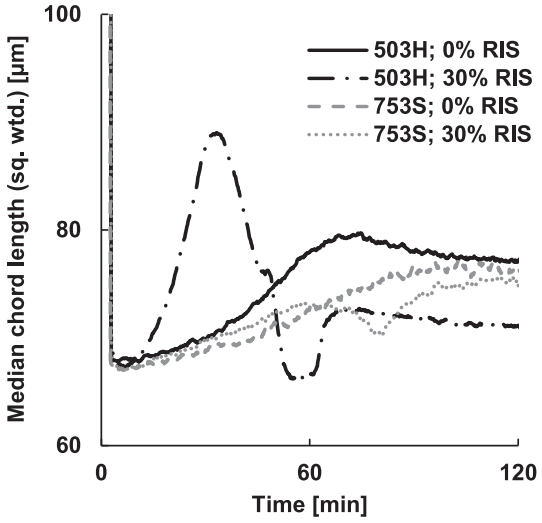

Fig. 10. Effect of PLGA grade and risperidone loading on the square weighted median chord length during the solvent evaporation process (200 mL batch size).

753S (44–50 °C) is similar to that of 503H (44–48 °C), Therefore, both PLGA grades solidified at approximately the same residual dichloromethane content. However, due to the higher lipophilicity of 753S compared to 503H, dichloromethane had an increased affinity to the PLGA matrix and was removed more slowly. The slower extraction of dichloromethane in the aqueous phase with the 753S was also reflected in the later crystallization of unencapsulated risperidone in the aqueous phase at 72 min (Fig. 11). This is 11 min later than with 503H (Fig. 6) due to a delayed decrease of both the dichloromethane content and thus the risperidone solubility in the aqueous phase with the 753S. The slower and even solidification of 753S favored the extraction of risperidone. Diafiltration increased the encapsulation efficiency to about 75 % (Fig. 9).

Dexamethasone, which is poorly soluble in dichloromethane and water, showed good encapsulation efficiency without diafiltration. At a theoretical loading of 10 %, diafiltration decreased the encapsulation efficiency (Fig. 9), by increasing dissolution capacity not only for dichloromethane but also for dexamethasone. At a low drug loading and low diafiltration rate, the faster drug extraction outweighed the more rapid PLGA solidification due to the opposing effect of improved dichloromethane and drug dissolution capacity on the encapsulation efficiency.

3.5. *Effect of diafiltration on drug release*

The in-vitro release of dexamethasone from PLGA 503H was investigated at theoretical loadings of 10 % and 30 %, which corresponded to

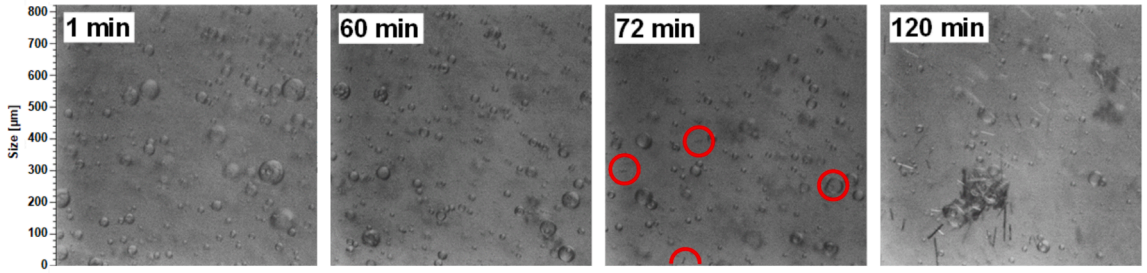

Fig. 11. Microscopic appearance (in-situ) during encapsulation of risperidone in PLGA 753S without diafiltration (Circles mark first appearance of needle-shaped risperidone crystals).

an actual drug loading of 8 ± 1 % and.

26 ± 0.7 %, respectively. The release of risperidone from PLGA 503H was also examined at a theoretical drug loading of 30 %, corresponding to an actual drug loading of 22.5 ± 1.5 %. For the release from PLGA 753S, the theoretical drug loading was varied between 30–40 % to compensate the strong effect of diafiltration and an actual drug loading of 27 ± 3 % was achieved. Since no comparable encapsulation in PLGA 753S was achieved for a process without modification of the extraction, a one-step dilution was carried out 15 min after emulsification as a comparison. An initial phase ratio of 1:200 resulted in large porous particles with a broad particle size distribution and low encapsulation efficiency due to the sudden extraction (data not shown). Emulsion equilibrium was reached, and a certain shrinkage could occur due to the delay of 15 min. This resulted in a particle size comparable to the microparticles without modified extraction and a high encapsulation efficiency of 85 %.

Increasing diafiltration rate did not affected the in-vitro release of risperidone from PLGA 503H or 753S and 10 % dexamethasone from 503H (Fig. 12). The release was completely controlled by the PLGA matrix, independent of morphological changes (Fig. 8). Compared to dexamethasone, risperidone showed a significantly faster diffusional release, due to its higher solubility in the matrix and the release medium. This increased further after a few days, as the polymer began to degrade, the micro-pH dropped, and risperidone became protonated and more soluble [32]. Dexamethasone was almost completely undissolved in the polymer. A drug loading of 30 % resulted in a burst release of more than 25 % within 48 h. This was followed by a plateau phase lasting about two weeks with subsequent rapid release due to the beginning erosion of the microparticles. The initial burst was caused by percolation, which occurs at high loadings (above the percolation threshold) with dispersed drug [33]. Increasing diafiltration rate increased the initial burst release from 30 % dexamethasone to more than 50 % in 48 h as the percolation network was expanded through pre-existing pores and cavities created by fast solvent extraction (Fig. 12). An increase in initial release was also observed due to porosity created by other means, e.g. by the W/O/W double emulsion process or the use of porogens [34,35].

3.6. Optimization of diafiltration-induced microparticle porosity and burst release

When diafiltration-controlled extraction was performed at elevated temperature, the inhomogeneous matrix structure of the microparticles hardly changed at 35 °C, but it became significantly denser at 45 °C (Fig. 13). Being close to the glass transition temperature of pure 503H of 44–48 °C, the microparticles remained deformable and shrinkable for a longer time.

For 30 % dexamethasone the encapsulation efficiency decreased from 90 % to 81 % (35 °C) or 77 % (45 °C). At 35 °C the initial burst release was slightly increased further due to the increased porosity caused by faster dichloromethane extraction (Fig. 14). At 45 °C the burst decreased due to the denser morphology, although not to the level of microparticles prepared without diafiltration. The subsequent plateau phase was significantly shortened. Such an earlier erosion-controlled final release was facilitated by an increased hydrolysis of PLGA during manufacturing. This might be caused by a longer surpassing of the glass transition temperature of the polymer–solvent system at higher process temperatures [36].

The residual water content of undried dexamethasone-loaded microparticles increased from 2.2 to 10.6 % by increasing the relative diafiltration rate from 0 to 20 %/min at 20 °C, supporting the assumption, that pores and cavities caused by accelerated microparticle solidification are filled with water during manufacturing. Increasing the process temperature increased the water content first to 12.7 % (35 °C) and then reduced it to 6.8 % (45 °C), which agreed with the visibly reduced porosity at 45 °C. The initial burst release within the first 48 h correlated directly with the residual water content before drying as an

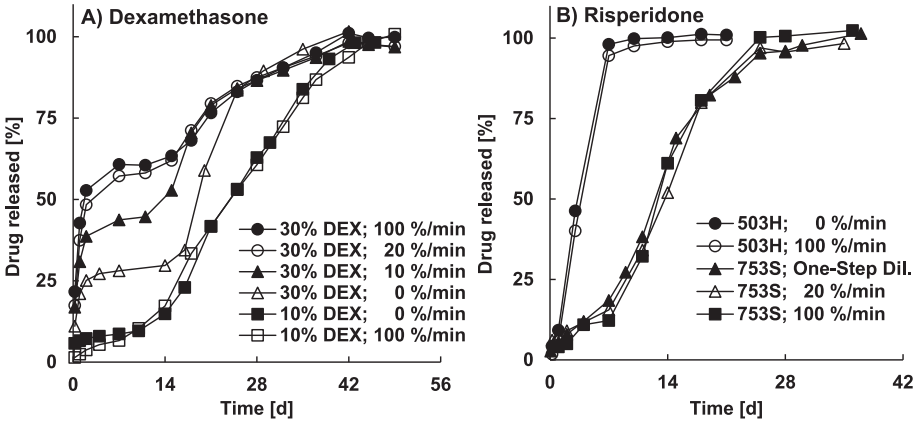

Fig. 12. Effect of relative diafiltration rate on the in-vitro drug release of (A) dexamethasone (PLGA 503H) as a function of theoretical drug loading and (B) risperidone as a function of the PLGA grade.

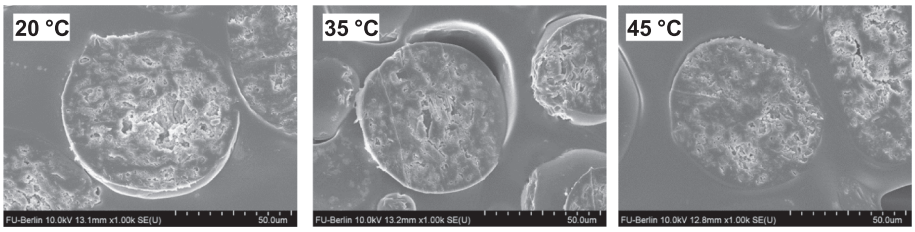

Fig. 13. Effect of temperature during diafiltration assisted preparation (20 %/min) of dexamethasone-loaded PLGA 503H microparticles (30 % theoretical drug loading) on the morphology of microparticle cross-sections.

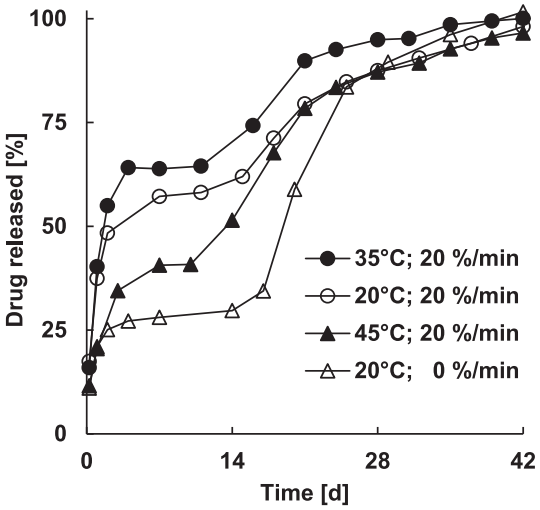

Fig. 14. Effect of temperature during diafiltration assisted preparation (20 %/min) of dexamethasone-loaded PLGA 503H microparticles (30 % theoretical drug loading) on the in-vitro drug release.

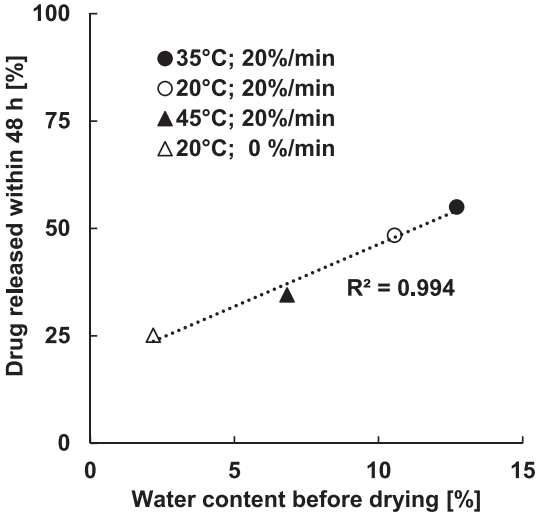

Fig. 15. Effect of temperature during diafiltration assisted preparation (20 %/min) of dexamethasone-loaded PLGA 503H microparticles (30 % theoretical drug loading) on the water content before drying and burst release within 48 h.

indicator of the porosity, confirming percolation theory for high dexamethasone loadings (Fig. 15).

4. Conclusion

The preparation of PLGA microparticles, i.e. the solvent extraction and the associated solidification, was effectively controlled by diafiltration in a single-vessel approach. In this way, the efficiency of microparticle manufacturing was increased by shortening the process time and keeping the volume of manufacturing equipment small. Further, the encapsulation efficiency of drug dissolved in the dispersed phase was significantly increased with the use of diafiltration. This effect was particularly evident when a more lipophilic slower solidifying PLGA grade was used. An increase in particle size and porosity due to the rapid extraction did not affect the in-vitro release of the drugs tested when their percolation threshold was not exceeded. If dispersed drug was processed in high concentrations, the increased porosity and associated burst caused by rapid solvent extraction could be counteracted partially by increasing temperature and thus delaying polymer solidification.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Florian Kias: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Conceptualization. **Roland Bodmeier:** Writing – review & editing, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We would like to acknowledge for the assistance with SEM by the Core Facility BioSupraMol, funded by the DFG.

References

[1] Y. Yeo, N. Baek, K. Park, Microencapsulation methods for delivery of protein drugs, Biotechnol Bioprocess Eng. 6 (2001) 213–230.
[2] A. Schoubben, M. Ricci, S. Giovagnoli, Meeting the unmet: from traditional to cutting-edge techniques for poly lactide and poly lactide-co-glycolide microparticle manufacturing, J. Pharm. Investig. 49 (2019) 381–404.
[3] F. Kias, R. Bodmeier, Accelerated removal of solvent residuals from PLGA microparticles by alcohol vapor-assisted fluidized bed drying, Submitted to Int. J. Pharm. (Unpublished results 2024).
[4] The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, Impurities: Guideline for Residual Solvents: ICH Q3C (R9), 2024.
[5] F. Kias, R. Bodmeier, Acceleration of final residual solvent extraction from poly (lactide-co-glycolide) microparticles, Pharm. Res. (2024).

[6] W.-I. Li, K.W. Anderson, R.C. Mehta, P.P. Deluca, Prediction of solvent removal profile and effect on properties for peptide-loaded PLGA microspheres prepared by solvent extraction/ evaporation method, J. Control. Release 37 (1995) 199–214.
[7] Y.-Y. Yang, T.-S. Chung, X.-L. Bai, W.-K. Chan, Effect of preparation conditions on morphology and release profiles of biodegradable polymeric microspheres containing protein fabricated by double-emulsion method, Chem. Eng. Sci. 55 (2000) 2223–2236.
[8] Y. Yeo, K. Park, Control of encapsulation efficiency and initial burst in polymeric microparticle systems, Arch. Pharm. Res. 27 (2004) 1–12.
[9] J. Wang, S.P. Schwendeman, Mechanisms of solvent evaporation encapsulation processes: prediction of solvent evaporation rate, J. Pharm. Sci. 88 (1999) 1090–1099.
[10] H. Katou, A.J. Wandrey, B. Gander, Kinetics of solvent extraction/evaporation process for PLGA microparticle fabrication, Int. J. Pharm. 364 (2008) 45–53.
[11] X. Luan, M. Skupin, J. Siepmann, R. Bodmeier, Key parameters affecting the initial release (burst) and encapsulation efficiency of peptide-containing poly(lactide-co-glycolide) microparticles, Int. J. Pharm. 324 (2006) 168–175.
[12] R.A. Mensah, S.B. Kirton, M.T. Cook, I.D. Styliari, V. Hutter, D.Y.S. Chau, Optimising poly(lactic-co-glycolic acid) microparticle fabrication using a Taguchi orthogonal array design-of-experiment approach, PLoS One 14 (2019) e0222858.
[13] Y. Bahl, H. Sah, Dynamic changes in size distribution of emulsion droplets during ethyl acetate-based microencapsulation process, AAPS PharmSciTech 1 (2000) E5.
[14] K.S. Soppimath, T.M. Aminabhavi, Ethyl acetate as a dispersing solvent in the production of poly(DL-lactide-co-glycolide) microspheres: effect of process parameters and polymer type, J. Microencapsul. 19 (2002) 281–292.
[15] W.-I. Li, K.W. Anderson, P.P. Deluca, Kinetic and thermodynamic modeling of the formation of polymeric microspheres using solvent extraction/evaporation method, J. Control. Release 37 (1995) 187–198.
[16] M. Li, O. Rouaud, D. Poncelet, Microencapsulation by solvent evaporation: state of the art for process engineering approaches, Int. J. Pharm. 363 (2008) 26–39.
[17] D.H. Lewis, Growth promoters for animals, U.S. 5,288,496, 1994.
[18] R.C. Mehta, R. Jeyanthi, S. Calls, B.C. Thanoo, K.W. Burton, P.P. Deluca, Biodegradable microspheres as depot system for patenteral delivery of peptide drugs, J. Control. Release 29 (1994) 375–384.
[19] R. Bodmeier, J.W. McGinity, Solvent selection in the preparation of poly(dl-lactide) microspheres prepared by the solvent evaporation method, Int. J. Pharm. 43 (1988) 179–186.
[20] S. Mao, Y. Shi, L. Li, J. Xu, A. Schaper, T. Kissel, Effects of process and formulation parameters on characteristics and internal morphology of poly(d, l-lactide-co-glycolide) microspheres formed by the solvent evaporation method, Eur. J. Pharm. Biopharm. 68 (2008) 214–223.
[21] R. Jeyanthi, B.C. Thanoo, R.C. Metha, P.P. Deluca, Effect of solvent removal technique on the matrix characteristics of polylactide/glycolide microspheres for peptide delivery, J. Control. Release 38 (1996) 235–244.
[22] H. Sah, Microencapsulation techniques using ethyl acetate as a dispersed solvent: effects of its extraction rate on the characteristics of PLGA microspheres, J. Control. Release 47 (1997) 233–245.
[23] J.W. Gibson, R.J. Holl, A.J. Tipton, Emulsion-based processes for making microparticles, U.S. 6,291,013, 2001.
[24] T. Suzuki, Y. Matsukawa, A. Suzuki, Method and apparatus for preparing microspheres, U.S. 7,011,776 B2, 2006.
[25] M.C. Operti, A. Bernhardt, V. Sincari, E. Jager, S. Grimm, A. Engel, M. Hruby, C. G. Figdor, O. Tagit, Industrial scale manufacturing and downstream processing of PLGA-based nanomedicines suitable for fully continuous operation, Pharmaceutics 14 (2022) 276.
[26] J.M. Ramstack, Method for preparing microparticles using liquid-liquid extraction, U.S. 6,884,372 (2005) B2.
[27] J.L. Atkinson, B.K. Chambers, Solvent extraction microencapsulation with tunable extraction rates, U.S. 8,703,843 B2, 2014.
[28] K. Vay, W. Frieß, S. Scheler, Understanding reflection behavior as a key for interpreting complex signals in FBRM monitoring of microparticle preparation processes, Int. J. Pharm. 437 (2012) 1–10.
[29] A.S. Zidan, Z. Rahman, M.A. Khan, Online monitoring of PLGA microparticles formation using Lasentec focused beam reflectance (FBRM) and particle video microscope (PVM), AAPS J. 12 (2010) 254–262.
[30] M. Muhaimin, A.Y. Chaerunisaa, R. Bodmeier, Real-time particle size analysis using focused beam reflectance measurement as a process analytical technology tool for continuous microencapsulation process, Sci. Rep. 11 (2021) 19390.
[31] J. Panyam, D. Williams, A. Dash, D. Leslie-Pelecky, V. Labhasetwar, Solid-state solubility influences encapsulation and release of hydrophobic drugs from PLGA/PLA nanoparticles, J. Pharm. Sci. 93 (2004) 1804–1814.
[32] F. Bach, S. Staufenbiel, R. Bodmeier, Implications of changes in physical state of drugs in poly(lactide-co-glycolide) matrices upon exposure to moisture and release medium, J. Drug Deliv. Sci. Technol. 80 (2023) 104115.
[33] S.H.S. Koshari, X. Shi, L. Jiang, D. Chang, K. Rajagopal, A.M. Lenhoff, N.J. Wagner, Design of PLGA-based drug delivery systems using a physically-based sustained release model, J. Pharm. Sci. 111 (2021) 345–357.
[34] T. Ehtezazi, C. Washington, Controlled release of macromolecules from PLA microspheres: using porous structure topology, J. Control. Release 68 (2000) 361–372.
[35] C. Zhang, Biodegradable microparticles and in situ forming implants/microparticles containing drugs in different physical states. Doctoral dissertation, Berlin, 2022.
[36] K. Vay, W. Frieß, S. Scheler, A detailed view of microparticle formation by in-process monitoring of the glass transition temperature, Eur. J. Pharm. Biopharm. 81 (2012) 399–408.