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Multifactorial Design of Poly(D,L-lactic-*co*-glycolic acid) Capsules with Various Release Properties for Differently Sized Filling Agents

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ABSTRACT: The hydrolytic degradation and corresponding content release of capsules made of poly(D,L-lactic-*co*-glycolic acid) (PLGA) strongly depends on the composition and material properties of the initially applied copolymer. Consecutive or simultaneous release from capsule batches of combinable material compositions, therefore, offers high control over the bioavailability of an encapsulated drug. The keynote of this study was the creation of a superordinated database that addressed the correlation between the release kinetics of filling agents with different molecular weights from PLGA capsules of alternating composition. Fluorescein isothiocyanate (FITC)–dextran (with molecular weights of 4, 40, and 2000 kDa) was chosen as a model analyte, whereas the copolymers were taken from various 50:50 PLGA, 75:25 PLGA, and polylactide blends. With reference to recent publications, the capsule properties, such as the size, morphology, and encapsulation efficiency, were further modified during production. Hence, uniform microdisperse and polydisperse submicrometer nanocapsules were prepared by two different water-in-oil-in-water emulsification techniques, and additional effects on the size and morphology were achieved by capsule solidification in two different sodium salt buffers. The qualitative and quantitative examination of the physical capsule properties was performed by confocal laser scanning microscopy, scanning electron microscopy, and Coulter counting techniques to evaluate the capsule size distribution and the morphological appearance of the different batches. The corresponding agent release was quantified by fluorescence measurement of the FITC–dextran in the incubation media and by the direct measurement of the capsule brightness via fluorescence microscopy. In summary, the observed agent release showed a highly controllable flexibility depending on the PLGA blends, preparation methods, and molecular weight of the used filling substances. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2013

KEYWORDS: biodegradable copolymers (PLGA); microcapsules, submicrometer / nanocapsules; FITC-dextran release; drug delivery system; biomedical applications

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INTRODUCTION

The entrapment of chemical agents and their subsequent release from various carriers at the microscale and nanoscale has been widely studied over the past decades. Nowadays, (bio)degradable carriers consisting of polyurethane, poly(ε-caprolactone), and poly(D,L-lactic-*co*-glycolic acid) (PLGA) copolymers can be produced by various techniques, including spray drying, nanoprecipitation, and solvent evaporation.[1–17] Among these, the focus has especially been on capsules made of PLGA because of a long list of approved properties, including biocompatibility, that have high relevance in the field of medical treatment. The biodegradation products of PLGA are nontoxic, nonimmunogenic, and noncarcinogenic metabolites; these can be eliminated either by entrance into the tricarboxylic acid cycle or via kidney excretion.[3] Compared to other natural biocompatible polymers that have been studied for drug release, such as bovine serum albumin, human serum albumin, collagen, gelatin, or hemoglobin, PLGA features lower costs and higher available purity.[4] In addition, PLGA copolymers have a glass-transition temperature above the physiological temperature of 37°C, which results in

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sufficient mechanical strength. This is important for sustaining the physical stress to which drug-delivery devices are exposed to during application in living organisms.[4] In addition to mechanical strength, the swelling behavior, capacity to undergo hydrolysis, and subsequent biodegradation rates of PLGA are directly influenced by the crystallinity of the polymer. The crystallinity, in turn, can be influenced by variations in the type, molecular weight, and mixing ratios of the individual monomer components (lactide and glycolide) of the copolymer chain.[4] Hence specific application-related properties can be obtained by the choice of the right copolymer composition.

Although former studies have shown the possibility of incorporating depot drugs in bulk carriers made from PLGA such as bone replacements,[18–21] research efforts on controlled agent release for medical applications have mostly been based on small capsules. Along with general advantages such as easy fabrication, good reproducibility, and a chance to incorporate chemicals of various kinds, controlled release from capsules can greatly influence the bioavailability of drugs that have a short biological half-life, are instable, degrade quickly in the gastrointestinal tract, or are toxic in high concentrations.[3] Capsules made of thermoplastic aliphatic polyesters such as PLGA with an ideal size of less than 125 μm[4] can, therefore, be administered by needle injections via intramuscular, subcutaneous, or intravenous routes to reveal their potential for extended release applications. A capsule size of 3–8 μm would meanwhile be required to prevent capillary clogging and phagocytosis.[3]

Established production techniques of drug-carrying PLGA capsules are mostly based on water-in-oil (w/o) emulsions consisting of the copolymer solved in an appropriate solvent and the filling agent in an aqueous phase.[4] In a first step, the stirring of the emulsion produces the distribution of the filling agent in small water droplets within the polymer phase. Consecutive solvent evaporation and corresponding polymer precipitation is then followed by encapsulation of the filling agent in capsules of divergent morphology. The improved capsule homogeneity and acceleration of the precipitation process can be achieved by ejection of the w/o dispersion of a nozzle. The formation of small droplets with a large surface-to-volume ratio under high-pressure conditions forces their immediate solidification by fast solvent evaporation (spray drying).[10] Alternatively, the transfer of the w/o dispersion into a second aqueous phase of significantly larger volume leads to w/o droplets floating in a water-in-oil-in-water (w/o/w)-based solution, followed by capsule solidification due to the extraction of the solvent from the emulsion droplets into the surrounding aqueous phase.[1,2,11]

Model filling agents for previous release studies were taken from drug classes of vaccines, peptides, proteins, and micromolecules,[3,4] such as blue dextran 2000,[2] progesterone,[3] procaine hydrochloride,[6] bovine serum albumin,[7,12] benzocaine,[13] insulin,[14] and green fluorescent protein. Studies of the treatment of osteomyelitis have also been focused on the encapsulation of growth factors such as recombinant bone morphogenetic proteins (rhBMP-2).[15] Furthermore, poly(ethylene imine)–poly(ethylene glycol) copolymers carrying antisense oligonucleotides to induce dystrophin expression in the muscles cells of patients with Duchenne muscular dystrophy were encapsulated in an additional shell of PLGA to block the positive surface charge on the poly(ethylene imine)–poly(ethylene glycol)–antisense oligonucleotide capsules, which limits their biodistribution.[8]

Nonetheless, most research activities are still focused on the development and benefits of new production systems, their modifications[22–33] by the application of high- and low-molecular-weight PLGA blends,[22] polymer coatings,[24] surfactants and stabilizers,[25–27] double-emulsion techniques,[27,28] or core–shell hybrid microcapsules (MCs).[29,30] Despite these efforts, a decent optimization and evaluation of already existing techniques is often neglected. Hence, in this article, we present a general overview of how attributes such as the stability, shape, and size of capsules made from PLGA are correlated with the release efficiency of entrapped molecules of different sizes. We describe the release properties of PLGA capsules made from lactic acid to glycolic acid copolymer composition ratios of 50:50, 75:25, and 100:0. Moreover, each of the chosen PLGA compositions was examined as high- and low-molecular-weight polymer variants. Because both attributes are known to have a significant influence on the hydrolytic degradation rates of PLGA, the corresponding release behavior from the capsules was observed for FITC–dextrans with three different molecular weights (4, 40, and 2000 kDa). The study was extended further by two different capsule preparation techniques based on a w/o/w emulsification and subsequent solvent evaporation.

The aim of the first method was to yield PLGA capsules of improved homogeneity on the micrometer scale to ensure the reproducibility of the results. This was done through the application of a dispersion cell from Micropore Technologies (United Kingdom). Herein, the production of homogeneous capsules was achieved by the injection of the w/o emulsion through a metal membrane with a uniform pore size into a glass cylinder filled with an aqueous solution.[1] Despite the simple setup and comparability of the results, to our knowledge, these device-related benefits have never been used for the production of a database to summarize the release kinetics of various sized filling agents from homogeneous carrier batches with various properties. Because of its high value for improved efficiency in pharmacokinetic applications, in this article, we address the creation of such a database. To extend the range of the study, capsules of submicrometer/nanoscale size were produced by the ultrasound dispersion of a PLGA/FITC–dextran emulsion in an alternative process, as described elsewhere.[9]

Optional modifications of the both capsules types were realized by the application of various emulsifiers, including poly(vinyl alcohol) (PVA) and poloxamers (Pluronic F127) during the preparation process. Moreover, the final capsule solidification took place in one of two different kinds of sodium salt buffers, either pure phosphate-buffered saline (PBS; 150 m*M*, pH 7.4) or a 1:1 mix of 150 m*M* PBS and 100 m*M* 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.2). All of the observed parameters were chosen with regard to typical applications. The corresponding examinations were focused on the time-dependent swelling, degradation, or bursting of the capsules and the associated release behavior of FITC–dextran during incubation at 37°C.

EXPERIMENTAL

The dispersion cell was purchased from Micropore Technologies. The PLGA copolymers (RG 502, RG 504, RG 750 S, RG 752 S, R 203 H, and R 207 S) were obtained from Evonik Röhm GmbH, Germany. FITC–dextran (4 kDa, 40 kDa and 2000 kDa) was obtained from TdB Consultancy AB (Sweden). A Spurr low-viscosity embedding kit was purchased from VWR International GmbH (Germany). An InSpeck microscope image intensity calibration kit was purchased from Invitrogen (Germany). Common laboratory materials, including dichloromethane (DCM), PBS, sodium chloride (NaCl), and PVA, were purchased from Carl Roth GmbH & Co. KG (Germany).

Capsule Production

The capsules were generally prepared via solvent evaporation by two w/o/w emulsification techniques. To reduce bleaching of the fluorophores, all glassware and Falcon tubes used during the experiments were covered with aluminum foil in advance.

Microcapsule (MC) Production. The MCs were prepared in the dispersion cell obtained from Micropore Technologies. Mainly following instructions in the literature,[1] we prepared a primary w/o emulsion (i.e., the discontinuous phase) containing 1.8 g of PLGA copolymers dissolved in 14 mL of DCM (7 mL in the cases of RG 502 and RG 752 S) and 3 mL of FITC–dextran (0.5 g/mL dissolved in 150 m*M* PBS) using a dispersant (MICCRA D-9, level D) at 25,000 beats/min for 5 min in a 100-mL beaker. Under regular experimental conditions, 150 mL of a solution of 1% w/v PVA (molecular weight = 70 kDa), NaCl (40 g/L), and DCM (25 mL/L) solved in aqua distilled water (dest). (i.e., the continuous phase) were poured into the glass cylinder of the dispersion cell and stirred at 600 rpm. In the preceding step, a metal membrane with a pore size of 40 μm was placed on top of the bottom cavity between the injection nozzle and the glass cylinder of the dispersion cell.

A Teflon tube that was attached to a peristaltic pump (Watson Marlow, flow rate = 0,5 mL/min) was then used to inject the discontinuous phase through the nozzle inside the bottom cavity of the dispersion cell. After the bottom cavity was completely filled, the discontinuous phase started to leak through the membrane pores; this led to the formation of small droplets on the opposite surface exposed to the continuous phase. Here, the droplets were detached from the membrane surface due to mechanical stress under stirring conditions at 600 rpm. Accordingly, small viscous MCs were shaped out of the discontinuous phase and kept floating in the continuous phase to form a w/o/w emulsion. After complete injection of the discontinuous phase into the dispersion cell, the resulting MC w/o/w emulsion was poured from the glass cylinder into a 600-mL beaker. Solidification of the MCs took place during extraction and evaporation of the solvent out of the emulsion by shaking at 150 rpm overnight. Finally, the samples were washed with 150 m*M* PBS three times and stored in 10-mL aliquots of 150 m*M* PBS inside Falcon tubes for incubation and further evaluation.

Submicrometer/Nanocapsule (NC) Production. NC production was performed via ultrasound homogenization according to recent publications.[9] As described for the MC production, first a discontinuous phase was prepared containing 0.4 mg of PLGA solved in 4 mL of DCM (RG 504 and R 203 H) or 2 mL (RG 752 S), 0.65 mL of FITC–dextran (0.5 g/mL solved in 150 m*M* PBS) and with a dispersant (MICCRA D-9, level D) at 25,000 beats/min for 2 min in a 50-mL beaker. Afterward, the discontinuous phase was transferred into a modified continuous phase containing Pluronic F68 (10%) dissolved in 6 mL of aqua dest. and immediately exposed to ultrasound with an ultrasonic probe (Bandelin Sonoplus HD 2070, 70 Watt, 90% capacity) for 2 min. The resulting emulsion was then poured into a 600-mL beaker containing 100 mL of 100 m*M* HEPES buffer to balance the ionic interactions and, therefore, prevent fusion of the very small emulsion drops. Shaking at 150 rpm overnight led to the evaporation of the solvent and stabilization of the NCs. The samples were washed only two times with 150 m*M* PBS and stored in 10-mL aliquots of 150 m*M* PBS inside Falcon tubes for incubation and further evaluation.

Additional Modifications. To investigate the influence of the individual production steps on the general capsule properties, the following parameters were varied in accordance with previously published works:

1. The use of various PLGA blends for the discontinuous phase.[22]
2. The preparation of discontinuous phases of different emulsification grades by a change of mixing devices (dispersing staff and stirring rod).
3. The addition of 1% w/v surfactants such as poloxamers (Pluronic F68/F127) into the continuous phase inside the glass cylinder of the dispersion cell.[25–27]
4. The variation of the stirring speed (600 and 1800 rpm) and membrane pore size (40 and 5 μm) inside the dispersion cell.[1]
5. The solvent evaporation and implied solidification of MCs overnight in various buffers (150 m*M* PBS or a 1:1 mix of 150 m*M* PBS and 100 m*M* HEPES).

An overview of all of the batch modifications is given in Table I. For samples prepared with Pluronic F68, only blank capsules without any encapsulated agent were obtained. In all other combinations, blank capsules and capsules containing FITC–dextran with a molecular weight of 4, 40, or 2000 kDa were produced. In addition, NCs from the RG 504, RG 752 S, and R 203 H polymers and filled with FITC–dextran (at 4, 40, or 2000 kDa) were produced according to the previous instructions. Afterward, all of the listed sample variations were incubated simultaneously at 37°C.

Capsule Analysis

For final analysis, aliquots were taken from the capsule batches after 1, 7, 21, 42, and 84 days of incubation. The applied examination methods were based on optic devices such as microscopes [with fluorescence microscopy (FM), confocal laser scanning microscopy (CLSM), and scanning electron microscopy (SEM)], fluorescence intensity detection via a plate reader, Coulter counting, flow cytometry, and pH monitoring. In particular, SEM images were taken at different magnification levels (LEI mode, 5 kV, at 250, 1000, or 5000×) and were used to

Table I. Overview of the MC Batch Production Conditions Tested for Each Polymer (Composition)

Pretreatment	Dispergator	Magnetic stirrer				
Membrane pore size		40 μm			5 μm	
Stirring speed		600 μm				1800 μm
Additives	—	—	F68	F127	—	—
RG 502	•					
RG 504	•	•	•	•	•	•
RG 502 + RG 504 (1:1)	•					
RG 750 S	•	•	•	•		
RG 752 S	•					
RG 750 S + RG 752 S (1:1)	•					
R 203 H	•					
R 207 S	•	•	•	•		•
R 203 H + R 207 S (1:1)	•					

obtain visual information about the surface texture and the shape of the capsules. Additionally, the internal structure of the capsules was examined after drying and embedment in 1 mL of an ultra-low-viscosity resin per a sample that was prepared according to the manual instructions.[34] After the hardening of the 2-μm resin, slices were cut with a microtome diamond blade and evaluated via SEM.

CLSM (Zeiss Axiovert 200 M) layer scans of the capsules divided in sections of 2-μm thickness were made to illustrate the distribution of the filling agent within the carriers in solution (Plan-Apochromat Objectives, MCs: 20×, NCs: 63×). Excitation of the FITC–dextran took place via beam splitters at 488 nm, whereas emissions were sent through BP 505–550-nm filters.

The release behavior was quantified by fluorescence intensity measurement of the incubation buffer via a plate reader (BMG Labtech: PoloStar Galaxy). As a preceding step, a 1-mL aliquot was filtered through syringe filters (pore size = 0.2 μm) to dispose capsule debris. The retained capsules were resuspended in 1 mL of fresh PBS. Afterward, 100 μL aliquots were used for fluorescence detection, whereas the rest were taken for pH monitoring. As the degradation of PLGA capsules exposed acidic carboxyl groups to the solution, the corresponding decrease in the pH was monitored and used for correction of the obtained FITC fluorescence intensities.

MC-Specific Examination. FM (Olympus IX 51) pictures were used to observe the release of the filling agent by comparison of the MC fluorescence brightness after 500 ms of excitation between different incubation time intervals. Consistent measurement conditions were assured by the usage of a microscope image intensity calibration kit from InSpeck that contained 6-μm microspheres with a fixed emission intensity (here, 10% of the maximum intensity). The calibration check was performed at the beginning and end of each session. The recorded FM images were converted into an 8-bit grayscale format and evaluated with open-source ImageJ software to quantify the brightness of the FITC–dextran-loaded MCs.

Size evaluation of the MCs was performed after solidification by a Coulter counter (Beckman Coulter Multisizer 3) with an aperture with an effective size ranging between 20 and 240 μm. Analysis of the capsule distribution was based on the measurement of 10,000 particles per batch.

NC-Specific Examination. Unlike the MCs, the NCs were suitable for neither brightness nor size determination by FM imaging or Coulter counting techniques because of their low diameter. These measurements were, therefore, replaced by the additional evaluation of the samples in a flow cytometer (Cytomics FC 500, Beckman Coulter). Accordingly, the capsule fluorescence intensity and size distribution of 10,000 particles taken from 1-mL sample aliquots were determined at each incubation time interval of 1, 7, 21, 42, and 84 days. FITC-loaded calibration beads were again used to ensure consistent measurement conditions of the examined NCs.

RESULTS AND DISCUSSION

The content release from PLGA capsules depends on various parameters, including size, material composition, production technique, and applied surface modification of the capsules. Accordingly, previous studies have usually focused on the release studies of a model analyte from capsules that were obtained by individual strategies.[1–16,18,22–29] However, a direct comparison of multiple release-affecting parameters that are measured simultaneously is usually neglected. Hence, we decided to elaborate a comprehensive study for the drug-release behavior from PLGA capsules with divergent copolymer formulations that were prepared under various conditions and resulted in alternating physical and chemical properties. Furthermore, we observed how the physical capsule properties, such as surface morphology, volume, and pore size, were correlated with their chemical attributes, as marked by release of FITC–dextran of different molecular weights (4, 40, and 2000 kDa) as a filling agent.

PLGA capsule properties are known to strongly depend on the polymer composition. Therefore, six different copolymers were used for productions that differed in their mixture ratio and inherent viscosity (Table II). Because of the strong sensitivity of

Table II. Relative PLGA Degradation Properties Depending on the Copolymer Mixture Ratio and Inherent Viscosity Referring to Manufacturer Listings and Visual Control during Incubation in a Buffer Solution at 37°C

	PLGA ratio[a]				
	50_x:50_y	75_x:25_y	100_x:0_y	+++ ++ +	Fast degradation
Product name	RG 502	RG 752 S	R 203 H		
Inherent viscosity (dL/g)	0.16-0.24 (+++)	0.16-0.24 (+++)	0.25-0.35 (−)	−	Slow degradation
Inherent viscosity (dL/g)	0.45-0.60 (++)	0.8-1.2 (−−)	1.3-1.7 (−−−)	− −	
Product name	RG 504	RG 750 S	R 207 S	−−−	

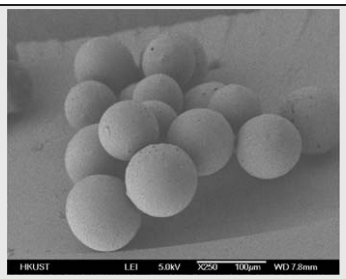
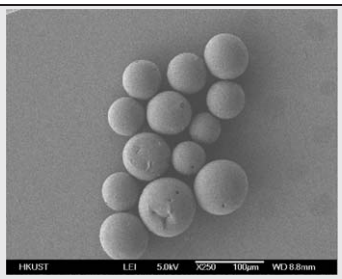
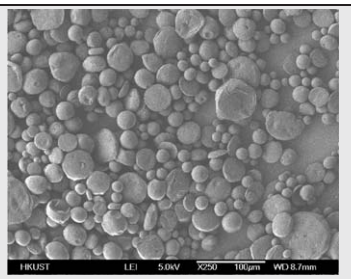
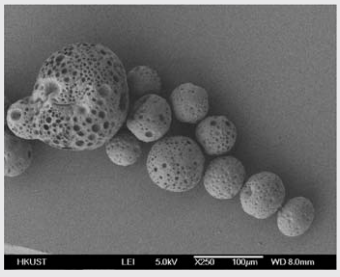
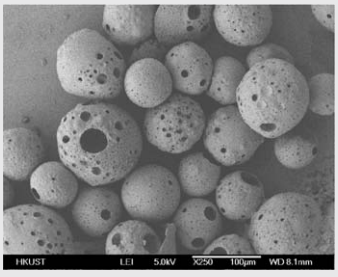
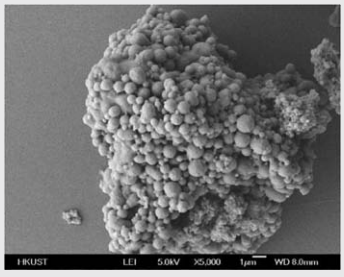
Advanced degradation within: +++, 1 week; ++, 3 weeks; +, 6 weeks; −, 12 weeks; −−, 24 weeks; −−−, more than 24 weeks.
[a] *x*, lactic acid; *y*, glycolic acid.

glycolide monomers to hydrolysis, capsules made from 50:50 PLGA copolymers were prone to a comparatively faster degradation in aqueous solution than those that contained a higher fraction of lactide monomers, such as 75:25 PLGA or pure polylactide (PLA). This effect could be increased further by a low inherent viscosity of the copolymer, which resulted in capsules with less stability. A direct comparison of the PLGA composites with fixed monomer ratios but different inherent viscosities revealed that appropriate choices allowed us to fine-tune the polymer degradation sensitivity. Nonetheless, the exploitation of these characteristics was limited by the current commercial availability of the respective substances.

Capsule Morphology

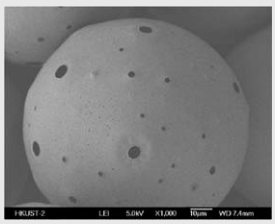
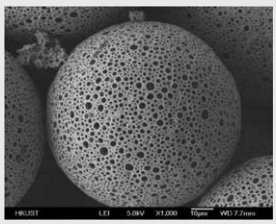
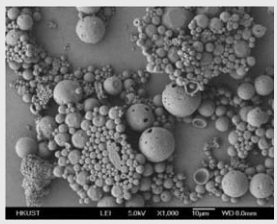
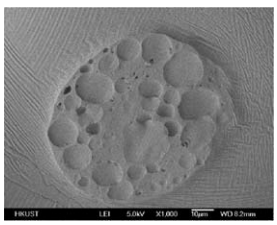
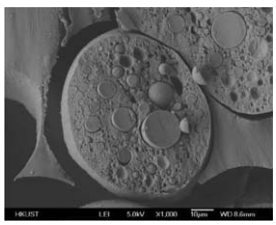
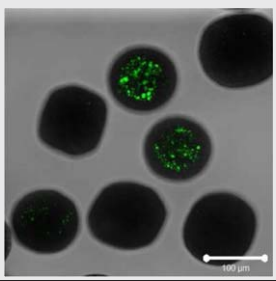
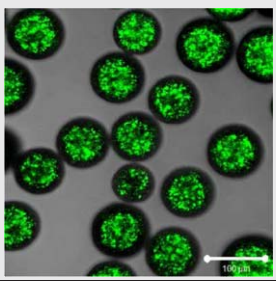
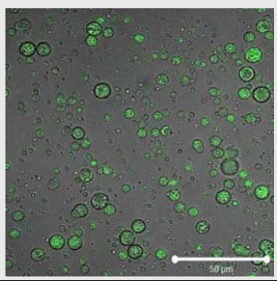
The surface and inner structure of the capsules were examined by SEM, FM, and CLSM. In general, capsule batches made out of the same composite material and with the same fabrication conditions had similar physical attributes. In particular, the size and homogeneity of the MCs could be controlled by the membrane pore size and stirring speed applied in the dispersion cell. Consequently, changes in the given conditions had significant influence on the resulting attributes. Although MCs produced with a spin rate of 600 rpm and a membrane pore size of 40 μm appeared almost uniform in shape and diameter, a change in the membrane pore size to 5 μm resulted in rather

Table III. SEM Images of the Resomer RG 504 MCs and Resomer RG 752 S Nanocapsules

MCs	MCs	MCs
		
s = 600 rpm, m = 40 μm	s = 600 rpm, m = 5 μm	s = 1800 rpm, m = 5 μm
MCs	**MCs**	**NCs**
		
s = 600 rpm, m = 40 μm, additive = Pluronic F68	s = 600 rpm, m = 40 μm, additive = Pluronic F127	w = 70, c = 90%, t = 2 min

s, spin rate of the stirrer within the dispersion cell; m, membrane pore size; w/c/t, Watt/capacity/time exposition to ultrasound. Scale bar = 100 μm for the MCs and 1 μm for the nanocapsules.

Table IV. Microscopic Images of the Resomer RG 504 MCs and NCs Filled with FITC–Dextran (40 kDa)

Image type	Capsule type		
	MCs		NCs
	Emulsification of the discontinuous phase with a stirring rod at 600 rpm	Emulsification of the discontinuous phase with a dispergator at 25,000 beats/min	Emulsification of the discontinuous phase with a dispergator at 25,000 beats/min
Outer morphology (SEM images)			
Inner morphology (SEM images)			Not applicable
FITC-dextran distribution (CLSM images)			

Scale bar for the CLSM images: 100 μm for the MCs, 50 μm for the NCs. Scale bar for the SEM images: 10 μm for all.

inhomogeneous MCs with an smaller average diameter down to 50% on the basis of microscopic control (Table III). The simultaneous use of a 5-μm membrane pore size and an increased spin rate of 1800 rpm formed MCs with an even higher diversity (Table III). Correlated with the described assembling process, these capsules partially shrunk down to 10% of the size yielded under standard conditions (600 rpm and 40 μm). The presence of surfactants such as poloxamers in the discontinuous phase of the dispersion cell, moreover, forced the formation of notches (Pluronic F68) and deep cavities (Pluronic F127) on the surface of the MCs that were made under standard conditions (Table III). In addition, these capsules also appeared to be divergent in size and spherical shape. Because poloxamers are known to interact between the interfaces of w/o/w emulsions,[35] the surfactant seemed to had a contraproductive effect during the formation of emulsion droplets and subsequent capsule assembly on the membrane surface. For reproducibility reasons, all further modifications and final test runs were, therefore, performed on MCs made under standard conditions without the addition of surfactants to the discontinuous phase.

In the following, the PLGA capsules described were derived from a discontinuous phase and emulsified under different shearing conditions with a regular magnetic stirrer or a dispergator. The shearing force experienced by the PLGA droplets during the fabrication process had an impact on the surface morphology, inner cavity, and distribution of the loaded FITC–dextran molecules within the resulting PLGA capsules. Thus, the mixing of the discontinuous phase with a magnetic stirrer at 600 rpm caused moderate shearing forces, which offered insufficient energy input to obtain a dispersion of high homogeneity. In addition, only small amounts of the surrounding gas phase were absorbed. Subsequent injection into the dispersion cell consequently resulted in the appearance of only a few pores on the capsule surface by inclusions of gas and an aqueous solution during the solidification process of the capsules. Moreover, only a small fraction of the large inner cavities of many capsules were filled with FITC–dextran (40 kDa) because of the nonhomogeneous agent distribution within the liquid polymer phase of the discontinuous phase (Table IV). The nonhomogeneous agent distribution might also have been related to a

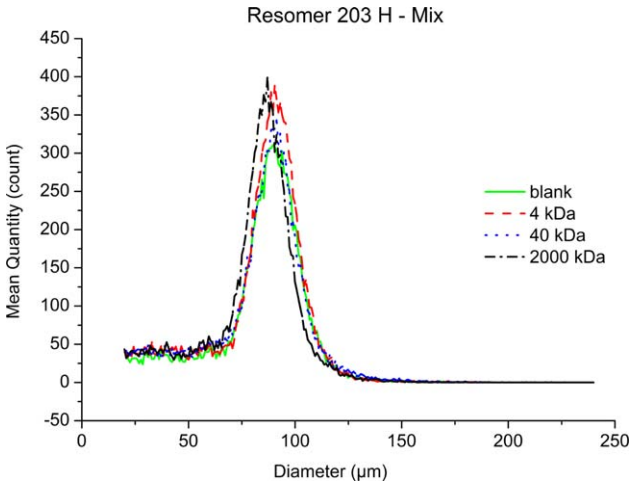


Figure 1. Average size of microcapsules made of Resomer 203 H, filled with FITC-dextran (4, 40 and 2000 kDa) and solidified in 150 mM PBS as measured by the Coulter counter. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

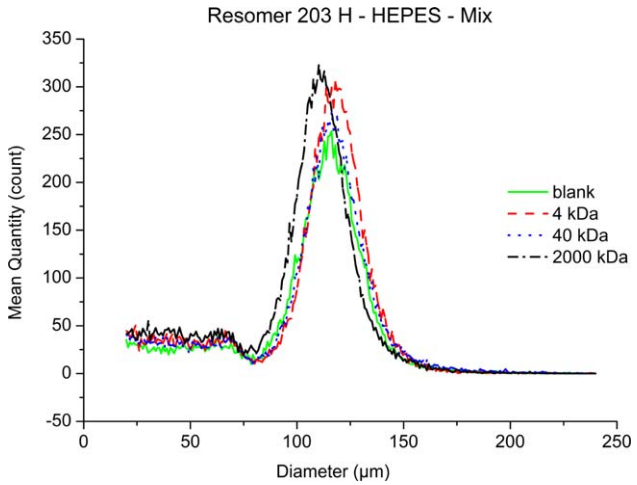


Figure 2. Effect of HEPES used as a solidification buffer on the average size of the Resomer 203 H MCs filled with FITC-dextran (4, 40 and 2000 kDa) as measured by the Coulter counter. Here an increase of about 60% in diameter compared to those MCs that were solidified in 150 mM PBS buffer could be observed. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

replacement of the aqueous phase within the large cavities by diffusion processes upon solidification of the capsules in buffer. At the beginning of this process, the FITC–dextran (40 kDa) molecules could partially diffuse into the still liquid polymer phase that surrounded the droplets of aqueous solution. In contrast, the mixing of the discontinuous phase with a dispergator at 25,000 beats/min caused a massive energy input into the emulsion. Hence, increased gas inclusion such as air bubbles and a high dispersity of the discontinuous phase led to a pore carpet covering the MC surface and homogeneous analyte distribution within the large amount of variously sized inner cavities of the capsules after solidification (Table IV). Most of the encapsulated FITC–dextran (40 kDa) should, therefore, have been located within the marginal area of the numerous comparatively small cavities. Differences between the capsules that were incubated in 150 m*M* PBS or 1:1 150 m*M* PBS/100 m*M* HEPES buffer during solidification manifested in an overall increased capsule diameter of up to 60% (Figures 1 and 2) and an enhanced number of surface pores and inner cavities for the latter buffer composition because of the extended and more fragile capsule scaffold.

Beyond that, the batches of NCs showed similar morphological properties, such as a high diversity in size, rather blank surfaces, and homogeneous filling agent distribution (Tables III and IV). The diversity in size was caused by imbalanced ultrasound spreading and a decreased energy input to the discontinuous phase at greater distances of the ultrasonic probe. The subsequent inhomogeneous emulsification grade formed polymer solution drops of various sizes, whereas only samples taken from batches produced with the Resomer RG 752 S copolymers showed the appearance of capsules down to the nanometer scale under SEM (Table III). However, unlike with mechanical stirring, the exposition of the discontinuous phase to ultrasound had a degassing effect on the fluid. This explained the improved entrapment of the model analyte and the missing pores on the capsule surface.

Size-Dependent Release Behavior

The dextran molecules were uncharged and were chosen as a model agent because of their relatively inert properties, which reduced the probability of interactions with the PLGA capsules in their environment. The degree of substitution for all of the tagged FITC–dextran molecules was 0.003–0.008 according to information provided by the manufacturer; this was relatively too insignificant to make an impact on the overall properties of the dextran molecules. The fluorescence intensity retained within the individual PLGA capsules was a function of the corresponding encapsulation efficiency, whereas the size of the PLGA capsules influenced the total amount of includable FITC–dextran. Therefore, the *relative encapsulation efficiency* was defined as the fluorescence intensity per unit diameter of the capsules.

Figure 3 shows that larger PLGA capsules displayed a lower encapsulation efficiency of FITC–dextran (40 kDa) with an almost linear trend. Furthermore, it was remarkable that this trend significantly increased for comparatively bigger capsules, which were solidified in 1:1 150 m*M* PBS/100 m*M* HEPES buffer at pH 7.2. Although generally more filling agent should be entrapped within larger sized capsules, this effect was balanced by their higher surface porosity, which allowed parts of the FITC–dextran (40 kDa) to escape during the solidification process and in the first hours of the incubation of the capsules. Some exceptions were found for MCs consisting of PLGA copolymers with a low inherent viscosity. These capsules showed a low encapsulation efficiency, despite their relatively small diameter. This could be explained by the rather loose capsule scaffold density, which corresponded to the low inherent viscosity and, thereby, the low molecular weight of the used PLGA. As a result, the capability of entrapping and holding the FITC–dextran molecules (40 kDa) decreased.

Figure 4 shows the time that was required to release 50% of the FITC–dextran (40 kDa) content from differently sized capsules.

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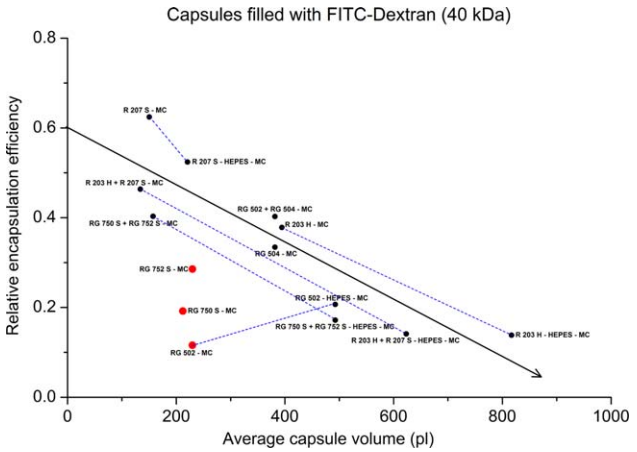

Figure 3. Decreasing trend of the capsule size and corresponding encapsulation efficiency presented by the brightness per unit volume (arrow: trend direction, red dots: samples off the trend, and dashed lines: influence of the HEPES application during capsule solidification). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The mark of 50% release was chosen for two reasons. On the one hand, this time interval is important, for example, for pharmacokinetic applications that require an immediate but continuous agent release from an administered carrier. On the other hand, a significantly faster filling-agent release was measured during the first hours to days of incubation, whereas the time required for 50% of the content to be released from the capsules increased linearly in response to the rising capsule volume. The first effect was probably related to the primary leaking of FITC–dextran out of interconnected pore channels or areas close to the capsule surface rather than the release of more deeply enclosed FITC–dextran by the degradation of whole capsule parts within the first days of incubation. The second effect could be explained by the lower surface-to-volume ratio, which led to a slower hydrolytic degradation of the capsules and longer distances for the analyte to leak out of the pores. These observations were reduced for capsules that were solidified in the 1:1 150 m*M* PBS/100 m*M* HEPES buffer and resulted in the

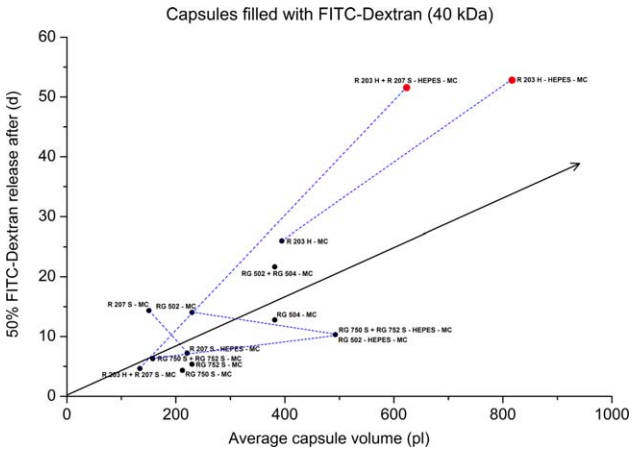

Figure 4. Increasing trend of the capsule size and correlated time-dependent content release (with formatting analogous to that in Figure 3).). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

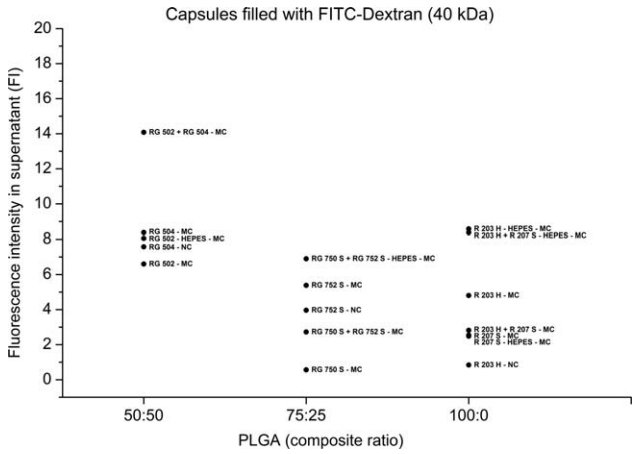

Figure 5. Effects of the polymer compositions on the general encapsulation efficiency and subsequent release behavior within 3 months in the capsules filled with FITC–dextran (40 kDa).

previously described less dense capsule scaffold. Exceptions were found for batches consisting of the PLA R 203 H polymer solidified in the 1:1 150 m*M* PBS/100 m*M* HEPES buffer. Here, the FITC–dextran (40 kDa) released from the capsules decreased because of the comparative insensitivity of the polymer toward hydrolytic degradation.[36] Additionally, its low inherent viscosity, combined with the use of the mixed solidification buffer, induced an expansion of the capsule diameter. Because the size of the nanocapsules was not within the detection range of the Coulter counter, these batches were excluded from the comparison.

PLGA Compositions and Molecular Weights of the Filling Agents

The effects of the polymer compositions and molecular weights of the filling analyte on the general encapsulation efficiency and subsequent release behavior are presented in Figures 5 and 6. Figure 5 clearly shows that the composition ratio of PLGA had a strong influence on the release behavior of FITC–dextran (40 kDa) from the capsules within an incubation time of 3 months at 37°C. Accordingly, all capsule batches made from the

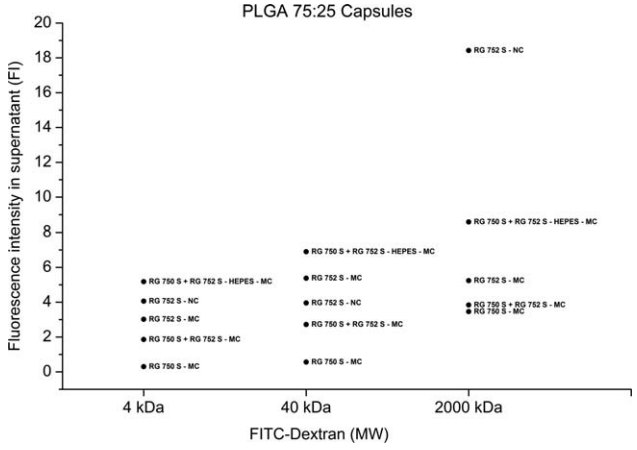

Figure 6. Effects of the molecular weight of the filling agent on the general encapsulation efficiency and subsequent release behavior within 3 months in the capsules made of PLGA 75:25 compositions.

Table V. Corresponding Release Efficiency (Ratio/Rate) of the Applied Capsule Modifications

Capsule modification	Release ratio	Release rate
HEPES (used during solidification)	+	No effect (except trend deviations)
PLGA composition ratio (higher glycolide fraction)	+	No effect
PLGA inherent viscosity (lower)	+	No effect (except R 203 H: slow)
Capsules (increasing size)	—	—
Filling agent (increasing molecular weight)	+	No effect
Discontinuous phase (increased dispersion grade)	+	+

comparatively quickly degrading PLGA 50:50 composites were fully degraded within this time interval (Figure S1, Supporting Information). Regardless of the polymer's inherent viscosity, the complete release of the capsule content was detectable in the supernatant of the incubation buffer. Consequently, capsules made from the more stable 75:25 PLGA mixtures with an average inherent viscosity showed an overall minor release of the filling agent. FITC–dextran (40 kDa) molecules fixed inside the pores were not able to leak out of the capsules, whereas these remained intact. Although the material showed a slower capsule degradation, the marginal release was affected by the generally lower encapsulation efficiency in the 75:25 PLGA derivatives with a high inherent viscosity. This correlated to the faint capsule brightness observed under the fluorescence microscope (Figure S2, Supporting Information). Finally, capsules made from the pure PLA showed an average release behavior between those of the 50:50 and 75:25 PLGA composite ratios. For these batches, control over the degradation could be achieved within a wide range because of the low sensitivity of the polymer toward hydrolysis combined with a strong diversity of the inherent viscosity derivatives, which yielded capsule scaffolds of various stabilities. In particular, the combination of 1:1 150 m*M* PBS and 100 m*M* HEPES as a solidification buffer with a polymer of lower inherent viscosity had a strong influence on the increased release of FITC–dextran (40 kDa) to the supernatant. The same applied to capsules made of 50:50 and 75:25 PLGA composite ratios. All of release study conclusions of FITC–dextran (40 kDa) from various PLGA capsule compositions described to this point were further confirmed by the corresponding data for the 4 and 2000 FITC–dextran release; they are available in the Supporting Information (Figures S3–S8).

Figure 6 shows the influence of the model analyte size on its release behavior. The release of FITC–dextran with molecular weights of 4, 40, and 2000 kDa was observed after encapsulation in 75:25 PLGA. The 75:25 PLGA was chosen because of its generally slow degradation and rather constant release of the encapsulated agent from the capsules, as previously discussed. Briefly, the results demonstrate that a higher filling agent volume led to a higher amount of measureable FITC–dextran in the supernatant. The data were correlated with the fact that the microscopic fluorescence control of uniformly sized MCs revealed decreased intensities proportional to the molecular weight of the entrapped FITC–dextran (Figures S9–S13, Supporting Information). Because all of the FITC–dextran derivatives (4, 40, and 2000 kDa) had the same fluorescence intensity in solution (Figure S14, Supporting Information), it was likely that this effect was caused by a generally lower encapsulation efficiency of FITC–dextran with low molecular weight, that is, a significant loss of filling agent through the pores of the MCs during their solidification process and washing steps. The use of the 1:1 150 m*M* PBS/100 m*M* HEPES mixture as a solidification buffer, once again, had a significant influence on the increased amount of released filling agent to the supernatant regardless of the molecular weight of the FITC–dextran derivatives. Further confirmation of the observed release study conclusions is available for the 50:50 PLGA and 100:0 PLA composites as Supporting Information (Figures S15 and S16). A summary of the correlation between the applied capsule modifications and the corresponding release efficiency is shown in Table V.

CONCLUSIONS

In this study, we observed the relation between the molecular weight and release properties of differently sized FITC–dextran molecules entrapped in various compositions of PLGA MCs and NCs to create a superordinated database for pharmacokinetic applications. We demonstrated the great effects of the capsule size, PLGA composition and molecular weight of the filling agent, dispersion grade of the outgoing w/o emulsion, and selection of appropriate buffers for capsule solidification to allow the manufacturing of tailor-made capsules with different release kinetics. Accordingly, mixed batches of selected capsules improved control over the bioavailability of an encapsulated drug by consecutive degradation of capsules with slow, medium, and fast release kinetics.

Related publications within the scope of our study have mainly focused on the development of faster and more efficient PLGA capsule production techniques with a higher accuracy or the encapsulation of a specific drug.[10,14,15,24,28,29,37] Further research has been dedicated to the effect of the process parameters on the morphology and release kinetics of PLGA capsules but usually addresses only one preselected copolymer blend or model analyte at a time.[38]

This study extended the published work, therefore, not only through the discussion of new PLGA copolymer blends, such as Resomer R 203 H and RG 752 S. It also provided a simultaneous overview on multiple release kinetics affecting parameters with limited production effort on a new scale. This could ease the adaptation to individual requirements of pharmaceutical or clinical interests.

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