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Microparticle preparation by a propylene carbonate emulsification-extraction method

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ABSTRACT

The use of various harmful organic solvents for microparticle formulations is still widespread. Here, an alternative low toxicity solvent (propylene carbonate; PC) is proposed for the preparation of poly(lactic-co-glycolic-acid) (PLGA) microparticles. Based on the classical emulsification-solvent extraction methodology, the use of PC offers the unique advantage of an additional solvent extraction step using hydrolytic solvent cleavage during microparticle preparation. Spherical, rough-surfaced microparticles were obtained with a volume median diameter range from 20 to 60 µm. The residual PC content has been identified to be the major factor for the solidification hindrance, leading to polymeric Tg shifting due to a plasticizing effect. When applying the enhanced PC extraction step, the residual PC content was lowered from 8.8% to 2.7% and subsequently Tg values shifted from 8.2 to 37.7 °C. Additionally, the hydrolytic solvent cleavage confirmed to have no impact on the PLGA stability. This method presents a significant advancement towards replacing of conventional solvents in the microparticle preparation due to more efficient solvent extraction.

1. Introduction

Various pharmaceutical formulations nowadays still rely on the use of organic solvents. This is particularly true for microparticulate parenteral formulations intended for controlled drug release of small molecules or protein drugs. The microencapsulation of these substances is usually based on an emulsification – solvent elimination approach (Ao et al., 2011; Rosca et al., 2004; Shao et al., 2017). In general, an initial oil-in-water emulsification step is employed, followed by the elimination of the inner organic phase performed by either extraction or evaporation (depending on the vapour pressure of the organic solvent) (Katou et al., 2008; Vay et al., 2012).

Different organic solvents are used for the formulation of microparticulate drug carrier systems (Song et al., 2006). Among the most current ones are non-halogented solvents, like ethyl acetate or isopropanol, but also halogenated solvents like 1,2-dichloromethane. However, according to ICH guidelines for residual solvents Q3C(R5), halogenated solvents possess potential toxic properties belonging to the class II solvents (ICH, 2016). Formulations prepared with class III solvents such as acetone or ethanol typically are allowed to contain more "parts per million" residual solvent, but the final removal below the permitted threshold after microparticle preparation can be technically challenging (Bitz and Doelker, 1996; Herberger et al., 2003).

Potentially toxic solvents are needed to dissolve hydrophobic polymers like PLGA or PLA, despite using moderate preparation conditions which are appropriate for sensitive drugs (Bitz and Doelker, 1996). As an alternative, non-toxic solvents could be advantageous because they can overcome the safety-related issues. Hence, the use of non-toxic polymer solvents for multiparticulate systems can be suggested to avoid the issue of a complete residual solvent removal. These solvents possess a considerable advantage, since they can remain within the formulation after preparation of the microparticles due to their low toxicity. Solvents like dimethyl sulfoxide, glycofurol and liquid polyethylene glycols have been previously used in this manner (Ali and Lamprecht, 2013; Allhenn and Lamprecht, 2011; Viehof et al., 2013). However, the use of these solvents involves formulation issues such as high viscosity, low drug solubility, potential stability problems, etc. Previous reports suggested that using ester-type solvents like methyl propionate (Kim et al., 2016) and ethyl formate (Sah, 2000), both being partially water-soluble, can be a good alternative for the production of microparticles, while exhibiting low toxic properties.

Here, we propose a new formulation technique based on propylene carbonate (PC) as an alternative low toxic ester-type organic solvent for microparticle preparation intended for parenteral administration. PC is a member of cyclic organic carbonates, miscible with most organic solvents like acetone, ethanol, chloroform etc. (Fujinaga and Izutsu,

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1971; Raymond et al., 2009). Also, it is freely miscible with water at concentrations up to 20% (Shaikh and Sivaram, 1996). The ability to dissolve a wide range of polymers makes PC an attractive alternative to commonly used solvents.

However, in the context of alternative safe solvents, plasticization of the polymeric matrix has been identified to be the major issue involved in microparticle design (Jain et al., 2000; Katou et al., 2008; Sah, 1997). This is especially pronounced for water miscible or partially miscible solvents like glycofurol or ethyl acetate (Allhenn and Lamprecht, 2011; Sah, 1997). Consequently, solvent-based plasticization is the major hindering factor for microparticle solidification if the residual solvent quantity is not lowered.

In terms of safety considerations, the non-toxicity of PC is underlined in various reports (Beyer et al., 1987; Das et al., 2017; Quintanar-Guerrero et al., 1996; Sommer et al., 1990). PC undergoes two degradation pathways either by acid/base-induced hydrolysis (Shaikh and Sivaram, 1996) or enzyme-catalyzed hydrolysis in vivo (Yang et al., 1998). In both cases, cyclic organic carbonates produce carbonic acid and 1,2-diols, where the type of the produced diol is dependent on the type of cyclic organic carbonate, confirming the safe degradation of PC into carbon dioxide and propylene glycol (Clements, 2003). Accordingly, we were able to enhance the solvent extraction from the polymeric matrix by the chemical degradation of PC, making PC much more suitable as a polymer solvent compared to non-toxic solvent approaches that have been reported before.

2. Materials and methods

2.1. Materials

PLGA [Poly(DL-lactide-co-glycolide)] (Resomer® RG 502H) was obtained from Boehringer Ingelheim (Germany). Propylene carbonate (PC) was purchased from Merck (Darmstadt, Germany). Glycofurol, sodium carbonate, methanesulfonic acid, lactic acid, glycolic acid, sodium hydroxide and hydrochloric acid were obtained from Sigma-Aldrich (Steinheim, Germany). Thymol blue, sodium dihydrogen phosphate, sodium hydrogen carbonate and sodium sulfate were purchased from Roth (Karlsruhe, Germany). Polysorbate 80 was obtained from Caelo (Hilden, Germany). All other chemicals were of analytical grade.

2.2. Methods

2.2.1. In-situ drop to microparticle transformation

The drop to microparticle transformation was microscopically evaluated using a Leica DM 2700 M microscope (Leica Microsystems, Wetzlar, Germany) equipped with a QImagingMicroPublisher 5.0 Real-Time Viewing camera (QImaging, Surrey, BC, Canada) and recorded using QCapture Suite software. Two experimental approaches have been used: drop transformation during microparticle preparation and detailed observation of a single droplet during PC diffusion. Both observations were done without enhancement of the PC extraction. For the first approach, samples were drawn at different time points during the preparation and directly observed. The experimental setup of the second approach consisted of a polystyrene petri dish with a microscope glass slide which was mounted on the microscope stand. The glass slide was used in order to prevent the instant droplet collapse due to the high affinity of PC for polystyrene. A 1% PLGA in PC solution was prepared and mixed with nile red as a lipophilic stain. 40 ml of a 0.004% polysorbate 80 solution (corresponding to the final polysorbate concentration in the extraction medium during microparticle preparation) is added to the petri dish. Using a 1 ml syringe with a 30 G needle, a small drop of the nile red stained PLGA – PC solution was introduced into the petri dish and recorded during 30 min.

2.2.2. PC hydrolysis tracking

The PC hydrolysis tracking was accomplished using thymol blue (TB) as a pH shift indicator which occurs during PC hydrolysis. The major analytical drawback for the hydrolysis tracking of PC is the optical inertness which it exhibits both in UV and VIS region (Fujinaga and Izutsu, 1971; Grizić et al., 2016). For this reason, an indirect detection method was employed by using the ability of thymol blue (TB) to exhibit pH-dependant color transitions in the regions between pH < 8.0 (yellow) and pH > 9.6 (blue). During PC hydrolysis using aqueous sodium hydroxide, ring opening of PC (cyclic ester) occurs, which leads to the formation of propylene glycol and sodium hydrogencarbonate. If excess amounts of sodium hydroxide are present, sodium carbonate is formed. For this reason, we evaluated aqueous solutions of these potentially forming substances in stoichiometric identical concentrations which are formed during the actual microparticle preparation using TB and retrieved the respective spectra (Fig. S1). The end-point of PC hydrolysis gives a solution with two absorption maxima at 434 nm and 597 nm, respectively. In brief, 5 ml of 2% Na_2CO_3, 1.5% $NaHCO_3$, 0.15% $NaHCO_3$ and 2% PC were mixed with 0.05 ml 0.1% ethanolic TB solution and analyzed using a UV–VIS spectrophotometer (Lambda 12, PerkinElmer UV–Vis spectrophotometer, MA, USA), recording their spectra from 400 to 700 nm. Secondly, the optimal process parameters regarding the hydrolysis of PC (dropping speed and concentration of sodium hydroxide) which at the end could affect the stability of the excipients, had to be found. A constant amount of PC (100 mg) and varying concentrations of sodium hydroxide, expressed as the percentage of the maximum stoichiometric amount which is needed for a complete reaction (39.18 mg sodium hydroxide), were used. The analysis was performed using a 1 cm quartz cuvette, filled with a mixture of 50 µl 0.1% TB solution and 2 ml 5% PC. Immediately after adding the sodium hydroxide solution, continuous time-dependent measurements at 434 nm and 597 nm were performed, measuring the absorbance every 2 sec during 30 min. This procedure was repeated for all sodium hydroxide concentrations. Different concentrations of sodium hydrogencarbonate (the major product during PC hydrolysis) gave different intensities, but always the same intensity ratio between the two absorption maxima, which was 0.66. This value was the fixed end-point in all further investigations. The influence of the sodium hydroxide concentration on the speed of hydrolysis was evaluated (the linear relationship is shown in Fig. S2), giving the insight into the needed hydroxide ion concentration which has the shortest residence time in the solution (2 ml of a 2 M sodium hydroxide solution dropped at a dropping speed of 20.0 µl/min and a dropping rate of 1 drop/45 s).

2.2.3. Hydrolytic profiling of PC, PLGA and polysorbate 80

Using the TB-based hydrolysis tracking method, hydrolytic profiles of PC, PLGA and polysorbate 80 were evaluated. The hydrolysis of PC and polysorbate 80 was evaluated directly in a quartz cuvette by adding 0.24 ml of a 0.002% sodium hydroxide solution into the premixed PC/TB and polysorbate 80/TB solutions and immediately measuring the color transition at 597 nm over 3 h. For PLGA, 5 mg of the polymer was dispersed in 2 ml of water and mixed with 50 µl 0.1% TB solution. 0.24 ml of a 0.002% sodium hydroxide solution was added and the suspension was filtrated (0.2 µm) at predetermined time intervals and analyzed at 597 nm also for 3 h. It is important to note that the final sodium hydroxide concentration for microparticle preparation and for the hydrolytic profiling were stoichiometric identical (0.00024%).

2.2.4. Microparticle preparation

An emulsification – solvent extraction method was employed for the preparation of all microparticle samples. In brief, 100 mg of PLGA 502H was dissolved in 10 ml of propylene carbonate, giving a 1% PLGA/PC solution. Thereafter, 25 ml of a 0.1% aqueous polysorbate 80 solution was added, leading to a biphasic system. Subsequently, this mixture was stirred by a propeller stirrer (IKA RW 20 digital, 4-bladed stirrer,

shaft size 8 mm × 200 mm, stirrer diameter 35 mm) at 400 rpm for 2 min, leading to the formation of an o/w emulsion. The formed emulsion was immediately added to 500 ml of distilled water which was kept stirring at 250 rpm for 100 min. In order to improve the microparticle solidification, an enhanced PC extraction was integrated in the preparation procedure using hydrolytic treatment of the formed emulsion by adding 2 ml of sodium hydroxide (2 M) drop wise using a peristaltic HPLC pump (with a dropping speed of 20.0 µl/min and a dropping rate of 1 drop/45 s). Additionally, microparticles which were not subjected to the enhanced PC extraction step (no addition of sodium hydroxide), were also prepared. The obtained suspension was centrifuged at 800 rpm during 3 min, the supernatant removed and the pellet washed with distilled water. Finally, the microparticles were collected by filtration and dried in a desiccator overnight.

2.2.5. Particle size distribution

Laser diffraction (Helos, Sympatec®, Clausthal, Zellerfeld, Germany) was employed in order to investigate the size change during the drop to microparticle transformation, expressed as the volume distribution of the particles. For this purpose, deionized water (as during microparticle preparation) was used for the analysis, while the optical concentration was maintained at 3%. All samples were analyzed in triplicate.

2.2.6. Scanning electron microscopy

A scanning electron microscope (Hitachi SU3500, Tokyo, Japan) was used to evaluate the microparticle morphology of all samples, at 10 kV. Firstly, all microparticle samples were mounted on aluminum supports using double-adhesive tape and gold-coated using a sputter-coater (Polaron SC7640 Sputter Coater, Quorum Technologies Ltd., Newhaven, UK). Finally, the samples were placed onto the sample holder of the scanning electron microscope and analyzed.

2.2.7. Confocal laser scanning microscopy

A Nikon® Eclipse Ti Al Laser Scanning Confocal Imaging System (Nikon Corporation Inc., Tokyo, Japan) equipped with a modular laser system and an inverted Nikon® microscope was used to analyze the microparticles. The argon laser was run at 488 nm with a pinhole size of 1.5 A.U. In order to investigate the distribution of residual PC, sodium fluorescein was chosen since it dissolves in propylene carbonate, but does not stain PLGA. Samples with pre-stained propylene carbonate were prepared without and with the enhanced PC extraction step and analyzed.

2.2.8. HPLC evaluation of PLGA degradation

PLGA degradation was evaluated using HPLC, based on previous findings which showed that the tracing of PLGA monomers gives a good representative image of the overall degradation profile of the polymer (Li et al., 2012). For this purpose, pure PLGA, PLGA mixed with either polysorbate 80 or PC and a microparticle preparation mixture (PLGA, polysorbate 80 and PC) were used. At the end of the hydrolytic treatment, aliquots were withdrawn and analyzed using an Acclaim™ OA, 250 mm × 4 mm, 5 µm (Thermo Scientific) column. The analysis was performed at 30 °C using a flow of 0.6 ml/min of the mobile phase, which consisted of 100 mM Na_2SO_4 adjusted to pH 2.65 with methanesulfonic acid. 25 µl of pre-filtered and degassed sample was injected in each run and detected at 210 nm. The obtained calibration linearity range was between 5 and 1000 µg/ml (R = 0.9994 for glycolic acid and 0.9999 for lactic acid). The mean retention times for glycolic acid and lactic acid were 4.5 min and 5.7 min, respectively.

2.2.9. Differential scanning calorimetry

DSC examinations were carried out using a Mettler Toledo DSC2 instrument (Columbus, OH, U.S.A.), which was calibrated using indium as a standard. All samples (PLGA, PC, PLGA microparticles without the enhanced PC extraction step, PLGA microparticles with the enhanced PC extraction step and microparticles with the enhanced PC extraction step for stability study) were placed in non-hermetically sealed aluminum pans and equilibrated at −60 °C for 5 min. Afterwards, all samples were heated from −60 °C to 250 °C at a rate of 10 °C/min. The samples were again cooled down to −60 °C and after a repeated equilibration at −60 °C, the heating cycle was repeated. The results were analyzed using STAR^{e}SW 13.0 software. Additionally, microparticle stability was evaluated at three storage conditions: 25 °C ± 2 °C/60% RH ± 5% RH, 5 °C ± 3 °C and 40 °C ± 2 °C/75% RH ± 5% RH according to ICH guideline Q1A(R2) using a potential Tg shift investigation.

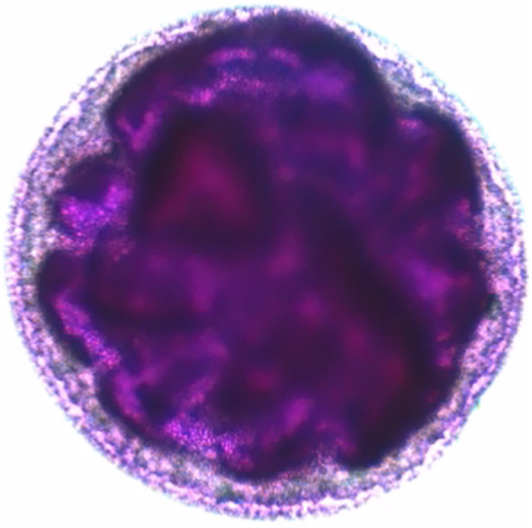
2.2.10. Quantification of residual PC

10 mg of microparticles were dissolved in 100 µl of glycofurol and then 900 µl of distilled water were added to the clear solution to precipitate PLGA. After filtration through a 0.2 µm polypropylene membrane, the clear aqueous filtrate was assayed for PC content as described previously (Grizić et al., 2016). The limit of quantification (LOQ) for the used analytical method was 3.1 ± 1.4 µg/ml, allowing the quantification of the residual PC content in the microparticles. All results were expressed as percentage [m/m].

3. Results

The microparticle preparation was based on an emulsification – solvent extraction method, where PC along with the dissolved PLGA acted as the inner phase and aqueous polysorbate 80 as the outer phase. After an o/w emulsion was formed, an excess amount of water was added leading to solvent extraction. A gradual transformation initiated by droplet shrinkage and finished with an apparently complete solidification was observed at consecutive time points (Fig. 1A–C).

Additionally, the drop to microparticle transformation was observed using a single droplet setup in order to assess the detailed inner and outer morphology change during microparticle solidification which lasted typically for 15–20 min (Video 1).



Video 1. Drop to microparticle transformation observation using a single droplet microscope setup reveals the detailed inner and outer morphology change during microparticle solidification. The observation was done without enhancement of the PC extraction. Initial experiments which employed drying of the apparently solid microparticles which were not subjected to the enhanced PC extraction step resulted in microparticle coalescence and aggregation, eventually leading to polymeric film formation (Fig. 2A). The product was further evaluated in terms of residual PC, revealing a content of 8.8 ± 0.1%. On the other hand, the application of the enhanced PC extraction step during preparation inhibited the microparticle aggregation and film formation (Fig. 2B), leading to the obtainment of dry microparticles. In this case just 2.7 ± 1.3% of

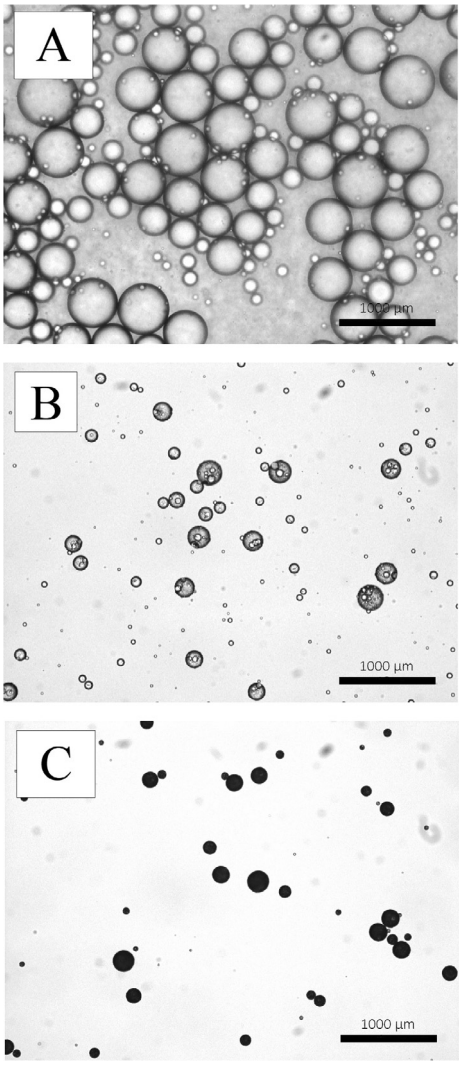


Fig. 1. Microscopic images of the drop to microparticle transformation process during (A) emulsification, (B) solvent extraction after 5 min and (C) solvent extraction after 30 min. Sample formulation was done without the enhanced PC extraction step during preparation. The scale bar represents 1000 µm.

residual PC was present. Even though both samples had the same extraction time of 100 min, differences in the physical appearance were significant. Consequently, residual PC was identified to have a significant impact on the possibility to obtain a non-aggregated product.

In order to evaluate the internal structure of the microparticles regarding a possible residual PC localisation, confocal laser scanning microscopy was used. Using fluorescein, a hydrophilic dye which stains PC while leaving PLGA unstained, a localisation pattern was observed. While an almost continuous PC distribution throughout the microparticle matrix could be observed for the sample which was untreated (Fig. 3A), the enhanced PC extraction led to PC depletion and localisation mainly in the interior cavities (Fig. 3B).

The transition from emulsion droplet to microparticle during the preparation step was distinctly slower in absence of the enhanced PC extraction step and led additionally to an increased droplet diameter until sufficient particle solidification took place (Fig. 4A). Oppositely, the application of the enhanced PC extraction step resulted in the final size distribution already at the first data point (Fig. 4B).

In order to exclude the possibility that the enhanced PC extraction step will accidentally degrade other compounds than PC, namely PLGA or polysorbate 80, their respective hydrolysis was experimentally assessed (Fig. 5A). PC hydrolysis was completed when PLGA hydrolysis

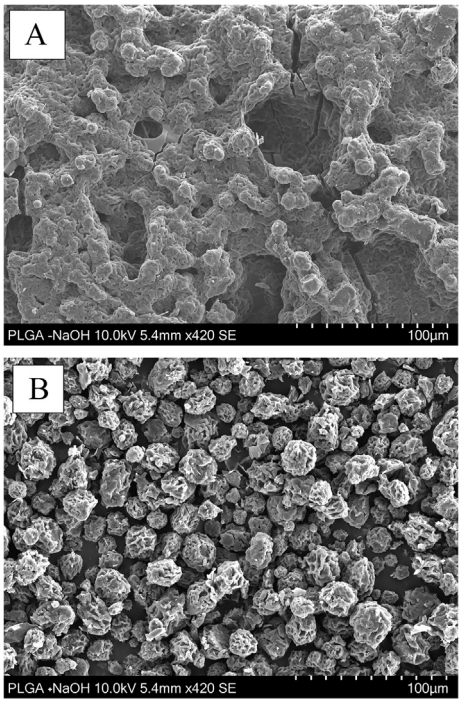


Fig. 2. Investigation of the microparticle morphology and coalescence tendency of PLGA microparticles prepared without (A) and with (B) the enhanced PC extraction step. Both samples had an extraction time of 100 min. The scale bar represents 100 µm.

only initiated, allowing the selective hydrolysis of the solvent only. Also, it was observed that polysorbate 80 had a profoundly higher hydrolytic resistance towards the alkaline solution compared to PC, where PC hydrolysis was completed when polysorbate 80 hydrolysis did not even initiate.

In addition, the feasibility investigation of the enhanced PC extraction step was finalized by assessing sample aliquots obtained during microparticle preparation for degradation-based acidic monomers. For this purpose, pure PLGA, PLGA with the addition of polysorbate 80, PLGA with the addition of PC and a full microparticle composition (PLGA, polysorbate 80 and PC) were subjected to the enhanced PC extraction step as described in the microparticle preparation Section 2.2.4. After the total amount of sodium hydroxide was added drop wise, the supernatants were analyzed using HPLC (Fig. 5B). Pure PLGA showed a high amount of degradation products (lactic and glycolic acid) after being treated. PLGA degraded in a lesser extent when just polysorbate 80 was present and no PC was added. Finally, PC containing samples (alone or with polysorbate 80) did not result in PLGA degradation, pointing to the high reactivity of PC compared to PLGA and polysorbate 80.

The influence of the duration of the enhanced PC extraction step on microparticle solidification was evaluated for periods of 0.5 h, 1.5 h and 4.5 h, respectively. Changes in terms of morphology were identified using SEM (Fig. 6). It could be observed that samples with a longer enhanced PC extraction ($\geq$1.5 h) showed a complete solidification, while the shorter lasting extraction (0.5 h) resulted in solidified and unsolidified microparticles (which can also be noticed by the decreased number of solid microparticles in the 0.5 h sample). Additionally, these samples were also tested for PLGA degradation products and the results showed that no degradation products were present, pointing to the integrity of the matrix even at longer extraction times (data not shown).

The plasticizing effect of residual PC which has a strong impact on PLGA was assessed by DSC measurements, focusing on the Tg shift. Due to the fact that PC has a low Tg (−114.6 °C), it represents a potential plasticizing agent. The absence of the enhanced PC extraction step

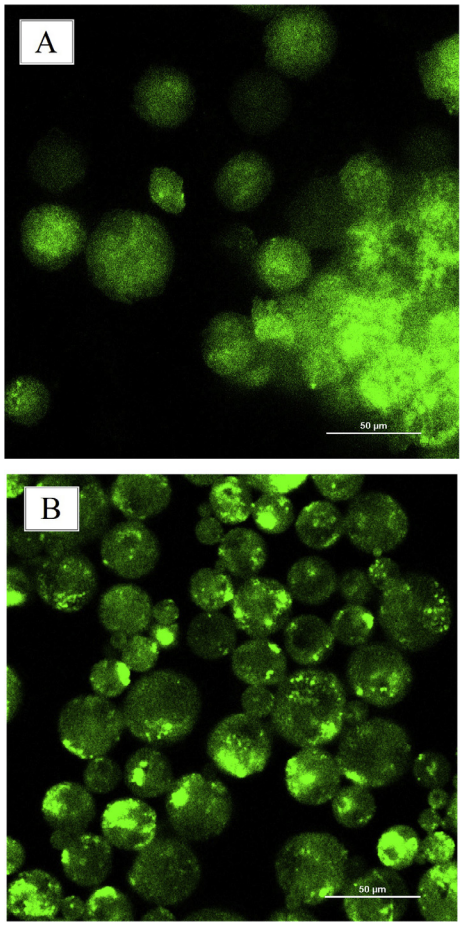


Fig. 3. Localisation of residual PC in the microparticle matrix of microparticles without (A) and with (B) the application of the enhanced PC extraction step; bright fluorescent spots throughout the microparticle matrix depict the stained residual PC. The scale bar represents 50 µm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

showed a significant decrease of the glass transition temperature in untreated microparticles (8.2 °C) compared to PLGA microparticles with the enhanced PC extraction step (37.7 °C) (Fig. 7). Consequently, this clearly points to the high plasticizing effect of PC and also the importance of the enhanced PC extraction step for the obtainment of solid dry microparticles.

Finally, microparticle stability has been analyzed in terms of a potential Tg shift of PLGA during storage at 5 °C ± 3 °C (Fig. 8A) and 25 °C ± 2 °C/60% RH ± 5% RH (Fig. 8B). Samples stored at 5 °C ± 3 °C for 12 months had a constant Tg value, and no significant Tg shift was observed. On the other hand, samples stored at 25 °C ± 2 °C/60% RH ± 5% RH had a constant Tg value (37.0 ± 0.8 °C) during the first 3 months, while after 6 months the Tg value gradually declined to 17.5 °C and was not observable after 12 months due to liquefaction. Samples stored at 40 °C ± 2 °C/75% RH ± 5% RH were showing signs of liquefaction after one week (data not shown) pointing at the instability of the samples at such conditions.

4. Discussion

Polyester microparticles are regarded to be an attractive formulation approach in terms of biodegradability as well as biocompatibility (Anderson and Shive, 2012; Ignatius and Claes, 1996). Since they are typically used as a parenteral drug delivery formulation, low toxicity is a major requirement that expands to all involved excipients, including

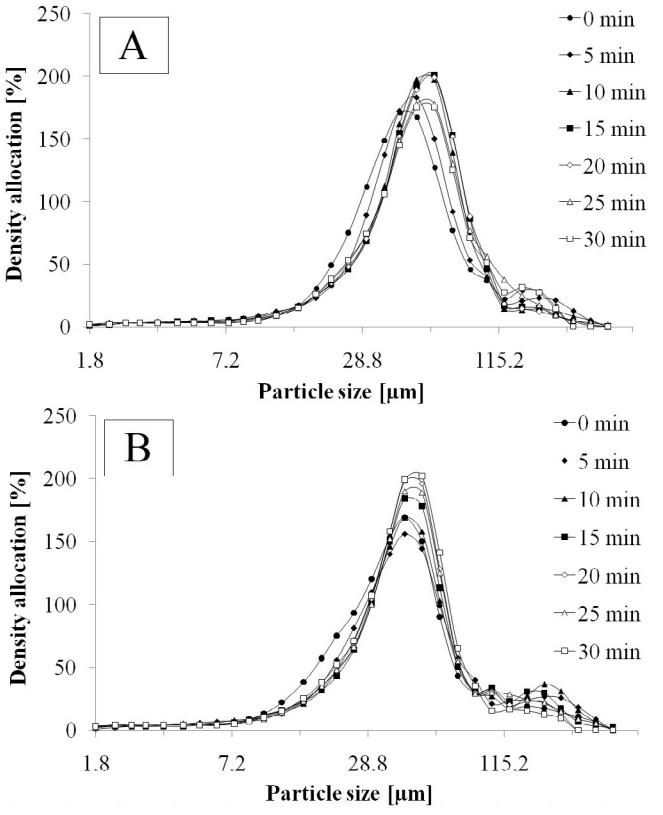


Fig. 4. Droplet size measurements in the o/w emulsion over time (A) without and (B) with the enhanced PC extraction step.

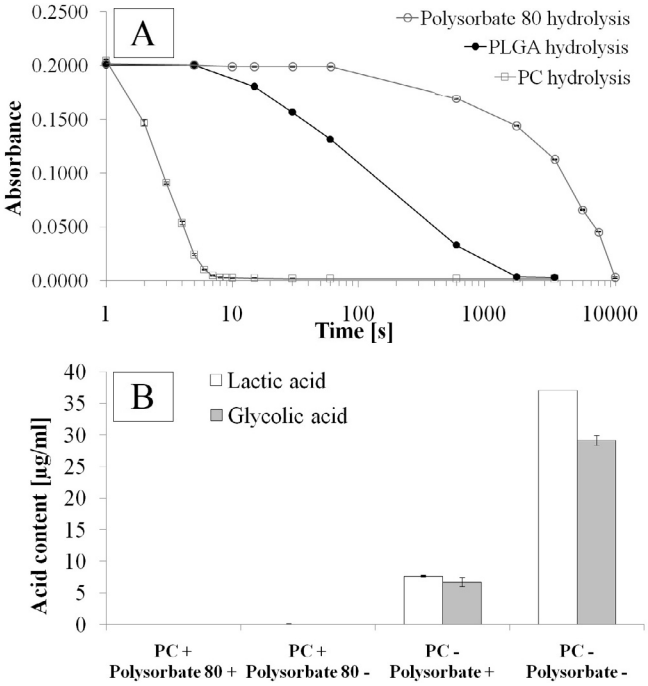


Fig. 5. Hydrolytic treatment of (A) PC (empty squares), PLGA (full circles) and polysorbate 80 (empty circles) and comparison of their respective hydrolytic profiles; (B) microparticle preparation mixtures evaluating the specific PLGA degradation products lactic and glycolic acid at the end of the production procedure (mean ± SD, n = 3).

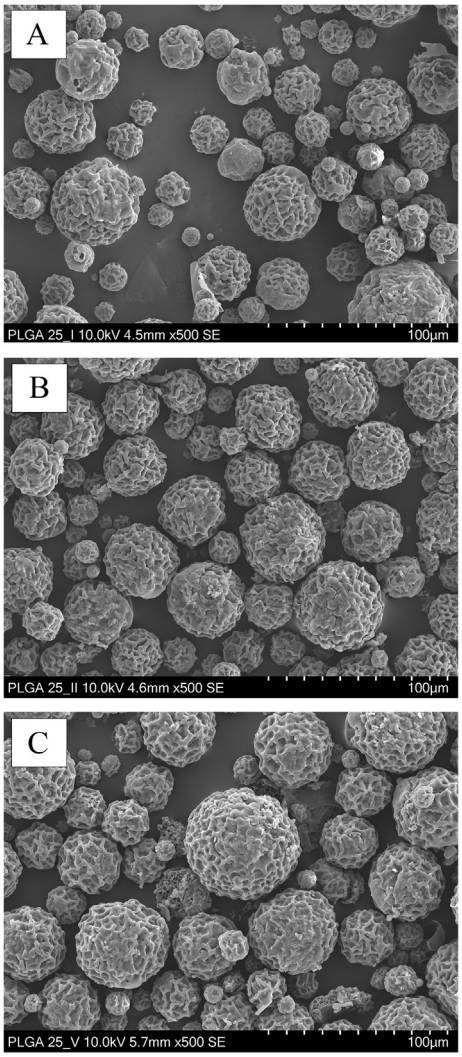


Fig. 6. Microparticle morphology observed after (A) 0.5 h, (B) 1.5 h and (C) 4.5 h of the enhanced PC extraction step. The scale bar represents 100 µm.

organic solvents applied during the preparation step (Osterberg and See, 2003). In this context, distinct efforts have been made to replace standard organic solvents, for example with liquid polyethylene glycols (Ali and Lamprecht, 2013; Viehof et al., 2013). Despite having low toxic properties, polyethylene glycols show formulation issues such as high viscosity, slow PLGA solubility and potential stability issues due to the formation of peroxyl radicals (Gullapalli and Mazzitelli, 2015; Schou-Pedersen et al., 2014). In addition, it was reported that the nucleophilic side groups of low molecular polyethylene glycols (namely PEG 300) tend to form block-copolymers with PLGA (Schoenhammer et al., 2009). For this reason, the use of propylene carbonate as a non-toxic and partially water-miscible organic solvent can, on one hand, fulfill the safety requirements for such formulations and on the other hand provide more suitable physicochemical properties such as low viscosity or enhanced polymer solubility.

A PC/water emulsion system was stabilized with polysorbate 80 forming an o/w emulsion as microparticle precursors similar to other conventional methods previously described (Elkharraz et al., 2011; Jeyanthi et al., 1996). The solvent extraction from the PLGA – rich droplet was initiated using distilled water as the extraction phase. In our case, this phenomenon was firstly tracked using nile red as a contrast agent during drop to particle transformation. Since nile red exhibits very lipophilic properties, it will not leak from the inner polymer phase during PC diffusion (shown in the Video Supplement data). It was observed that the PC diffused out of the microparticle very fast (in form of a convective flow), leaving solidified porous particles with a rough surface. This clarified the observed roughness of the microparticle surface, which appeared during the drop to particle transformation and not as a result of drying. In addition, using water-soluble solvents (e.g. glycofurol or DMSO), may build porous microparticles due to the water intake (Allhenn and Lamprecht, 2011; Boimvaser et al., 2012). This porosity and the overall microparticle roughness could affect the degradation speed of the polymer matrix (Boimvaser et al., 2012) and finally have an impact on drug release kinetics.

The high solubility of PLGA in PC is a major advantage of this method allowing for fast polymer solution preparation. However, it also represents one significant obstacle in view of its plasticizing effects which strongly affects the microparticle solidification. So far, different studies pointed to such plasticizing influences of different organic solvents (Jain et al., 2000; Katou et al., 2008; Marquette et al., 2014).

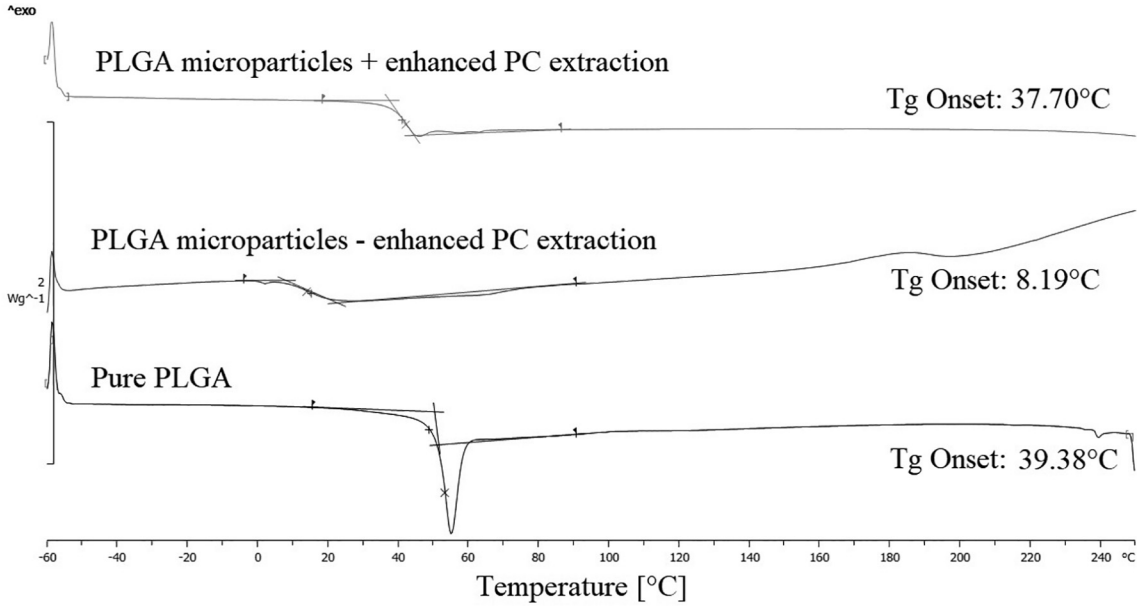


Fig. 7. Impact of the enhanced PC extraction step on the PLGA Tg shift; lower curve: pure PLGA, middle curve: PLGA microparticles without the enhanced PC extraction step; upper curve: PLGA microparticles with the enhanced PC extraction step.

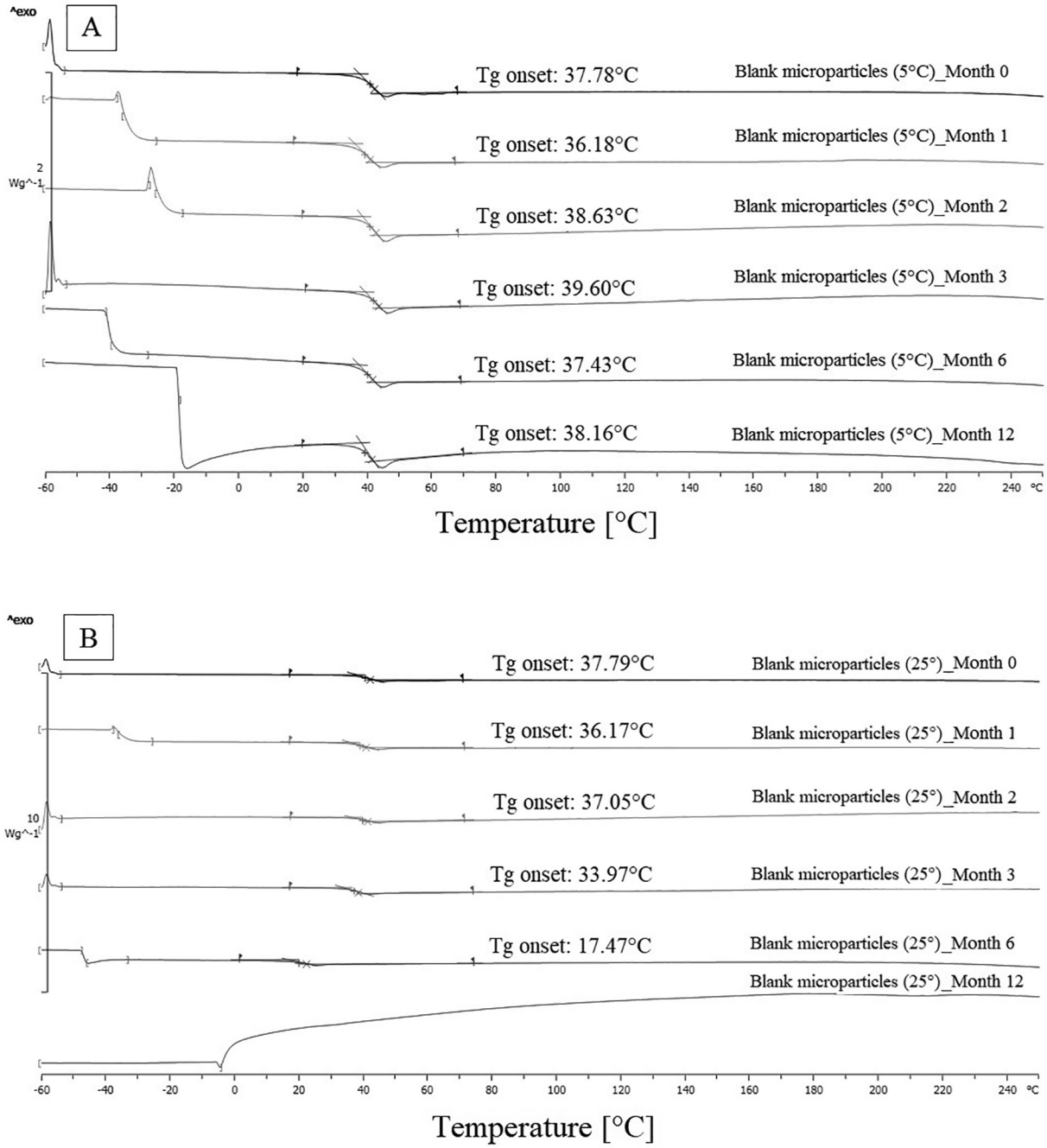


Fig. 8. Stability evaluation of PLGA microparticles at (A) 5 °C ± 3 °C and (B) 25 °C ± 2 °C/60% RH ± 5% RH during 12 months. All microparticle samples were prepared with the enhanced PC extraction step.

Precedent findings employing water-miscible organic solvents such as glycofurol and ethyl acetate showed very pronounced plasticizing effects, reflected in a significant Tg shift to lower temperatures (Allhenn and Lamprecht, 2011; Sah, 1997).

In the case where a classical solvent extraction step was applied (without the enhanced PC extraction), a higher residual PC content was impeding the formation of non-aggregated microparticles by its plasticizing effect. The resulting microparticle aggregation after drying was also observed for other solvents such as ethyl acetate (Sah, 1997) or ethyl formate (Sah, 2000) and could be prevented by lowering the residual solvent content (Marquette et al., 2014; Matsumoto et al., 2008; Vay et al., 2012).

During the extraction step, the intraparticulate solvent content is in equilibrium with the solvent amount present in the extraction phase (Katou et al., 2008). This equilibrium can, however, be shifted by an increased solvent elimination from the extraction phase (Katou et al., 2008). Different from the existing approaches, i.e. evaporation of the solvent or further extraction by increased external phase volume, it was achieved here by integrating an enhanced solvent extraction from the PLGA-rich droplet. The additional hydrolytic treatment triggers a PC mass transfer out of the particle matrix leading to residual PC concentrations far below typical values with similar solvents (Ali and Lamprecht, 2013; Allhenn and Lamprecht, 2011; Viehof et al., 2013). The application of the hydrolytic treatment simultaneously enhances PC diffusion into the external phase and leads to the formation of propylene glycol. Moreover, this rapidly formed propylene glycol is a PLGA non-solvent, accelerating the PLGA solidification. It is also noteworthy that the toxicity of propylene glycol is not an issue (McMartin,

2014). The instant PC degradation was observed during the drop to particle transformation investigation, where the surrounding of the droplets showed changes in light refraction when sodium hydroxide was present. The observed faster particle solidification in laser diffraction measurements further confirmed the enhanced PC diffusion/extraction out from the microparticle matrix. In addition, this analysis was performed after the emulsion formation during the PC extraction step. The apparent particle size increase for the sample without the enhanced PC extraction step is due to droplet collision and leads to a general increase in particle diameter (Fig. 4A) while the enhanced extraction step leads to a more or less immediate solidification of the particle by building a polymer crust on the droplet surface. Accordingly, the particle diameter does not change during the entire preparation period (Fig. 4B).

Even though the high hydrolytic reactivity of alkaline solutions towards PLGA (Croll et al., 2004) and polysorbate 80 (Kerwin, 2008) is well known, this study showed that the PC hydrolysis occurred faster and confirmed that all other excipients remained chemically unaffected. Even though acid-terminated PLGA (PLGA 502H) was used, which is known to be more hydrophilic and prone to degradation compared to the end-capped PLGA (Ding and Schwendeman, 2004), no degradation was observed. The quantification of PLGA degradation products (namely lactic and glycolic acid) under microparticle preparation conditions revealed that in the presence of PC, no PLGA degradation occurred and that polysorbate 80 also reduced the hydrolysis of the polymer. Besides, the overall duration of the enhanced PC extraction could be varied between 1.5 h and 4.5 h without affecting microparticle morphology or PLGA degradation, which states clearly the applicability and robustness of this methodology for the obtainment of solid microparticles.

The fact that residual amounts of PC were still present in the matrix after the enhanced PC extraction was confirmed both by quantification and visualization. It became clear that PC is localized inside small droplet-shaped cavities inside the particle matrix, which were formed during the microparticle solidification. A similar localisation pattern was reported from earlier studies when glycofurol was employed for microparticle preparation (Allhenn and Lamprecht, 2011). However, the amounts present in the particle interior did not affect the mechanical stability of the dry microparticles. It should be mentioned however, that the presence of residual amounts of non-toxic solvents can also possess advantages such as solubility and dissolution rate improvement, which was previously described for liquisolid formulations (Spireas and Sadu, 1998).

In case residual solvents still remain present after microparticle solidification, storage stability evaluation was previously suggested (Marquette et al., 2014). In this context, it was shown that residual ethyl acetate content of 36,400 ppm (3.64%) shifted the initial polymeric Tg from 53.9 °C to 37.2 °C. Interestingly, during storage at 5 °C ± 3 °C, 25 °C ± 2 °C/60% RH and 40 °C ± 2 °C/75% RH for 12 weeks, the Tg shifted back to higher temperatures, which was attributed to the volatility of ethyl acetate and the subsequent migration and depletion during storage (Marquette et al., 2014). As PC is non-volatile, the need for stability assessment in terms of a potential Tg shift is even higher. The observable microparticle liquefaction after twelve months at 25 °C ± 2 °C/60% RH ± 5% RH is in accordance with previous findings showing significant polymer degradation after 12 months at 25 °C (Dunne et al., 2000). Regardless of the present residual PC content in our study, short term storage (3 months) at 25 °C ± 2 °C/60% RH ± 5% RH and long-term storage at 5 °C ± 3 °C (> 12 months) showed acceptable stability profiles underlining the feasibility of the microparticle formulation approach.

5. Conclusion

PC revealed excellent properties as a low toxic organic solvent for polyester microparticle preparation. Especially, the unique technical option of an enhanced PC extraction step enabled a viable preparation method and solved the problems caused by high viscosity and residual solvent content encountered with other non-toxic solvents. Accordingly, the enhanced PC extraction step can be considered as a major advancement for the techniques employing low toxic cyclic ester-based solvents. The fact that no degradation issues were identified when other ester-based excipients were used underlines the robustness of the method and suggests numerous pharmaceutical applications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpharm.2018.03.062.

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