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Encapsulation of water-soluble drugs by an o/o/o-solvent extraction microencapsulation method

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ABSTRACT

A new o/o/o-solvent extraction microencapsulation method based on less toxic solvents is presented in this study. The drug is dissolved/dispersed into a poly(D,L-lactide)/or poly (D,L-lactide-co-glycolide) (PLGA) solution in a water-miscible organic solvent (e.g., dimethylsulfoxide or 2-pyrrolidone) (o_1), followed by emulsification into an oil phase (o_2) (e.g., peanut oil). This emulsion is added to the external phase (o_3) to solidify the drug-containing polymer droplets. The polymer solvent and the oil are extracted in an external phase (o_3) (e.g., ethanol), which is a nonsolvent for the polymer and miscible with both the polymer solvent and the oil. One major advantage of this method is the reduced amount of solvent/nonsolvent volumes. In addition, very high encapsulation efficiencies were achieved at polymer concentration of 20%, w/w for all investigated polymers and o_1/o_2 phase ratios with ethanol as the external (o_3) phase. The encapsulation efficiency was very low (<20%) with water as external phase. The particle size of the microparticles increased with increasing polymer concentration and o_1/o_2 phase ratio and larger microparticles were obtained with 2-pyrrolidone compared to dimethylsulfoxide as polymer solvent (o_1). After an initial burst, in vitro drug release from the microparticles increased for the investigated polymer as follows: Resomer® RG 506 > RG 756 > R 206. A third more rapid release phase was observed after 6 weeks with Resomer® RG 506 due to polymer degradation. Similar drug release patterns were obtained with the o/o/o and w/o/w multiple emulsion methods because of similar porous structures. This new method has the advantages of less toxic solvents, much lower preparation volume and solvent consumption and high encapsulation efficiencies when compared to the classical w/o/w method.

1. Introduction

Solvent evaporation/extraction or organic phase separation methods are popular techniques for the encapsulation of macromolecular drugs within biodegradable PLGA polymers (Ahmed et al., 2008). In the solvent evaporation/extraction method, the drug is dissolved or dispersed as powder or emulsified as aqueous solution in an organic polymer solution. Depending on the drug properties (e.g., solubility, stability), the drug-containing polymer phase is then emulsified into an external oil (o/o or w/o/o) (Sturesson et al., 1993; Wang et al., 1991; Yeh et al., 1995; Blanco and Alonso, 1998) or aqueous phase (o/w or w/o/w) (Bodmeier et al., 1991; O'Donnell and McGinity, 1997; Cleek et al., 1997; Couvreur et al., 1997; Freytag et al., 2000). The polymer droplets harden into solid microparticles by solvent extraction/evaporation. In the phase separation techniques (Thomasin et al., 1996; Nihant et al., 1995; McGee et al., 1995; Jain, 2000), an organic nonsolvent is added to the polymer solution containing the dispersed drug, resulting in the formation of coacervate droplets around the dispersed drug. The droplets are hardened with suitable organic nonsolvents for the polymer and drug.

Major problems of both microencapsulation methods are the use of toxic solvents (e.g., methylene chloride, chloroform, acetonitrile, etc.) and residual solvents in the microparticles (Brannon-Peppas and Vert, 2000). Methylene chloride is the most frequently used solvent in the solvent extraction/evaporation method. It has a good solvation power for a broad range of biodegradable polymers and its high volatility facilitates easy removal by evaporation. Because of toxicity issues, the solvent selection for the microparticles preparation has received some attention (Bodmeier and McGinity, 1988; Arshady, 1990) and a number of non-chlorinated solvents (e.g., ethyl acetate or acetonitrile) have been evaluated. However, the replacement of the toxic and environmentally harmful methylene chloride, which is a group 2 solvent according to the ICH guidelines (solvents to be limited), by the less toxic ethyl acetate (a group 4 solvent) lead to a decreased drug encapsulation efficiency (Herrmann and Bodmeier, 1995; Guideline for residual solvents, 2009).

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According to ICH guideline, 50 mg or less per day (corresponding to 5000 ppm or 0.5%) of class 3 solvents are acceptable for human use without justification (Guideline for residual solvents, 2009). There are several examples of class 3 solvent applications in the development of novel drug delivery systems. For instance, the in situ implant forming technology was developed by dissolving PLA- or PLGA-polymers in water-miscible solvents, such as N-methyl-2-pyrrolidone (NMP), dimethylsulfoxide (DMSO), 2-pyrrolidone or PEG 400 (Dunn et al., 1990; Tipton and Fujita, 1991; Shah et al., 1993; Lambert and Peck, 1995; Kranz and Bodmeier, 2007, 2008; Kranz et al., 2008). Viadur®, a subcutaneously implanted leuprolide acetate-containing device uses 104 mg DMSO as solvent (Strickley, 2004).

In this study, a new solvent extraction microencapsulation method based on less toxic and biocompatible solvents (DMSO and 2-pyrrolidone) was developed to overcome toxic residual solvent problem. The influence of solvent, polymer type and concentration, drug loading, phase ratio and external phase were investigated with regard to micromeritic, encapsulation efficiency and in vitro drug release properties of the microparticles.

2. Materials and methods

2.1. Materials

The following materials were used as received and were at least of reagent grade: antisense oligonucleotides (MW 6347.5 Da) (Isis Pharmaceuticals Ind., Carlsbad, CA, USA); poly(D,L-lactide) (PLA) (Resomer® R 206, MW 125.000 g/mol), poly(D,L-lactide-co-glycolide 50:50) (PLGA) (Resomer® RG 506, MW 100,000 g/mol), poly(D,L-lactide-co-glycolide 75:25) (Resomer® RG 756, MW 89,000 g/mol) (Böhringer Ingelheim KG, Ingelheim, Germany); polyvinyl alcohol (Mowiol® 40–88, Clariant GmbH, Frankfurt, Germany); 2-pyrrolidone (Soluphor®, BASF AG, Ludwigshafen, Germany); peanut oil (Henry Lamotte GmbH, Bremen, Germany); dimethylsulfoxide, ethanol, methylene chloride, n-hexane, sodium azide (Merck KGaA, Darmstadt, Germany).

2.2. Preparation of microparticles

2.2.1. o/o/o method

Antisense oligonucleotides (11–45 mg) were dissolved or dispersed in a polymer (Resomer® RG 506, RG 756 or R 206) (100–400 mg) solution in 0.90 ml dimethylsulfoxide (DMSO) or 2-pyrrolidone by shaking (phase o_1), respectively. This phase was emulsified into peanut oil (phase o_2) at ratios of 1:1, 1:2 and 1:3 for 30 s with an Ultra-Turrax (Ultra-Turrax T 25, Janke & Kunkel GmbH & Co., IKA Labortechnik, Staufen, Germany) at 9500 min^{-1} resulting in the primary o_1/o_2 emulsion. This emulsion was emulsified into 400 ml ethanol (phase o_3) under propeller (Heidolph Elektro, Kehlheim, Germany) stirring (250 rpm) for 30 min to extract both the solvent and peanut oil. After polymer solidification, the microparticles were separated by wet sieving and washed at least three times in 30 ml ethanol by stirring (75 rpm) for 30 min. The microparticles were collected by filtration, air-dried for 2 days and stored in a desiccator. A schematic representation of the ($o_1/o_2/o_3$) solvent extraction method is shown in Fig. 1.

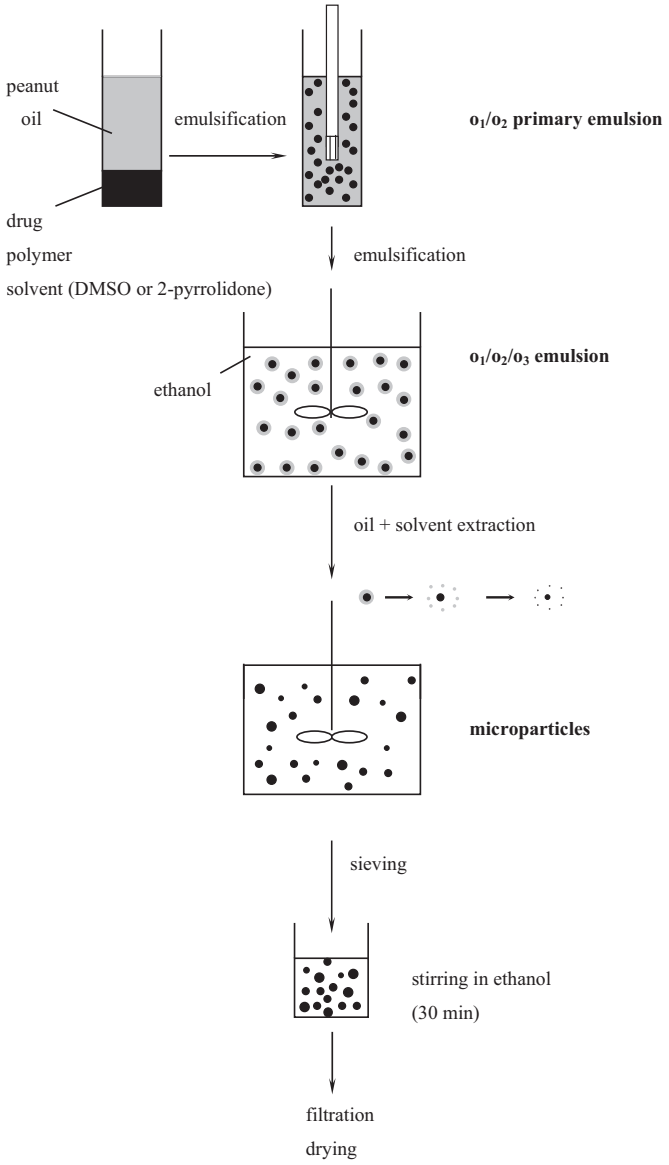

Fig. 1. Schematic representation of the o/o/o solvent extraction method.

2.2.2. o/o/w method

The primary o_1/o_2 emulsion (prepared as described above) was emulsified in 400 ml water under propeller stirring (Heidolph Elektro, Kehlheim, Germany) for 5 min and then further agitated for 1 h with a magnetic stirrer (Janke & Kunkel GmbH & Co., IKA Labortechnik, Staufen, Germany) to extract the solvent resulting in the solidification of the polymeric particles. The microparticles were separated by wet sieving and washed at least three times in 100 ml n-hexane by stirring for 15 min. The resulting microparticles were air-dried for 2 days and stored in desiccator.

2.2.3. w/o/w method

23 mg oligonucleotides were dissolved in 0.2 ml water and emulsified into a solution of 200 mg Resomer® RG 506 in 5.5 ml methylene chloride by probe sonication (Sonoplus® HD 250, Bandelin Electronic, Berlin, Germany) for 30 s under ice-cooling. The resulting primary w/o-emulsion was dispersed into an external phase (500 ml 0.25%, w/v PVA containing 0.25 M NaCl) under propeller stirring (Heidolph Elektro, Kehlheim, Germany) for 5 min and then further agitated for 1 h with a magnetic stirrer (Janke and Kunkel GmbH & Co., IKA Labortechnik, Staufen, Germany) for the solidification of the polymeric particles by solvent extraction/evaporation. The microparticles were separated from the external aqueous phase by wet sieving, washed with water, collected by filtration and dried in a desiccator for 3 days at room temperature.

2.3. Viscosity measurement

A rotational rheometer (Rheostress RS 100, Haake Meß-Technik GmbH, Karlsruhe, Germany) was used to measure the viscosity of the polymer solutions. 10, 20, and 30%, w/w Resomer® R 506 solutions in DMSO or 2-pyrrolidone were analyzed using a plate/cone geometry (20 mm diameter, 1° angle) with a controlled shear rate of 5 s^{-1} at 24 °C ($n = 3$).

2.4. Particle size

The particle size distribution of the microparticles was analyzed by laser light scattering including Polarization Intensity Differential Scattering (PIDS) with a Coulter® LS 230 using the small volume module (Coulter Electronics, Krefeld, Germany).

2.5. Determination of the actual drug loading and encapsulation efficiency

Microparticles (10–12 mg, accurately weighed) dissolved in 8 ml 0.5 M NaOH, followed by agitation in a horizontal shaker (IKA HS 501 digital horizontal Shaker, Janke & Kunkel & Co IKA Labortechnik, Staufen, Germany) for 12 h ($n = 3$). The drug concentration in the aqueous phase was determined by UV-spectrophotometry at $\lambda = 260$ nm (Shimadzu UV 2101 PC UV–vis scanning spectrophotometer, Kyoto, Shimadzu Japan) (2). The polymer did not interfere at the wavelength used. The actual drug loading and encapsulation efficiency were calculated as follows:

$$\text{Actual drug loading}(\%) = \left(\frac{\text{drug mass in microparticles}}{\text{mass of microparticles}}\right) \times 100$$

$$\text{Encapsulation efficiency}(\%) = \left(\frac{\text{actual drug loading}}{\text{theoretical drug loading}}\right) \times 100$$

2.6. Drug release

10 mg microparticles were placed in 8 ml pH 7.4 phosphate buffer (USP XXIV) containing 0.1%, w/v sodium azide and shaken at 37 °C in a horizontal shaker (GFL 3033, Gesellschaft für Labortechnik, Burgwedel, Germany) at 80 rpm. At predetermined time intervals, 2 ml samples were withdrawn and replaced with fresh medium. The drug concentration was detected UV-spectrophotometrically at $\lambda = 260$ nm ($n = 3$).

2.7. Determination of residual solvent

20–25 mg placebo PLGA 506 microparticles were dissolved in 3 ml 0.5 M NaOH. After polymer hydrolysis, the pH of the solution was adjusted to approximately pH 7.4 by dropwise addition of 1 N HCl. The solvent (DMSO) concentration in the buffer medium was measured with a Shimadzu-HPLC system at 220 nm (UV-detector) at room temperature. A 40 μl sample volume was injected onto a LiChrospher-100 RP 18.5 μm vertex column (Knauer GmbH, Berlin, Germany) using an acetonitrile/pH 7.4 phosphate buffer mixture (10:90, v/v) as the mobile phase at a flow rate of 1.0 ml/min (Kranz, 2000). Solutions of DMSO in phosphate buffer pH 7.4 of known concentrations were checked with respect to linearity ($r > 0.99$), sensitivity (2 μg/ml), precision (<1.3 RSD) and accuracy (<5% RSD).

2.8. Scanning electron microscopy (SEM)

The external and internal morphology of the microparticles was studied by scanning electron microscopy (Philips SEM 515, type PW 6703, Philips Optical Electronics, Eindhoven, Netherlands). The microparticles were dispersed in a solvent-free glue (UHU GmbH, Bühl, Germany) followed by cutting the dried matrix with a razor blade in order to observe the internal structure. The microparticles were coated for 230 s with gold–palladium under an argon atmosphere using a gold sputter module in a high-vacuum evaporator (Sputter coater device 040, Blazers Union, Liechtenstein). The coated microparticles were then observed with a scanning electron microscope.

2.9. Differential scanning calorimetry (DSC)

Thermograms of the polymers, solvents and microparticles were obtained using a computer-interfaced differential scanning calorimeter (DSC 821, Mettler Toledo AG, Gießen, Germany) equipped with a low temperature environmental chamber. Dried samples (6–8 mg) were sealed in aluminum pans. Samples were heated to 80 °C, cooled to 20 °C and reheated to 80 °C. The second heating run was evaluated. The scanning rate was 10 °C/min for the heating and 40 °C/min for the cooling cycles. All tests were carried out in a nitrogen atmosphere.

3. Results and discussion

The classical solvent evaporation method mostly requires toxic organic solvents such as methylene chloride to dissolve the PLGA polymers. The purpose of this study was to develop a microencapsulation method with less toxic solvents and high encapsulation efficiencies for highly water-soluble drugs, possibly also without the use of water as internal or external phase. In the proposed o/o/o solvent extraction microencapsulation method, the drug is dissolved or dispersed in a solution of the polymer in a water-miscible organic solvent (e.g., dimethylsulfoxide and 2-pyrrolidone). This phase (o_1) is dispersed into peanut oil (o_2) to form the primary emulsion o_1/o_2. The solidification of the polymer droplets then occurs by extraction of both solvent and oil into an external phase (o_3) (ethanol) under continuous stirring. The solvent for the polymer does not have to be volatile or water-immiscible, as usually required in the solvent evaporation methods.

3.1. Micromeritic properties of the microparticles

Microparticles prepared by the classical w/o/w-method are spherical with a smooth external surface (Fig. 2A). The internal structure is composed of a dense polymeric shell with a porous or sponge-like center. Microparticles prepared by the o/o/o-method have a smooth surface and are highly porous with finger-like cavities in the center of the microparticles (Fig. 2B).

Microparticles were smaller and with a narrower size distribution with DMSO as polymer solvent when compared with 2-pyrrolidone (Table 1 and Fig. 3A). The lower solution viscosity of the DMSO-polymer solution when compared to 2-pyrrolidone (Table 1) resulted in smaller droplets during emulsification. As expected, higher polymer concentrations in DMSO (10–30%) resulted also in larger particles (Table 1 and Fig. 3B). The particle size decreased and the size distribution was narrower with increasing phase ratio o_1/o_2 (Table 1 and Fig. 3C). The reason of the observed effects is that the obtained microparticles size depends on the diameter of initial emulsion droplets, which are smaller and more finely dispersed in a large oil amount. Therefore, the droplet coalescence was probably less with a larger oil volume.

3.2. Encapsulation efficiency

Among the three methods investigated to prepare microparticles (RG 506), the o/o/o-method resulted in the highest encapsulation efficiency (Table 2). A high encapsulation efficiency was achieved at the higher polymer concentration 20% (w/w) and

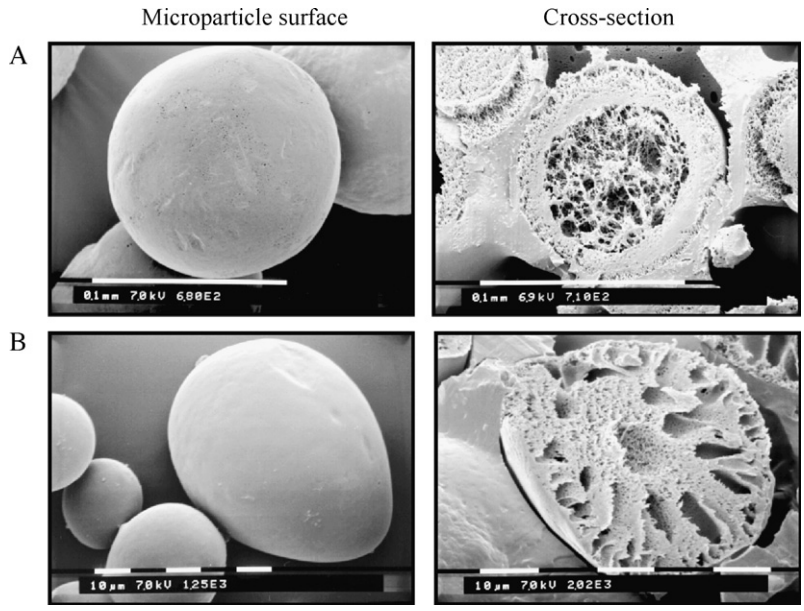

Fig. 2. Scanning electron microscopy of microparticles prepared by [A] w/o/w solvent extraction/evaporation method (methylene chloride, RG 506 3.5%) and [B] o/o/o solvent extraction method (DMSO, RG 506 20%).

Table 1
Effect of formulation parameters on the average particle size of microparticles prepared by the o/o/o solvent extraction method (RG 506).

Solvent type	Polymer concentration (%w/w)	Viscosity of polymer solution (Pa s)	o_1/o_2 phase ratio	Average particle size (μm)
DMSO	10	0.09 ± 0.02	1:3	19.87 ± 0.48
	20	1.84 ± 0.07	1:1	71.28 ± 1.02
			1:2	56.29 ± 0.78
			1:3	41.55 ± 1.32
			1:4	35.08 ± 0.92
	30	19.54 ± 0.78	1:3	139.1 ± 2.12
2-Pyrrolidone	20	16.20 ± 1.65	1:3	90.66 ± 1.62

Table 2
Encapsulation of oligonucleotide within PLGA microparticles prepared by different methods (DMSO; polymer:oil = 1:3).

Method		Polymer		Drug in polymer solution	Theoretical loading (%)	Actual loading (%)	Encapsulation efficiency (%)
		Type	Conc. (w/w%)				
o/o/o Extraction		RG 506	10	Dissolved	10.5	4.7	44.8 ± 0.4
			20	Dissolved	10.2	10.2	100.0 ± 0.8
			30	Partially dissolved	10.3	8.3	80.6 ± 0.6
		RG 756	20	Dissolved	10.3	10.2	99.0 ± 0.5
		R 206	20	Dissolved	10.6	10.5	99.1 ± 0.4
o/o/w Extraction		RG 506	20	Dissolved	10.1	1.9	18.8 ± 0.4
w/o/w Extraction	Evaporation	RG 506	3.5	Emulsified	10.6	8.1	76.4 ± 0.5

30% (w/w) (Table 2), while only 44.8% were encapsulated at the lower polymer solution concentration of 10%. Obviously, the 20% (w/w) polymer solution had a higher viscosity resulting in a more dense skin layer formation, which prevented drug diffusion. This was not the case for the 10% (w/w) polymer solution (Table 1). Microparticles prepared with 30% polymer solution had a slightly lower encapsulation efficiency compared to those prepared with 20% polymer solution (Table 2). This is caused by incomplete dissolution of the drug in the 30% polymer solution. The undissolved drug was not encapsulated.

The encapsulation efficiency was found to be independent of the investigated polymer types. The Resomers RG 506, RG 756 and R 206 as 20% polymer solution in DMSO showed the same encapsulation efficiencies close to 100% (Table 2).

Microparticles with high encapsulation efficiency (close to 100%) were also obtained by the o/o/o solvent extraction method irrespective of the o_1/o_2 phase ratios used (1:1, 1:2 and 1:3) (data not shown). This is due to the drug insolubility in both peanut oil (o_2) and the external ethanol phase (o_3).

The encapsulation efficiency was lowest with microparticles prepared with DMSO with an external aqueous phase (18.8%, o/o/w method) and also lowers with microparticles prepared by solvent extraction/evaporation method (76.4%, w/o/w) (Table 2). This is because of the high water solubility of the oligonucleotide and thus it is partitioning into the external aqueous phase.

3.3. Residual solvent in the microparticles

The efficiency of DMSO extraction with the nonsolvent (ethanol) during microparticle preparation was investigated by determining the residual DMSO in the microparticles by HPLC. Increasing the polymer solution concentration resulted in higher residual DMSO (Table 3) because of the higher solution viscosity and thus larger particle size (Fig. 3B) and thus lower surface area available

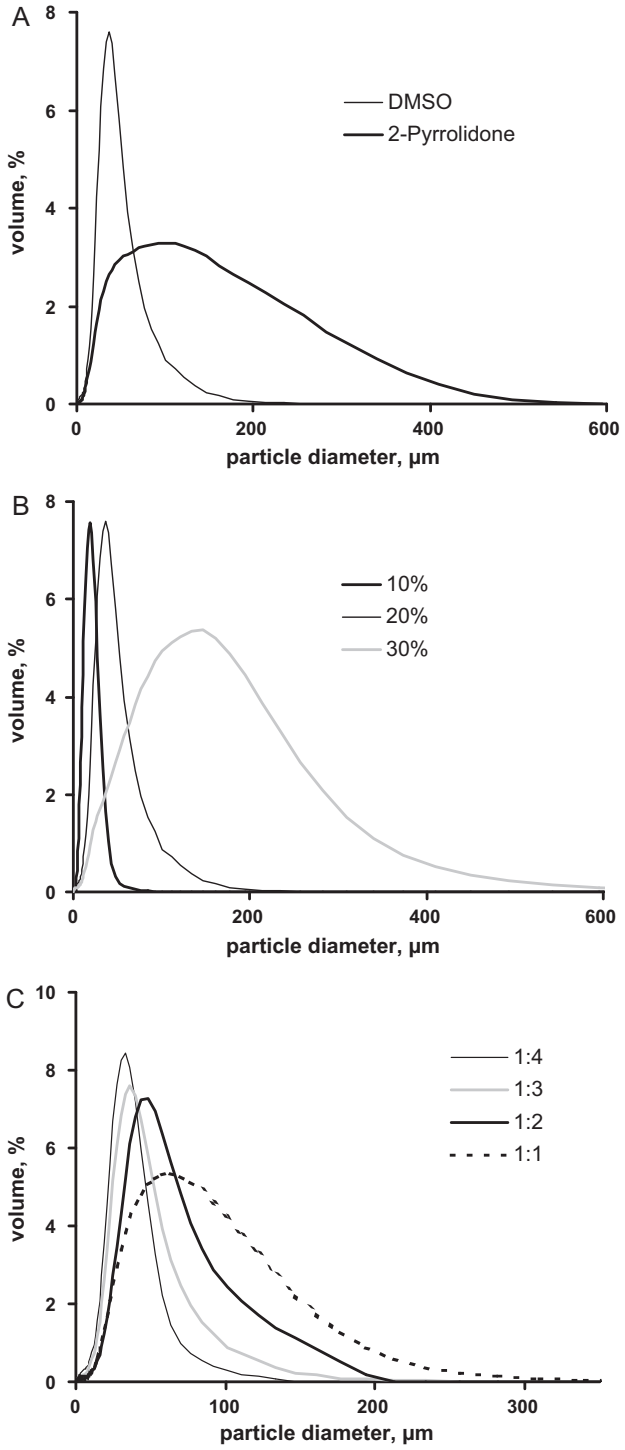

Fig. 3. Effect of formulation parameters on the particle size distribution of microparticles prepared by the o/o/o solvent extraction method: [A] solvent type (RG 506 20%, o_1/o_2 ratio 1:3), [B] solvent concentration, (DMSO, RG 506, o_1/o_2 ratio 1:3) and [C] o_1/o_2 ratio (DMSO, RG 506 20%, o_1/o_2 phase ratio = 1:3).

for DMSO diffusion in the external ethanol phase. Increasing the stirring time decreased the residual DMSO content to less than 0.2% for microparticles prepared with the highest polymer concentration of 30% (Table 3). This is an acceptable level according to ICH-guidelines (Guideline for residual solvents, 2009). The longer stirring times did not result in a decrease in drug content because of the insolubility of the oligonucleotide in ethanol.

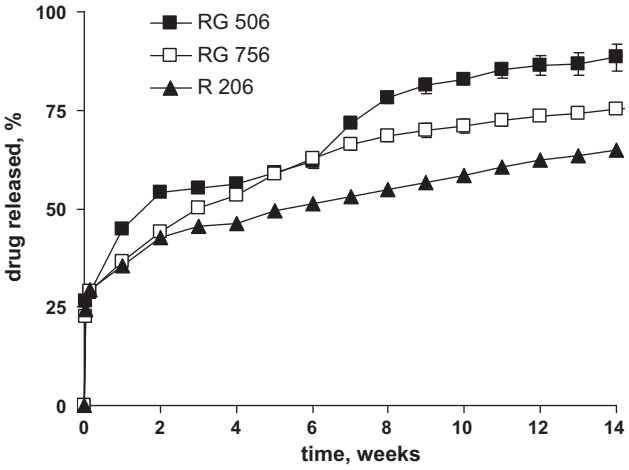

Fig. 4. Effect of the polymer type on oligonucleotide release from microparticles, prepared by the o/o/o solvent extraction method (DMSO, drug loading 10%, polymer concentration 20%, w/w, o_1/o_2 phase ratio = 1:3, particle size 50–100 µm, pH 7.4, $n = 3$).

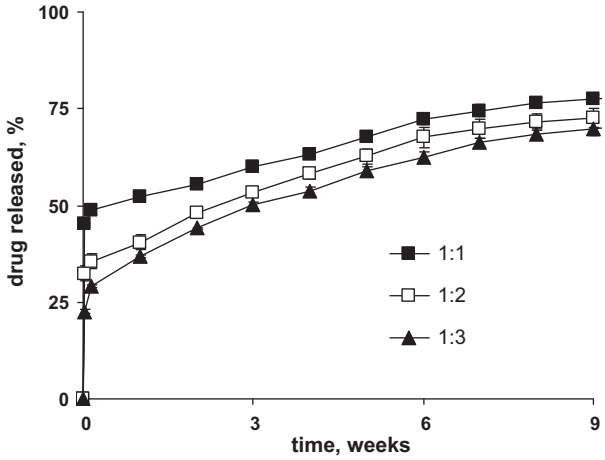

Fig. 5. Effect of the o_1/o_2 phase ratio on oligonucleotide release from microparticles, prepared by the o/o/o solvent extraction method (Resomer® RG 756 20%, DMSO, particle size 50–100 µm, pH 7.4, $n = 3$).

3.4. In vitro drug release

An initial burst release (approx. 25% within 24 h), followed by extended drug release over 14 weeks was observed for microparticles prepared with all investigated polymers (20%, w/v polymer solution in DMSO) (Fig. 4). The initial burst can be attributed to the drug available on the surface and dissolution through pre-existing pores and channels, which formed during the solvent extraction process. The remaining drug was released because of slow polymer erosion. This can be explained by the observation that rapid morphological changes at the surface of the microparticles occurred during the first 24 h (data not presented). A skin layer (film) with decreased porosity was observed around the microparticles after 24 h of incubation (Ahmed, 2003). During the drug release test the release medium penetrated into the microparticles and acted as plasticizer (Ahmed and Bodmeier, 2010). Consequently, the glass transition temperature (Tg) of the polymer decreased and as a result the microparticles swelled and softened. Therefore, pores, especially those present at the surface of the microparticles were filled and a non-porous skin around the microparticles was formed. This skin layer becomes more and more dense, leaving almost no pores behind and after 24 h it act as diffusion barrier. A similar mechanism

Table 3
Effect of the polymer concentration and stirring time on DMSO extraction from microparticles (RG 506, placebo microparticles, o_1/o_2 phase ratio = 1:3).

Polymer concentration (% w/w)	Viscosity of polymer solution (Pa s)	Stirring time (min)	Residual solvent (%)
10	0.09	15	0.02
		75	0.02
		150	0.01
20	1.84	15	0.15
		75	0.08
		150	0.04
30	19.54	15	0.70
		75	0.17
		150	0.13

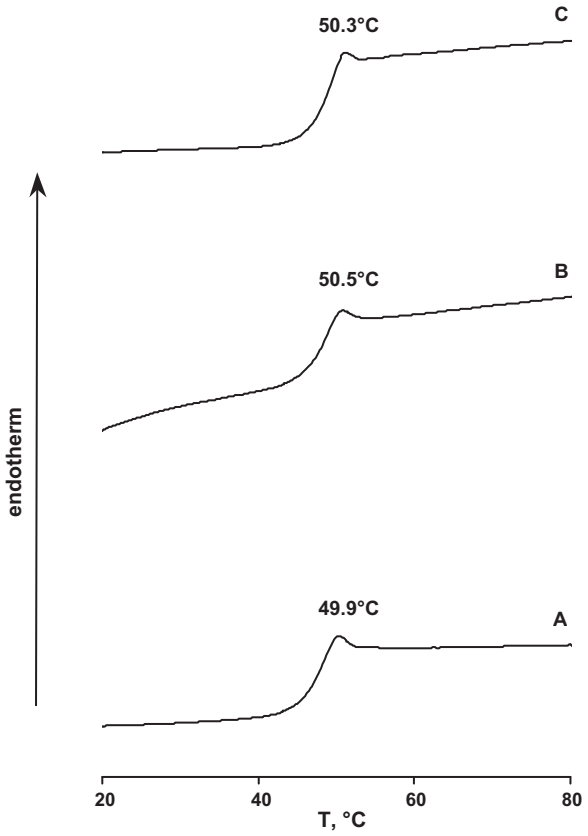

Fig. 6. Differential scanning calorimetry (DSC) thermograms of [A] polymer Resomer® RG 506 as well placebo microparticles prepared with Resomer® RG 506 by [B] w/o/w solvent evaporation and [C] o/o/o solvent extraction methods.

of morphological changes at the surface of microparticles during a drug release test was observed and reported by Wang et al. (2002).

The release rate after the burst release increased slightly with increasing glycolide content of the polymer due to its increasing hydrophilicity (Resomer® RG 506 > RG 756 > R 206). After 6 weeks, a third release phase with polymer RG 506 was observed caused by erosion of the polymer.

The initial burst decreased with increasing peanut oil portion (decreasing phase ratio o_1/o_2 from 1:1 to 1:3) (Fig. 5). This is due to reduced rate of the solvent extraction caused by increased amount of peanut oil and consequently decreased the porosity of microparticles (Kranz, 2000).

3.5. Comparison of o/o/o and w/o/w methods

The preparation of microparticles by solvent extraction/evaporation methods (e.g., o/o or w/o/w) usually requires water-immiscible and volatile solvents, which are toxic or flammable (e.g., acetone, methylene chloride, chloroform, etc.).

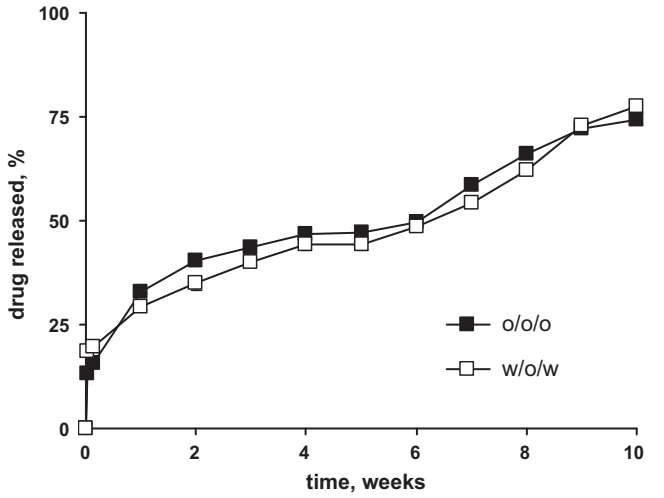

Fig. 7. Oligonucleotide release from Resomer® RG 506 microparticles, prepared by the w/o/w solvent evaporation and the o/o/o solvent extraction methods (drug loading 8%, particle size 50–100 μm, pH 7.4, $n = 3$).

In contrast, less toxic and nonflammable solvents (e.g., dimethylsulfoxide and 2-pyrrolidone) are used in the proposed o/o/o method.

Another advantageous aspect of the o/o/o vs. w/o/w method is the higher polymer concentration in solution, namely 20% vs. 3.5%, w/w, respectively (Table 2). The higher polymer concentration results in a smaller preparation volume and lower solvent consumption.

The encapsulation efficiency was almost 100% vs. 76.4% for microparticles prepared by the o/o/o vs. the w/o/w solvent extraction/evaporation method, respectively (Table 2). The low encapsulation efficiency in the case of w/o/w method was due to high water solubility of the oligonucleotide and its partitioning into aqueous external phase. In case of o/o/o-method, high encapsulation efficiency of oligonucleotides was achieved because oligonucleotide is practically insoluble in ethanol used as external phase to extract DMSO and peanut oil.

No changes in glass transition temperature (Tg) between original starting polymer (Resomer® 506, Tg = 49.9 °C) and microparticles prepared by different methods were observed (Fig. 6). A possible plasticization effect on the polymer during microparticles preparation (caused by e.g., residual solvent or oil, etc.) can be excluded.

The oligonucleotide release from microparticles prepared by the o/o/o and the w/o/w techniques was similar with an initial burst release of approx.15% and 20% after 24 h followed by sustained release (Fig. 7). After six weeks, a third phase of drug release was observed due to polymer degradation (RG 506) (Fig. 7). The similar morphology of microparticles prepared by o/o/o and w/o/w methods, i.e. smooth external surface and porous internal structure explained the similar drug release (Fig. 2).

4. Conclusion

The highly water-soluble oligonucleotide was encapsulated very efficiently into biodegradable polymeric microparticles by the o/o/o extraction method. This microencapsulation method has the following advantages over the w/o/w method: use of less toxic solvents, lower preparation volume and solvent consumption and high encapsulation efficiency.

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