



## Review

## Principles of encapsulating hydrophobic drugs in PLA/PLGA microparticles

Christian Wischke<sup>1</sup>, Steven P. Schwendeman\*

Department of Pharmaceutical Sciences, University of Michigan, 428 Church Street, Ann Arbor, MI 48109-1065, USA

## ARTICLE INFO

## Article history:

Received 17 March 2008

Received in revised form 29 April 2008

Accepted 29 April 2008

Available online 7 May 2008

## Keywords:

PLGA

Biopharmaceutical classification system

Microencapsulation

Controlled release

Microparticle

Hydrophobic drug

## ABSTRACT

Injectable biodegradable and biocompatible copolymers of lactic and glycolic acid (PLGA) are an important advanced delivery system for week-to-month controlled release of hydrophobic drugs (e.g., from biopharmaceutical classification system class IV), which often display poor oral bioavailability. The basic principles and considerations to develop such microparticle formulations is reviewed here based on a comprehensive study of papers and patents from the beginnings of hydrophobic drug encapsulation in polylactic acid and PLGA up through the very recent literature. Challenges with the diversity of drug properties, microencapsulation methods, and organic solvents are evaluated in light of the precedence of commercialized formulations and with a focus on decreasing the time to lab-scale encapsulation of water-insoluble drug candidates in the early stage of drug development. The influence of key formulation variables on final microparticle characteristics, and how best to avoid undesired microparticle properties, is analyzed mechanistically. Finally, concepts are developed to manage the common issues of maintaining sink conditions for in vitro drug release assays of hydrophobic compounds. Overall, against the backdrop of an increasing number of new, poorly orally available drug entities entering development, microparticle delivery systems may be a viable strategy to rescue an otherwise undeliverable substance.

© 2008 Elsevier B.V. All rights reserved.

## Contents

1. Introduction .....	299
2. Drug properties relevant for microencapsulation and release .....	300
2.1. Recent trends in drug discovery and their implications on microencapsulation candidates .....	300
2.2. Drug solubility in aqueous and organic media .....	300
2.3. Drug stability .....	301
2.4. Drug-polymer interactions .....	301
2.5. Drug solid-state properties .....	301
3. Microencapsulation techniques for hydrophobic drugs .....	302
3.1. o/w emulsion technique .....	302
3.2. s/o/w technique .....	302
3.3. o/o method .....	303
3.4. w/o/w method .....	303
3.5. In situ forming microparticles .....	303
3.6. Salting out/phase separation .....	304
3.7. Melting techniques .....	304
3.8. Methods using supercritical fluids (SCF) .....	304
3.9. Spraying techniques .....	305
3.10. Ammonolysis .....	305
3.11. Rationale to select an encapsulation technique .....	305

\* Corresponding author. Tel.: +1 734 615 6574; fax: +1 734 615 6162.

E-mail address: [schwende@umich.edu](mailto:schwende@umich.edu) (S.P. Schwendeman).<sup>1</sup> Present address: Center for Biomaterial Development and Berlin-Brandenburg Center for Regenerative Therapies, Institute of Polymer Research, GKSS Research Center Geesthacht GmbH, Kantstr. 55, 14513 Teltow, Germany.

4.	Solvents/cosolvents .....	305
4.1.	Dispersed phase solvents .....	305
4.1.1.	Solubility of polymer solvent in continuous phase .....	305
4.1.2.	Solubility of water in the polymer phase .....	307
4.1.3.	Solvent removal rate .....	307
4.1.4.	Solvent toxicity and regulatory considerations .....	308
4.2.	Cosolvents .....	309
5.	Polymer types .....	311
5.1.	Criteria for polymer selection .....	311
5.2.	Polymer degradation behavior .....	311
5.3.	Polymer mixtures and alternative PLGA copolymers .....	312
5.4.	Impact of drug properties and preparation procedure on polymer characteristics .....	312
6.	Controlling the polymer microparticle size .....	312
6.1.	Emulsification procedure .....	312
6.2.	Formulation parameters .....	313
6.3.	Particle size analysis .....	313
7.	Encapsulation efficiency .....	314
7.1.	Methods to determine the encapsulation efficiency .....	314
7.2.	Increasing the encapsulation efficiency .....	314
7.2.1.	Drug particle size .....	314
7.2.2.	Emulsifiers and drug solubility in the continuous phase .....	314
7.2.3.	Mass transfer of o-phase solvents and microparticle hardening .....	315
8.	Drug release from the microparticles .....	315
8.1.	In vitro assays—rationale for using sink conditions .....	315
8.2.	In vitro assays—media to maintain sink conditions .....	315
8.3.	In vitro assays—experimental set-up .....	316
8.4.	Controlling drug release .....	317
8.4.1.	Typical release pattern from PLGA microparticles .....	317
8.4.2.	Burst release .....	317
8.4.3.	Effect of particle size and porosity on the release rate .....	318
8.4.4.	Drug diffusion through the particle polymer matrix and effect of drug loading on release .....	318
8.4.5.	Onset of erosion-controlled release and attempts to obtain zero-order profiles .....	320
9.	Conclusions and future outlook .....	320
	Acknowledgments .....	320
	References .....	320

## 1. Introduction

The modern microencapsulation of bioactive substances continues to be an important formulation strategy since its inception about 70 years ago. Starting first with the aim to protect vitamins from oxidation (Taylor, 1938) it took some decades until polylactic acid (PLA) and later its copolymers, e.g., poly(lactic-co-glycolic acid) (PLGA), were evaluated as biodegradable and biocompatible polymers for drug delivery (Kulkarni et al., 1971; Cutright et al., 1971; Brady et al., 1973; Yolles and Sartori, 1980; Laurencin and Elgendy, 1994; Ignatius and Cleas, 1996; Anderson and Shive, 1997). Although already patented (Boswell and Scribner, 1973) and initially described by others (Nuwayser et al., 1977; Gardner et al., 1977), Beck, Tice, and coworkers were among the first to intensively study the encapsulation of hydrophobic drugs, i.e., steroids, and focus on their efficiency in vivo (Beck et al., 1979, 1980, 1981, 1983a,b; Tice and Lewis, 1983; Hahn et al., 1983; Cowsar et al., 1985). Despite the clear significance of these findings, these very early papers commonly did not focus in detail on both the experimental methods and the underlying concepts and principles of drug encapsulation. At the same time, the initial patents and reports on the delivery of peptide therapeutics, mostly for luteinizing hormone-releasing hormone analogs, were also becoming available (Chang, 1976; Sanders et al., 1984; Redding et al., 1984; Kent et al., 1986; Okada et al., 1987; Shimamoto, 1987; Ogawa et al., 1988a).

In the present literature of polymeric drug delivery devices, most publications focus on the encapsulation of larger molecules, e.g., peptides, proteins, and DNA/RNA for potential use as vaccines or as long-acting release (LAR<sup>®</sup>) drug formulations. Importantly, some

of these initiatives led to important pharmaceutical products and most of them are still on the market (e.g., Lupron Depot<sup>®</sup>, Zoladex<sup>®</sup>, Decapeptyl<sup>®</sup>, Eligard<sup>®</sup>, Enantone<sup>®</sup>, Trenantone<sup>®</sup>, Nutropin Depot<sup>®</sup>, and Profact<sup>®</sup>). However, the vast majority of new chemical entities are neither peptides nor proteins, but molecules with a low molecular weight. Although no precise data are available, it has been estimated that up to 40% of all new chemical entities show poor solubility (Straub et al., 2005). Particularly with the development of BCS class IV drugs with a low solubility and a low permeability, which exhibit low oral bioavailability, companies are frequently faced with the choice to either develop or discard the early stage compound. In order to expedite this decision, the question of alternative delivery technologies needs to be discussed in the early stages of drug development. For certain drugs that (i) have a broad therapeutic window, (ii) require a low daily dose, and (iii) are going to be used for the long-term treatment of disease, injectable controlled release depots such as drug-loaded biodegradable polymer microparticles, may provide such an alternative delivery strategy, potentially rescuing an otherwise undeliverable drug.

Despite the literature focussing on the considerable challenges with injectable depots for biomacromolecules (e.g., peptide/protein stability, high encapsulation efficiency, and undesired initial burst release; Schwendeman et al., 1996; Sinha and Trehan, 2003; Jiang et al., 2005; Tamber et al., 2005), hydrophobic small molecules are an extremely significant class of drug substances and pose unique challenges in their own right. Therefore, this review focuses on hydrophobic drugs and seeks to develop some guiding principles to examine and solve key issues of their encapsulation in, and release from, injectable PLA and PLGA microparticles.

## 2. Drug properties relevant for microencapsulation and release

### 2.1. Recent trends in drug discovery and their implications on microencapsulation candidates

Although sometimes subject to variable bioavailability from numerous factors, e.g., food effects that may alter drug bioavailability (Myers-Davit and Conner, 2008), oral administration is generally the most desired administration, since it is typically simple, painless, and dosing of the medication can be easily adjusted or terminated. Therefore, small-molecule drug discovery programs strongly desire compounds with significant oral bioavailability. New compounds are subjected to a screening of key physicochemical parameters, i.e., solubility,  $pK_a$ , lipophilicity, permeability, and stability (Alsenz and Kansy, 2007). The 'rule of five' (RO5) is often used to estimate physicochemical drug properties from the structure, and suggests that poor absorption and permeability are more likely when one or more of the following are satisfied: the calculated logarithmic octanol–water partition coefficient ( $cLogKp^2$ ) is  $>5$ , the molecular weight (Mw) is  $>500$ , there are more than 5 H-bond donors, or 10 H-bond acceptors in the molecule (Lipinski et al., 1997). Others found a lower polar surface area (sum of polar atoms including H-bond donors and acceptors) and a reduced flexibility of the molecule (less rotatable bonds, as typically observed for lower Mw drugs), both being interrelated with the RO5 criteria, to be a good predictors for oral bioavailability (Veber et al., 2002).

Moving away from easy oral delivery, there is a growing trend towards discovery of new chemical entities with larger molecular weight and/or larger lipophilicity obtained by medical chemistry (Lipinski, 2000). Although the median  $cLogKp$  values of patented substances in 2001–2006 were variable across several pharmaceutical companies, a total of 30% of the patented compounds had  $cLogKp$  values  $>5$  (Leeson and Springthorpe, 2007). It also needs to be pointed out that the simple passing of the RO5 or showing RO5 violations does not guarantee certain properties of interest (drug-like vs. non-drug-like) such as high or low oral bioavailability or target selectivity, respectively, for a specific chemical entity (Lipinski, 2004). However, there is a high probability that new drugs considered for parenteral application in a microparticle formulation will show physicochemical properties that violate one or more of the RO5 criteria.

Besides physicochemical properties, the pharmacokinetics of the drug, i.e., its absorption, distribution, metabolism, and excretion (ADME), needs to be taken into account when discussing bioavailability. The absorption of small hydrophobic molecules is often related to their physicochemical properties that include drug dissolution from the (oral) formulation (dissolution rate vs. retention time in the intestine), drug solubility, and drug permeability (passive diffusion is a main mechanism for lipophilic compounds, but might be limited by high Mw) (Cao et al., 2008). The biochemical barrier serves the biological function to reduce potential toxicity from xenobiotics by hepatic and intestinal first-pass metabolism (Thummel et al., 1997) as well as intestinal efflux transporters such as P-glycoprotein (Pgp) (Ho and Kim, 2005; Katragadda et al., 2005), which may reduce bioavailability and, in turn, raise microencapsulation candidates that potentially have passed the RO5. However, there is some likelihood that drug conformity with a RO5 subset (Mw  $<400$ , H-bond acceptors  $<4$ , and certain ionization features) will result in drug candidates that are not substrates of Pgp (Didziapetris et al., 2003; Varma et al., 2006).

Finally, low-dose drugs that show good oral bioavailability should be considered for encapsulation in, and controlled release from, injectable microparticles if: (a) a more constant plasma concentration is required than obtained when administered orally, (b) a local delivery is desired, (c) the drug is indicated for the long-term treatment of diseases often associated with a low compliance, e.g., narcotic addiction (Chiang et al., 1984) or certain neurological disorders (Young et al., 1984; Remington and Adams, 1995), (d) if the embedding into microparticles will help to stabilize or target the drug, or (e) it would be more convenient to have a shot every couple months than following daily administration schedules.

### 2.2. Drug solubility in aqueous and organic media

The term "hydrophobic drugs" roughly describes a heterogeneous group of molecules that exhibit poor solubility in water but that are typically, but certainly not always, soluble in various organic solvents. Often, the terms slightly soluble (1–10 mg/ml), very slightly soluble (0.1–1 mg/ml), and practically insoluble ( $<0.1$  mg/ml) are used to categorize such substances (Martin, 1993; BP, 2001). Steroid drugs are an important class of poorly water-soluble drugs; however, their water solubility varies over at least two orders of magnitudes, as can be seen in Table 1. Other types of hydrophobic drugs show even a lower aqueous solubility of only a few ng/ml. Since insufficient solubility commonly accompanies undesired pharmacokinetic properties, the high-throughput screening of kinetic and thermodynamic solubility (Alsenz and Kansy, 2007) as well as the prediction of solubility (Faller and Ertl, 2007) are of major importance in discovery (lead identification and optimization) and development.

As microparticles are most often prepared by emulsion techniques that include aqueous phases, the solubility of the drug in these media is an important value that needs to be determined in the initial phase of every microencapsulation study. Such external phases are commonly aqueous solutions containing polyvinyl alcohol (PVA), the predominantly used emulsifier in emulsion-based encapsulation techniques. In the case of ionizable drugs the pH-dependency of the solubility needs to be carefully characterized and can be performed by micro solubility methods that address the limited availability of drug and have shown good agreement with conventional assays, as recently reviewed (Avdeef, 2007). Moreover, the effect of excipients, e.g., Tween<sup>®</sup> 20 or Tween<sup>®</sup> 80 non-ionic surfactants, which are often used in release buffers like PBST (phosphate buffered saline + Tween<sup>®</sup> surfactant) on the drug solubility should also be determined.

**Table 1**  
Solubility of selected steroid drugs in water (25 °C)

Drug	Water solubility ( $\mu\text{g/ml}$ ; 25 °C)	References
Hydrocortisone	280	Giunchedi et al. (1998) and Merck Index (2006)
Prednisolone	215	Kabasakalian et al. (1996)
Methylprednisolone	120	Drugbank (2008)
Dexamethasone	100	Merck Index (2006)
Trimacinalone	80	Florey (1972a)
Beclomethasone dipropionate	49	Drugbank (2008)
Triamcinolone diacetate	48	Florey (1972b)
Triamcinolone acetonide	40	Florey (1972c)
Bethamethasone dipropionate	$<40$	Ferrante and Ruby (1977)
Testosterone	27	Lunberg (1979)
Budesonide	10	Drugbank (2008)
17 $\alpha$ -Ethinylestradiol	9.2	Shareef et al. (2006)
Progesterone	7	Nandi et al. (2003)
17 $\beta$ -Estradiol	3	Salole (1986)
Levonorgestrel	2.05	Drugbank (2008)
Fluticasone propionate	0.51	Drugbank (2008)

<sup>2</sup> Commonly used  $cLogP$  is replaced with  $clogKp$  to avoid conflict with the definition of mass transfer (or permeability) coefficient ( $P$ ) later in the manuscript.

**Table 2**  
Properties of solvents (S) used for microencapsulation processes

Solvent (S)	Solubility <sup>a</sup> (%)		Boiling point <sup>a</sup> (°C)	Class of solvent (USP <sup>b</sup> )	Limit (ppm, USP <sup>b</sup> )	References
	S in water	Water in S				
Methylene chloride	1.32 <sup>c</sup>	0.20	39.8	Class 2	600	Maulding et al. (1986)
Butyl acetate	0.68	1.20	126.6	Class 3	5000 <sup>d</sup>	Herrmann and Bodmeier (1995, 1998)
Ethyl acetate	8.70	3.30	76.7	Class 3	5000 <sup>d</sup>	Choi et al. (2002)
Ethyl formate	13.60	4.50	54.7	Class 3	5000 <sup>d</sup>	Sah (2000)
Methylethyl ketone	26.80	11.80	79.6	Class 3	5000 <sup>d</sup>	Sah et al. (1996)

<sup>a</sup> Solubility in % (m/m) at 20 °C (methylene chloride: 25 °C) and boiling point according to Doolittle (1954).

<sup>b</sup> United States Pharmacopeia.

<sup>c</sup> Solubility of methylene chloride in water at 20 °C: 2.0% (Horvath, 1982).

<sup>d</sup> Values according to ICH (1997, 2003).

Most of the microencapsulation techniques for hydrophobic drugs employ volatile organic solvent to dissolve the matrix polymer and, if applicable, the drug as well. Therefore, it is essential to determine the drug solubility in common organic solvents like methylene chloride and ethyl acetate, in potential cosolvents like methanol, ethanol, acetone, and tetrahydrofuran, and in the solvent mixtures. The results of the solubility studies will form the basis of most considerations of choosing the appropriate encapsulation technique.

The octanol–water partition coefficient  $K_p$ , calculated or experimentally determined for new molecules to describe their lipophilic/hydrophilic nature and to make predictions on their behaviour in biological systems, can suggest how the drug will distribute in two-phase solvent systems. Because both octanol and water show some finite solubility in one another, the  $K_p$  value can only be estimated but not accurately calculated from the ratio of drug solubility in the two pure phases. During microencapsulation the effect of dissolved organic solvent on drug solubility in the continuous phase will be more pronounced, since commonly used solvents (Table 2) show much higher solubility in water than octanol (0.03% at 20 °C; Doolittle, 1954).

### 2.3. Drug stability

The most commonly used microencapsulation methods include organic phase emulsification, subjecting drug crystals or dissolved molecules to high local temperatures, shear forces, and the presence of the respective solvent. The toxicological and regulatory relevance of characterizing process-related or degradant impurities has been highlighted recently in a theme issue of *Adv. Drug Del. Rev.* (Basak et al., 2007).

Drug sensitivity to temperature-induced degradation can be determined easily by stress-tests at different temperatures in relevant solvents including release media from room temperature to accelerated storage conditions. Stability studies of new compounds should address the sensitivity of dissolved drug to acids, bases, and oxidation as well as solid-state humidity-related, thermal, and photo-degradation. Analysis may also include prediction of likely degradants from organic chemistry (Alsante et al., 2007; ICH, 1996, 2006). As it is known for PLGA (Reich, 1998), the use of ultrasound for emulsification might result in degradation of drugs, too, especially those that contain hydrolyzable bonds such as esters. Accumulation of PLGA degradation products inside the microparticles under release conditions results in an acidic microclimate (Mäder et al., 1998; Shenderova et al., 1999; Li and Schwendeman, 2005) that also may affect hydrolyzable bonds in the drug molecule. Amine groups in the drug, especially primary amines, may undergo acylation by PLGA degradation products as shown for peptides (Lucke et al., 2002; Na et al., 2003). During storage of microparticles, lomustine, an antineoplastic agent with hydrolytic degradation

pathways, was described to be destroyed due to the interaction with PLA (Benita et al., 1984). However, in that study the likely and important effect of residual water in the microparticles after vacuum drying was not considered. Overall, samples of forced drug degradation should be included when establishing drug determination assays, typically utilizing reverse phase HPLC, to ensure that degradation products will be distinguished from the intact molecule.

### 2.4. Drug–polymer interactions

If weak bases or acids are to be encapsulated, the presence of any drug-induced polymer degradation should be evaluated (Li et al., 1996; Frank et al., 2005). It is well established that amine drugs can catalyze degradation of the PLA/PLGA polyester (Maulding et al., 1986; Cha and Pitt, 1988, 1989), as discussed in Section 5. For significant drug-induced polymer hydrolysis to occur the drug is presumed to partition into the polymer phase.

The affinity of hydrophobic drugs to, or permeation in, plastic materials such as tubes used for sampling might cause serious systematic errors, especially at low drug concentrations, and should be determined by simple recovery experiments. Potential interaction of drugs with the matrix polymer should also be considered and may result in incomplete drug release. Adsorption to PLGA by hydrophobic interactions has been reported, particularly for proteins (Butler et al., 1999; Jeong et al., 2000). For certain basic compounds (and likely those that do not partition into the polymer phase or have restricted nucleophilicity), there have been reports of a reduced polymer degradation (contrary to drug-catalyzed degradation above) via ionic interaction of the drug with cationic PLGA end-groups (Miyajima et al., 1998; Klose et al., 2008). The question of adsorption and drug partitioning into the polymer may be addressed by simple uptake experiments in the PLGA powder (Miyajima et al., 1998) or films.

### 2.5. Drug solid-state properties

Before microencapsulation, the drug is typically in the solid state, and therefore, can be amorphous, crystalline, or combinations thereof. During microencapsulation, the drug will be dissolved or dispersed in a solvent and may be present in the microparticles as a solid solution, metastable molecularly dispersion, or may form amorphous or crystalline regions. If not already dissolved inside the polymer matrix, the drug needs to be dissolved during the last step before drug release, i.e., exposure to an aqueous medium after microparticle administration. This step is critical for hydrophobic drugs because of their typically low drug solubility, and therefore, slow dissolution rate. This dissolution rate may be reduced even further because of the anticipated poor mixing inside the polymer matrix where the drug is dissolving, giving rise to a substantial



unstirred boundary layer for diffusion out of the microparticle. Drug properties that may affect its dissolution in aqueous media from the crystalline state include the wettability of a crystal, the stability of the crystal structure (heat of fusion), or the surface area. The initial characteristics of the employed drug material may be subjected to alterations if the drug is dissolved, at least partially, and precipitates during the encapsulation procedure due to solvent removal. Drug polymorphism may become a serious problem if the polymorphs show strong differences in, e.g., solubility, and conversion to another form occurs during microencapsulation, storage, or under release conditions. Therefore, if solid drug is going to be encapsulated, classically the thermodynamically most stable polymorph is preferred for pharmaceutical development (Tong, 2008), although efforts have been made to engineer crystals by forming co-crystals or metastable polymorphs with altered dissolution behavior (Blagden et al., 2007).

When the drug is going to be encapsulated into the polymer by codissolving both substances in an appropriate solvent, the formation of true or metastable molecular dispersions is possible for low drug loadings. The latter state is undesired as it may be subjected to crystallization during storage which might severely affect the release properties of the formulation. Besides, due to limited mutual miscibility of certain drugs with the polymer, the state of the drug may depend on the loading of the particles, as it has been intensively studied for progesterone (Benoit et al., 1984, 1986a,b; Rosilio et al., 1991; Benoit, 1996; Hill et al., 1998). Therefore, it is strongly recommended to characterize the state of the drug inside the formulation by thermal analysis (Dubernet, 1995).

### 3. Microencapsulation techniques for hydrophobic drugs

#### 3.1. *o/w* emulsion technique

As a considerable number of hydrophobic drugs are soluble in various water-immiscible organic solvents and, of course, are poorly soluble in water, one of the simplest methods to encapsulate such drugs is by the oil-in-water (*o/w*) emulsion/solvent evaporation technique. By this method, both drug and biodegradable polymer are first dissolved in a solvent, e.g., generally methylene chloride is most desirable, and then the resulting organic oil phase is emulsified in an aqueous solution containing an appropriate emulsifier, the solution of which should have a low dissolving power for the drug. In general, volatile solvents can be removed from such emulsions by evaporation to a gas phase (Vrancken and Claeys, 1970a) or in any case by extraction to the continuous phase (Vrancken and Claeys, 1970b; Albayrak, 2005). It is important to point out that in the former case, the carrier solvent must first dissolve in the continuous phase before evaporation takes place (Wang and Schwendeman, 1999). The *o/w* methodology has been applied for the encapsulation of a large number of drugs, including: the neuroleptics thioridazine (Maulding et al., 1986; Fong et al., 1986), chlorpromazine (Suzuki and Price, 1985), and bromperidol (Kino et al., 1997), different local anesthetics (Wakiyama et al., 1981, 1982a,b; Nakano et al., 1984), the minor tranquilizer diazepam (Bodmeier and McGinity, 1987a), the synthetic opioid L-methadone (Cha and Pitt, 1988), the anticancer agents aclarubicin (Wada et al., 1988; Yoshikawa et al., 1989; Muranishi et al., 1991), lomustine (Benita et al., 1984; Benoit et al., 1984; Bissery et al., 1984), and paclitaxel (Burt et al., 1995; Demetrick et al., 1997; Liggins et al., 2000; Liggins and Burt, 2001; Wang et al., 1996, 1997; Gupte and Ciftci, 2004; Xie et al., 2007), the gestagens progesterone (Benita et al., 1984; Benoit et al., 1984; Bodmeier and McGinity, 1987a; Rosilio et al., 1991; Yang and Owusu-Ababio, 2000) and, at elevated temperature, levonorgestrel

(Beck et al., 1985), and the glucocorticoid dexamethasone (Thote et al., 2005).

For very high drug loading, e.g., 50% progesterone (Hill et al., 1998) or 70% testosterone propionate (Tice and Gilley, 1985) in PLA microparticles, crystallization of the drug has been observed during the solvent removal, which even may result in perforation of the particle wall by drug needles. The necessity to characterize the state of the drug inside the polymer has been emphasized in Section 2.3.

However, for drugs that do not show a high solubility in methylene chloride, e.g., the estrogen  $\beta$ -estradiol (Birnbaum et al., 2000), an alternative carrier solvent (i.e., solvent for the polymer and, in this case, the drug) may be considered. Table 2 lists some solvents that had been suggested to replace methylene chloride in microencapsulation processes but have not found general use for hydrophobic drugs. Alternatively, a cosolvent may be added to methylene chloride, or a different encapsulation technique may be employed.

#### 3.2. *s/o/w* technique

If the specific drug cannot be dissolved in a carrier solvent or solvent mixture, or extensive drug loss to the continuous phase cannot be avoided when employing cosolvent systems (see Section 4), then the *s/o/w* technique is usually used. With this method a preliminary formulation should be available for in vivo proof of concept studies in an appropriate time and the formulation can be later subjected to optimization. The majority of very early papers on hydrophobic drug encapsulation employed the *s/o/w* technique, e.g., for norethisterone as a contraceptive (Beck et al., 1979, 1980, 1981, 1983b; Cowsar et al., 1985; Cong and Beck, 1991) and multiple other drugs (Fong et al., 1986; Cavalier et al., 1986; Bodmeier and McGinity, 1987a; Tsakala et al., 1988; Gu et al., 1992). In the more recent literature, the *s/o/w* method was evaluated for hydrophobic drugs like levonorgestrel (Wang et al., 2005),  $\beta$ -estradiol (Birnbaum et al., 2000; Mogi et al., 2000), haloperidol (Kino et al., 1997), or camptecin and its derivatives (Shenderova et al., 1997, 1999; Ertl et al., 1999). Due to a low but distinct solubility of certain active agents in the organic solvent, a certain portion of the drug might also be in solution in *s/o/w* formulations. This is one reason to avoid storage of drug suspensions in the polymer phase, as crystal growth via Ostwald ripening may occur.

However, the *s/o/w* method requires a very low drug particle size in order to allow a complete encapsulation of the drug crystals. Due to the limited availability of micronized drug in the preclinical phases such material might need to be prepared on a lab-scale. A number of drug properties including the hardness and elasticity of the drug crystals, the melting point, the hygroscopicity, and the sensitivity to thermal or other decomposition reactions will impact the selection of a successful method. For non-hygroscopic drugs a simple set-up with a smooth mortar and pestle might be appropriate to grind the drug and cooling the mortar on dry-ice will even increase the efficiency due to a higher brittleness of the drug at lower temperature. With this cryogenic grinding, drug particles less than 1–10  $\mu\text{m}$  can often be obtained. Hygroscopic drugs should be subjected to grinding in a controlled atmosphere, such as inside an air-tight grinding jar of a ball mill with liquid nitrogen cooling. However, a lower recovery is typical for such mills.

Besides the necessity of small-sized drug material, other drawbacks of the *s/o/w* technique might be the tendency of the drug to show sedimentation (higher density than suspension medium) or flotation (caused by adhesion of gas bubbles to the hydrophobic surface due to low wettability) during the encapsulation process and, in the later stages of the product development, difficulties can also be expected during scaling up to large-scale manufacture. Alterations, which might result from changes in the drug synthe-

sis, e.g., in the drug crystal structure or the wetting behavior, are expected to affect the release profile from *s/o/w* particles. Moreover, differences in the release might appear compared to dense microspheres that were prepared by the *o/w* technique and show a homogeneous drug distribution. Especially if comparatively large drug material is incorporated, the presence of sparsely encapsulated drug crystals at the microparticle surface can increase burst release (Birnbaum et al., 2000). Therefore, some authors have suggested an extra coating step for *s/o/w* microparticles by slowly adding a polymer/chloroform solution to preformed microparticles suspended in 5% aqueous ethanol at 54 °C (Cong and Beck, 1991) (for coating procedures see also Section 3.7).

Following the dissolution of crystals in the vicinity of the polymer surface, large voids in the surface of the microparticles may appear, resulting in a faster mass transfer of the dissolution medium into the particles. Thus, the medium may access the whole payload if the drug crystals are not separated and homogeneously dispersed throughout the microparticles. Also, monolithic particles from, e.g., *o/w* techniques that have a uniform distribution of hydrophobic drug in the matrix can be expected to show higher matrix hydrophobicity and potentially lower water-uptake compared to *s/o/w* particles with larger crystals at similar drug loading. Both aspects may lead to faster release profiles from *s/o/w* microparticles.

### 3.3. *o/o* method

Although being classified as hydrophobic drugs, substances such as hydrocortisone exhibit an appreciable solubility in aqueous media (280 µg/ml water) like the external water phase (see Table 1). Therefore, *o/w* methods are expected to result in low encapsulation efficiencies due to a flux of the active agent from the dispersed phase to the larger volume of the continuous phase during the encapsulation process. In order to overcome this issue, *o<sub>1</sub>/o<sub>2</sub>* emulsion methods can be used. They include the extraction of the *o<sub>1</sub>*-phase solvent, e.g., acetonitrile, by a solution of an emulsifier (HLB typically <8; Jalil and Nixon, 1990a; Herrmann and Bodmeier, 1998) in oil, e.g., cottonseed oil or mineral oil (acetonitrile solubility in cottonseed oil ~10%; Leach et al., 2005), which should be a non-solvent for both the polymer and the drug (Jalil and Nixon, 1989, 1990a,b,c; Wada et al., 1990). The *s/o/o* technique combines the concepts of *s/o/w* and *o/o* methodologies (Janoria and Mitra, 2007). Also an *o<sub>1</sub>/o<sub>2</sub>/o<sub>3</sub>* technique has been described that may be applicable for certain hydrophobic drugs (Herrero-Vanell et al., 2000), which are soluble in fluorosilicone oil (*o<sub>1</sub>*-phase) but not in PLGA solvents like acetone (*o<sub>2</sub>*-phase). However, for methods carried out in oil the removal of the continuous phase requires a special treatment, e.g., washing of the particles with hexane or petroleum ether.

### 3.4. *w/o/w* method

Although initially afflicted with low encapsulation efficiency of hydrophilic molecules unless *w<sub>1</sub>*-phase solidification was performed (Okada et al., 1987), the *w<sub>1</sub>/o/w<sub>2</sub>* method has been described to result in extremely efficient loading of biodegradable microparticles with water-soluble compounds (Yamamoto et al., 1994) and is currently one of the most commonly used methods for peptide and protein encapsulation. Compared to *w/o*-based coacervation techniques that were being developed at about the same time for hydrophilic drug encapsulation into PLGA (Kent et al., 1986), the *w/o/w* method was described to overcome the issues of final oil removal by several washing steps and the tendency to particle aggregation during the preparation procedure (Yamamoto et al., 1994). In the literature there are a few cases where hydrophobic

drugs are stated to be encapsulated by a *w/o/w* method. However, a closer look at these methods revealed that either the drug was suspended in the inner water phase in a (*s + w*)/*o/w* complex dispersed system (Giunchedi et al., 1998) or solution of the active compounds, e.g., a mixture of ethinylestradiol and levonorgestrel, in an ethanol/water mixture was used as the *w<sub>1</sub>*-phase (Dhanaraju et al., 2003, 2004, 2006). The addition of some inner water phase in the (*s + w*)/*o/w* technique is expected to increase porosity in the particle core, which may influence drug release. However, employing an ethanolic drug solution as the *w<sub>1</sub>*-phase is expected to result in drug precipitation in *w<sub>1</sub>* due to mass transfer of ethanol (including some drug) from *w<sub>1</sub>* through the *o*-phase to the *w<sub>2</sub>*-phase, which may lower the encapsulation efficiency.

### 3.5. *In situ* forming microparticles

*In situ* forming depot systems, first designed as implants, were intended to overcome some drawbacks of conventional formulations such as reducing manufacturing costs and complexity and pain on injection through larger needles. They should be administered simply by injecting a polymer/drug solution or suspension in an appropriate solvent that would precipitate and form the implant at the injection site (Dunn et al., 1990, 1994, 2003; Tipton and Fujita, 1991; Shah et al., 1993), a concept that has been employed in FDA-approved LAR® products (Eligard® with the Atrigel® delivery system). Solvent partitioning into the tissue has been reported to cause mild transient burning at the injection side in about 20% of the cases (AGL9909 study, 2000). Besides Atrigel®, that uses solutions of PLGA in water-miscible solvents (e.g., NMP, DMSO), there are other methods for *in situ* precipitating PLGA implants such as Alzamer® (using partially water-miscible solvents like ethyl benzoate and triacetin) (Brodbeck et al., 2000) and the Saber® system (PLGA dissolved in sucrose acetate isobutyrate, a viscous sugar derivative, plus some organic solvent for easier injectability) (Tipton, 1999; Burns et al., 2000). There are also numerous strategies that employ matrices other than PLGA (Dittgen et al., 1998; Hafezi and Amsden, 2002; Packhaeuser et al., 2004), e.g., thermosensitive systems such as ReGel® low molecular weight PLGA-PEG-PLGA block copolymer micelles (Rathi et al., 2001; Zentner et al., 2001).

Soon thereafter, the idea of *in situ* forming depots was applied to microparticles in order to overcome issues associated with conventional microparticle formulations such as the cost-intensive preparation, drying, and potentially difficult resuspension. Also, in contrast to *in situ* implants, *in situ* microparticles showed lower myotoxicity (Kranz et al., 2001), easier injectability depending on the type of solvent and suspension medium (Im-Emsap, 2002), and a more reproducible surface area as opposed to the irregular shape obtained from such implants.

Starting with relatively large particles prepared by dropping aliquots of drug + PLGA/NMP into aqueous medium (Lambert and Peck, 1995), the formulations became injectable when preformed *o/o* emulsions stored until administration (Jain et al., 2000a,b,c; Jain, 2000), or two-compartment systems (syringes attached to each other with a syringe connector) with its content being dispersed at the bedside to form *o/w* or *o/o* emulsions (Bodmeier, 1998a; Voigt, 2006) were investigated. After injection, the partitioning of the biocompatible solvent (Royals et al., 1999) into the tissue causes the hardening of the emulsion droplets *in vivo* and is also responsible for a high burst release typically observed with such formulations (Jain et al., 2000c). Most authors have incorporated hydrophilic substances using *o/o* emulsions (Jain et al., 2000a,b,c; Kranz et al., 2001; Kranz and Bodmeier, 2007; Luan and Bodmeier, 2006a,b), and the external oil phase can be expected to act as a diffusion barrier that decreases the burst

release depending on its volume and viscosity (Luan and Bodmeier, 2006a).

However, safety issues will limit the type of oil that can be used. Paraffin/mineral oils are known to cause severe lipoid pneumonia (Perings et al., 2001; Simmons et al., 2007), and thus their presence in preparations for injection must be excluded. Vegetable oils are allowed as nonaqueous vehicles by the USP and Ph. Eur. (USP, 2007; Ph. Eur., 2002a) and peanut oil has been suggested by different pharmacopoeiae as a standard oil for injections. Although the extraction procedure of *Olea Herbaria* is designed to reduce allergenic protein impurities (Taylor et al., 1981; Ph. Eur., 2002b) and, e.g., peanut oil is presently used in several injectable formulations of steroids and other drugs, it must be recognized that a steadily rising number (>1%) of the children in the US and Europe (Sicherer and Sampson, 2007; Savage et al., 2007; Green et al., 2007) exhibit allergic symptoms with numerous peanut proteins (Bernard et al., 2007) and injection of contaminated peanut oil might cause fatal anaphylaxis. Since the standard refining procedure, thermal denaturation, might not always eliminate allergenicity (Burks et al., 1992), “peanut products should be treated as allergenic unless they have an analytically monitored non-allergenic specification” (EMA, 2004). Also, allergies are well-known for alternative oils such as sesame (Gangur et al., 2005), almond, and other oils (Roux et al., 2003). Medium chain triglycerides (MCT), as derived from the kernel of the Coconut Palm and the African Oil Palm (Ph. Eur., 2002c) and used in emulsions for parenteral nutrition in mixture with soybean oil (Discoll, 2006; USP, 2006), might, under exclusion of allergies of the respective patient, be an alternative nonaqueous  $o_2$ -phase for in situ formulations. However, in situ microparticle formation using MTC was limited to DMSO and propylene carbonate as  $o_1$ -phase solvent (Luan, 2006).

For hydrophobic drugs like indomethacin, o/w in situ formulations with water-immiscible solvents were reported, and, as expected, showed burst releases of up to 50% depending on the polymer concentration (Im-Emsap, 2002). Again, higher viscosities of the continuous water phase should reduce the diffusion of the drug during the particle hardening, but will impact the injectability of the emulsion. This issue can be overcome by adding substances to the water phase that change their viscosity when the ambient conditions are altered after injection into the tissue, e.g., by temperature or pH-dependent gelation (Bodmeier, 1998b, 2002). In myotoxicity studies o/w in situ formulations showed the best compatibility when ethyl acetate was employed as the drug/polymer solvent (Rungsevijitprapa et al., 2008).

### 3.6. Salting out/phase separation

Salting out is a method to precipitate dissolved polymers, i.e., most commonly proteins, by attracting water molecules to the salt ions and therefore decreasing the number of water molecules available for solvation of the polymer (simple coacervation). Although the term “salting out” might be misunderstood in case of water-insoluble polymers, the described method utilizes the controlled precipitation of PLGA from an organic phase of a water-miscible solvent while emulsified in a viscous PVA/salt solution. By adding water to the system, the  $o$ -phase solvent is slowly extracted, whereas the polymer is unable to follow the solvent and forms microparticles rather than nanoparticles (Rafler and Jobmann, 1997). However, this process seems to require a careful optimization of certain process parameters, e.g., the salt type and concentration, the type of polymer and solvent, and the ratios of these compounds in order to obtain microparticles at all.

Microparticle preparation by organic phase separation can be a temperature-induced process, but has mostly been performed by adding a coacervation agent (ternary system of

polymer + solvent + nonsolvent or second polymer) to a suspension or emulsion of a drug in a PLGA solution and solidifying the resulting liquid coacervate capsule in a hardening bath (Kent et al., 1986; Thomasin et al., 1998a,b). Some of the steps can be performed at a reduced temperature (Fong, 1979), e.g., the hardening in hexane cooled to  $-70^\circ\text{C}$  by a dry-ice/isopropanol mixture (Lapka et al., 1986). Such methods are commonly employed for water-soluble compounds (Nihant et al., 1995; Thomasin et al., 1997), but have been used for hydrophobic drugs, too (Nuwayser et al., 1977; Gardner et al., 1977; Leelarasamee et al., 1986).

### 3.7. Melting techniques

Melting techniques represent another strategy to encapsulate drugs into biodegradable polymers which, with some exceptions (Yolles et al., 1975; Yolles and Sartori, 1980), avoid the use of organic solvents but require the dispersion or melting of the drug in a polymer melt. In order to form microparticles the hot melt of the matrix polymer 1 can be dispersed in a second, molten, water-soluble polymer 2, which is immiscible with the matrix polymer 1. Then, the resulting emulsion will be solidified by cooling, and the polymer 1 microparticles will be collected after dissolving the continuous phase polymer 2 in water (Chenite et al., 2002). More commonly, the drug/matrix polymer melt is cooled down and then ground (Boswell and Scribner, 1973; Smith and Hunnyball, 1986) or jet-milled (Nykamp et al., 2002) to form non-spherical particles. To allow an easier grinding of otherwise unmanageable lumps of the congealed melt, it may be advantageous to extrude the material before complete solidification, especially for large batch sizes. Extrusion prior to grinding has also been used for the preparation of microparticles from pre-casted films (Gresser et al., 1978).

If spherical particles and a smaller size distribution are desired, the ground melt can be emulsified in a hot solution containing emulsifier (Wichert and Rohdewald, 1990) or a hot gel (Ruiz, 1992). Another approach to smooth the surface and prolong the in vitro release from ground, norethisterone-loaded poly-L-lactic acid microparticles was suggested (Anderson et al., 1976) that employs a coating of the particles with poly-D,L-lactic acid in benzene, a solvent avoided nowadays because of its carcinogenicity (for coating procedures see also Section 3.2). Also, clear drawbacks of the melting technique are the thermal treatment of the drug and the multitude of steps to obtain smooth microparticles. Moreover, the fear of residual solvents might be exaggerated, since lyophilization was shown to reduce solvent impurities to a safe value for an emulsion-based preparation technique (Wischke et al., 2006) and numerous commercial microparticle formulations are prepared with toxic carrier solvents, but have met regulatory standards. Lastly, it should be pointed out that melt-based encapsulation methods commonly develop highly nonporous polymer matrices (Zhou et al., 1998), which can lead to undesirably slow release profiles especially for hydrophobic drugs.

### 3.8. Methods using supercritical fluids (SCF)

Substances become supercritical fluids (SCF) when placed above their critical point (i.e.,  $T > T_c$  and  $p > p_c$ ). SCF exhibit the flow properties of a gas (low viscosity) and the dissolving power of a liquid. SCF can easily penetrate through materials because they do not show any surface tension, and their solvent power is related to their density, which experiences large changes in the vicinity of the critical point, and can be controlled by altering temperature and/or pressure (Williams et al., 2002). SCF have a variety of applications including their suitability for the extraction of substances from a large variety of materials, e.g., essential oils from plants (Pourmortazavi and Hajimirsadeghi, 2007). Most commonly  $\text{CO}_2$



is used for such purposes, as it has a low critical point and is an easily accessible, environment-safe gas. There are at least two techniques for the particle design by SCF (Jung and Perrut, 2001; Ginty et al., 2005), which can be roughly differentiated by their concept to dissolve and precipitate the polymer and the drug.

In two common scenarios the drug and matrix polymer might be either dissolved or melted in the SCF and afterwards form particles following the rapid expansion from supercritical solution (RESS) (Kim et al., 1996) or precipitate into particles from the gas-saturated solutions/suspensions (PGSS) (Whitaker et al., 2005) after spraying the melt and releasing the gas, respectively. By contrast, other techniques rely on the antisolvent properties of SCF for PLGA, a fact that has limited the usage of RESS and PGSS to low molecular weight PLA. Different antisolvent methods are known, for example, those which differ by the geometry of the nozzle used. These methods follow the concept of spraying the organic drug/polymer solution into a SCF, which extracts the organic solvent. Antisolvent protocols include particle precipitation by compressed antisolvents (PCA) (Falk et al., 1997; Martin et al., 2002), the aerosol solvent extraction system (ASES) (Bleich et al., 1994; Bleich and Müller, 1996), and the solution-enhanced dispersion by supercritical fluids (SEDS) (Ghaderi et al., 2000). Recently the supercritical fluid extraction of emulsion (SFEE), i.e., a classical o/w emulsion, has been described to reduce the time of solvent removal and polymer precipitation (Chattapadhyay et al., 2006). Methodological details, applications, and drawbacks of different SCF techniques for drug encapsulation have been noted in some current reviews (Tewes et al., 2006; Mishima, 2008; Davies et al., 2008).

It is obvious that the methods employing SCF require special equipment and that these techniques are therefore, not widely used on the bench scale. Both the fast extraction of the organic solvent and the partitioning of the SCF into the polymer beads, which afterwards expands during the decompression, may induce a high porosity and a faster drug release. Also one should consider that SCF can dissolve some, but not all, hydrophobic drugs (Vatanara et al., 2005) and that the extracted o-phase solvent acts as a cosolvent together with the SCF, so that a reduced encapsulation efficiency due to drug extraction from the polymer matrix has sometimes been observed (Bleich and Müller, 1996).

### 3.9. Spraying techniques

The preparation of microparticles by spray-drying (Gander and Merkle, 1997) or a cryogenic spray-congealing method (also known as Alkermes' ProLease®) (Gombotz et al., 1991; Johnson et al., 1997) has been intensively studied for protein encapsulation in order to improve the stability of these labile biomacromolecules (e.g., to obviate protein denaturation at o/w interfaces, during solution state micronization, and/or elevated temperature exposure). Spray-drying is also useful for hydrophobic drugs (Bodmeier and Chen, 1988; Pavanetto et al., 1993; Wagenaar and Müller, 1994; Benelli et al., 1998; Mu and Feng, 2001; Mu et al., 2005), particularly for large-scale production of microparticles. For example, this microencapsulation method can overcome the issue of large volumes of solvent-contaminated water phase that result from emulsion-based encapsulation methods. However, larger batch sizes are typically required compared to the emulsion methods and therefore, spray-drying is often more problematic on the economic bench scale or if very little drug is available in the early stages of the development of an experimental microparticle formulation.

### 3.10. Ammonolysis

Recently, risperidone was encapsulated into PLGA microparticles by an o/w emulsion technique that employed methyl

dichloroacetate in place of the traditional volatile carrier solvent to dissolve the polymer. By the addition of an ammonia solution the solvent was hydrolyzed into water-miscible products, i.e., methanol and dichloroacetamide, resulting in the precipitation of PLGA (Sah and Lee, 2006). Although the encapsulation efficiency was almost 100% for risperidone, the flux of methanol from the microparticle core to the water phase may result in a loss of methanol-soluble drugs. This theory is supported by a study, where the encapsulation efficiency of progesterone was as much as 15% lower when ammonolysis with methyl chloroacetate was compared to a standard encapsulation method with methylene chloride (Kim et al., 2007). In the preliminary studies, methyl dichloroacetate resulted in particle aggregation during vacuum drying and neither release nor precise gas chromatographic residual solvent analysis were provided as methyl dichloroacetate and methyl chloroacetate (Pohanish and Greene, 1996; USCG, 1999), like numerous traditionally used solvents for encapsulation, are toxic.

### 3.11. Rationale to select an encapsulation technique

Overall, there are a variety of methods that already have been used for the encapsulation of hydrophobic drugs in PLA/PLGA microparticles. A summary of these procedures including their challenges is given in Figs. 1 and 2.

However, for a pharmaceutical company the approval of the formulation for the market might be faster, easier, and cheaper, when techniques similar to those of already available commercial products are used. Table 3 shows PLGA-based microparticle products and provides information on the expected manufacturing procedure which we have assumed from the patent literature. For the hydrophobic drugs, risperidone and naltrexone, o/w solvent evaporation methods were described. Other substances, namely water-soluble drug salts, peptides, or proteins, were encapsulated by coacervation, double emulsion, or spraying techniques. As the o/w and s/o/w methods are most commonly used for small-scale microencapsulation studies, the following sections will focus on particles derived from these methods.

## 4. Solvents/cosolvents

### 4.1. Dispersed phase solvents

Organic solvents are used in emulsion-based microencapsulation techniques to dissolve the matrix polymer and, in the case of the o/w method, also the drug to be encapsulated. Often even hydrophobic drugs do not dissolve very well in the desirable carrier solvent, methylene chloride. Such drugs might be either encapsulated by the s/o/w technique or an alternative solvent might be used to prepare PLGA microparticles (Table 2). However, beside the ability of a solvent to dissolve both the polymer and the drug, other characteristics of the respective solvent need to be considered, since they commonly affect the size, morphology, drug release, or the residual solvent of the microparticles.

#### 4.1.1. Solubility of polymer solvent in continuous phase

The water solubility of the solvent will impact its initial extraction during microparticle preparation. In general, a fast precipitation of the polymer due to the initial efflux of the solvent to the external phase is considered to be advantageous for achieving high encapsulation efficiencies (Bodmeier and McGinity, 1988; Mao et al., 2008). However, if the solvent is too soluble in water, and/or a large volume of water is used, very fast solidification of the polymer may occur, forming a dense polymer shell around the



**Table 3**  
PLGA based microparticle depot formulations that have entered the market and their preparation procedure as expected from the literature<sup>a</sup>

Drug	Product <sup>b</sup>	Distributor	Indication	Encapsulation technique	References
<b>Peptides</b>					
Octreotide acetate	Sandostatin LAR <sup>®</sup> Depot	Novartis	Acromegaly; diarrhea associated with metastatic carcinoid tumors; Diarrhea associated with Vasoactive intestinal peptide secreting tumors	Coacervation <sup>c</sup>	Bodmer et al. (1996a, 1996b, 1997), Lambert et al. (2004) and Petersen and Ahlheimer (2007)
Leupro- lide acetate	Lupron Depot <sup>®</sup> (North America)	TAP Pharmaceuticals	Prostate cancer; endometriosis; uterine fibroids; central precocious puberty (indication may differ depending on the respective product and approving country)	w/o/w emulsion solvent evaporation	Okada et al. (1987, 1992, 1994), Okada (1997), Ogawa et al. (1988a,b,c, 1989), Ogawa (1992), Yamamoto et al. (1994) and Takechi et al. (2002)
	Enantone <sup>®</sup> ; Trenantone <sup>®</sup> (Europe) Prostap SR; Prostap 3 (GB)	Takeda Wyeth (licensed from Takeda)			
Triptorelin acetate	Decapeptyl <sup>®</sup> Depot <sup>d</sup>	Ferring Pharmaceuticals	Prostate cancer; endometriosis; uterine fibroids; central precocious puberty (indication may differ depending on the respective product and approving country)	Coacervation	Nerlich et al. (1996), Mank et al. (1996) and Klippel et al. (1999)
	Gonapeptyl <sup>®</sup> Depot (GB) Decapeptyl <sup>®</sup> SR (GB) Decapeptyl <sup>®</sup> Neo-Decapeptyl <sup>®</sup> Decapeptyl <sup>®</sup> Retard	Ipsen Technofarma <sup>e</sup> Ach�e <sup>e</sup> Sidus <sup>e</sup>			
Triptorelin pamoate	Telstar <sup>®f</sup>	Pfizer	Prostate cancer (US, Canada) Endometriosis (Canada)	Hot extrusion, cryogenic grinding <sup>g</sup>	Orsolini (1992, 1993) and Minkov et al. (2001)
	Telstar <sup>®f</sup>	Watson <sup>e</sup>			
Triptorelin embonate	Pamorelin <sup>®</sup> ; Pamrorelin <sup>®</sup> LA Pamorelin <sup>®</sup>	Ipsen <sup>e</sup> Rawfarma <sup>e</sup>	Prostate cancer	Not available	Not available
Lanreotide acetate	Somatuline <sup>®</sup> LA	Ipsen	Acromegaly; thyrotropic adenomas; neuroendocrinic tumours	Coacervation	Pellet and Roume (2001, 2002)
Buserelin acetate	Suprecur MP (Japan)	Mochida Pharmaceutical Co. (licensed from Aventis)	Endometriosis, uterine myoma	Spray-drying	Lill and Sandow (1995)
<b>Protein</b>					
Human growth hormone	Nutropin Depot <sup>®h</sup>	Genentech	Pediatric growth hormone deficiency	s/o cryogenic spray-congealing method (ProLease <sup>®</sup> , Alkermes)	Gombotz et al. (1991), Johnson et al. (1996, 1997), Lee et al. (1997) and Tracy (1998)
<b>Small molecules</b>					
Naltrexone	Vivitrol <sup>®</sup>	Cephalon	Alcohol dependence	o/w emulsion solvent extraction (Medisorb <sup>®</sup> , Alkermes)	Brittain et al. (2004) and Dean (2005)

Risperidone	Risperdal® Consta®	Janssen Pharmaceuticals	Schizophrenia	o/w emulsion solvent extraction (Medisorb®, Alkermes)	Rickey et al. (1998), Wright et al. (2001), Mesens et al. (2003), Ramstack et al. (2003) and Lyons and Wright (2004, 2005)
Minocycline hydrochloride	Arestin®	OralPharma	Periodontal disease	s/o coacervation	Lawter and Lanzilotti (1996)
Bromocriptine	Parlodol LA® <sup>i</sup> , Parlodel LAR® <sup>i</sup>	Sandoz (Novartis)	Parkinson disease; hyperprolactinemia; acromegaly	Spray-drying	Zdenek and Kissel (1986, 1989), Kissel et al. (1991) and Montini et al. (1993)

<sup>a</sup> Drug approval documents as available on the homepage of the FDA via <http://www.fda.gov/cder/drug/default.htm> do not allow conclusions on the preparation technique, because the respective paragraphs are withheld. As can be expected from the cited references, the stated preparation method at least initially had been used to produce the respective product. However, in most cases the stated encapsulation technique was not confirmed by the distributor or other authorities.

<sup>b</sup> Occasionally products are only available in certain countries as indicated.

<sup>c</sup> Older patents describe coacervation, double emulsion, and spray-drying techniques. Recent patents focus on the w/o/w technique, but state that silicone oil and heptane are impurities of Sandostatin LAR®, indicating the usage of a coacervation technique in the approved product.

<sup>d</sup> Product name may differ in certain countries, e.g., Decapeptyl® N 3.75 mg and Decapeptyl® GYN (Germany).

<sup>e</sup> Present licensees of Debiopharm (according to Debiopharm: <http://www.debiopharm.com/decapeptyl-trelstar-triptorelin.html>, February 7th, 2008).

<sup>f</sup> Available as Telstar™ Depot 3.75 mg and Telstar™ LA 11.25 mg.

<sup>g</sup> Statement by Minkov et al., 2001 ("prepared without organic solvents") indicates the use of a hot extrusion technique as patented (Orsolini, 1992, 1993).

<sup>h</sup> Marketing stopped in 2004 because of economic reasons.

<sup>i</sup> No longer on the market.

droplets that is unable to shrink, creating particles with a hollow core (Birnbbaum et al., 2000; Birnbbaum and Brannon-Peppas, 2003) or possibly collapsed particles when the remaining solvent is extracted from the core. On the other hand the fast flux of the solvent out of the o-phase can further disrupt the droplets and result in smaller microparticles as the emulsion droplets shrink accordingly, a typical observation for lower polymer concentrations when ethyl acetate instead of methylene chloride is used as the polymer solvent (Fig. 3). Beside the higher flux of ethyl acetate to the water phase, the lower interfacial tension of ethyl acetate–water ( $\gamma = 6.8$  nN/m; Adamson, 1999) compared to methylene chloride–water ( $\gamma = 27.8$  nN/m; Sah, 1999) will contribute to this phenomenon.

One way to overcome the issue of overly rapid solidification is a pre-saturation of the continuous phase with the respective solvent, so that the extraction of the solvent and the final hardening of the droplet surface are delayed and the particles are allowed to shrink (Soppimath and Aminabhavi, 2002). As can be seen from Fig. 3a and e, this step will be effective for high polymer concentrations, i.e., fast particle solidification. If rapid precipitation is the suspected cause of misshaped particles, the use of a lower polymer concentration in the respective solvent should be evaluated, too. For the o/w method a high solubility of the solvent in the hardening bath might be disadvantageous as this will increase the loss of the drug to the continuous phase and therefore, reduce the encapsulation efficiency. The extent of drug loss will be decreased by using a smaller volume of the w<sub>2</sub>-phase with an extended evaporation time and/or with an increased surface area (diameter of beaker) being available for the solvent evaporation. For ethyl acetate as the carrier solvent it was shown that the microstructure of the formed particles (hollow capsule vs. monolithic matrix) can be controlled by the o:w ratio when the emulsion is formed, followed by solvent evaporation or by solvent extraction after pouring in a larger volume of external phase (Sah, 1997).

#### 4.1.2. Solubility of water in the polymer phase

The solubility of water in the organic phase will typically affect the reverse flux of the continuous phase into the dispersed phase and therefore, the porosity of the microparticles. A higher porosity, as indicated above, will allow the release medium to penetrate the particles more easily and favor the drug to be released faster by pore-diffusion. However, a fast solidification of the particles will diminish water-uptake (Li et al., 1999). This can be achieved, for example, by increasing the polymer concentration or by subjecting the emulsion to evaporation under reduced pressure.

#### 4.1.3. Solvent removal rate

The removal of solvent from the hardening bath to the gas phase is required for solvent evaporation methods. It is obvious that the boiling point and vapour pressure of the respective solvent can affect the speed of evaporation, and depends on whether evaporation from the hardening bath is controlled by the unstirred boundary layer in the liquid or the gas (Wang and Schwendeman, 1999). Thus, solvent volatility can also influence the speed of the overall solvent removal from the particles. For methylene chloride the rate-limiting step in a beaker-method at controlled room temperature was shown to be the liquid-side transport, whereas the ethyl acetate evaporation was also restricted by the unstirred gas layer, and flushing the headspace of the beaker with another gas, e.g., N<sub>2</sub>, was found to efficiently increase the solvent removal in the latter case at this temperature (Wang and Schwendeman, 1999). However, for industrial applications in closed vessels a frequent replacement of the gas phase by intensively flushing the liquid surface has been suggested to be necessary for appropriate solvent evaporation rates (Takechi et al., 2002).

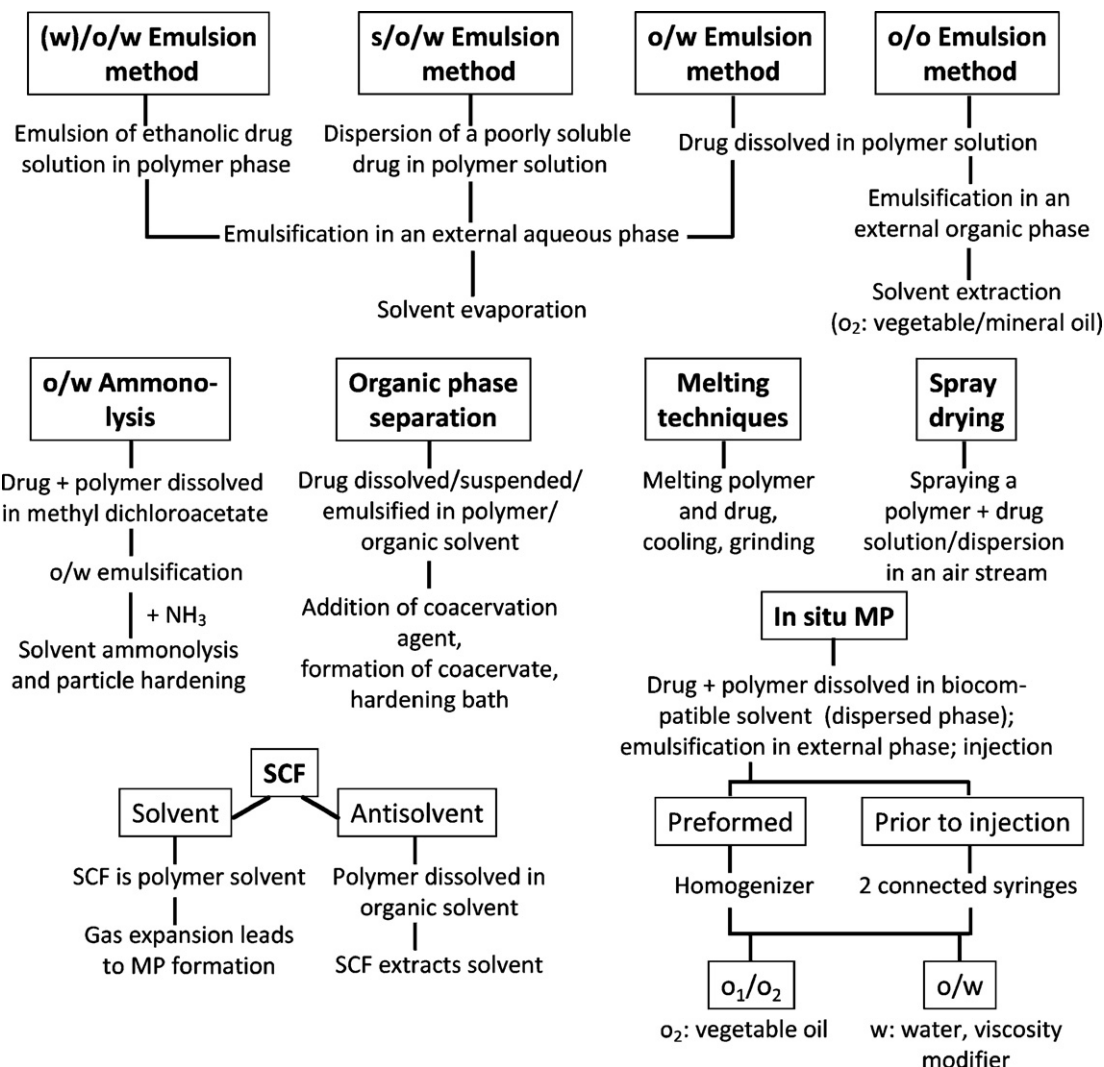


Fig. 1. Principle of microencapsulation techniques used for the incorporation of hydrophobic drugs into biodegradable microparticles (MP). SCF: Supercritical fluid.

Under common conditions of liquid-side mass transfer control and turbulent flow, the mass transfer (i.e., permeability) coefficient of the evaporation (i.e., carrier solvent flux out of the beaker divided by the carrier solvent concentration in water) during in-liquid hardening was related to the Kolmogorov length-scale of turbulence. This relationship and a few additional assumptions allowed the evaporation mass transfer coefficient ( $P$ ) to be predicted by 5 system variables (see Wang and Schwendeman, 1999 for details): impeller diameter,  $d$ ; rotational speed,  $\omega$ ; diffusion coefficient of the organic solvent in water,  $D$ ; and kinematic viscosity,  $\nu$ , and volume,  $V$ , of the water phase, as follows:

$$P \propto d^{-5/4} \cdot V^{1/4} \cdot \omega^{-3/4} \cdot \nu^{-5/12} \cdot D^{1/3} \quad (1)$$

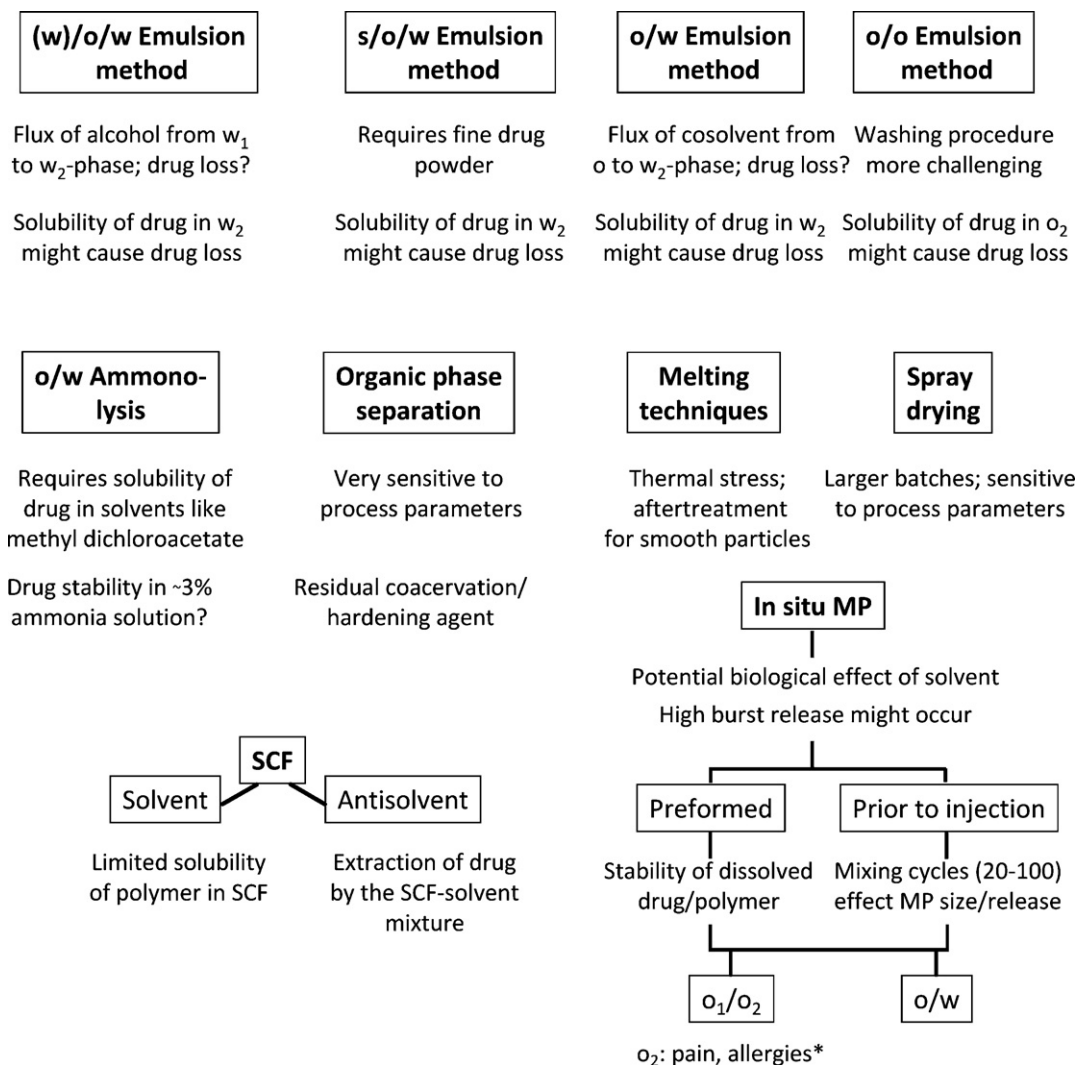
This prediction of evaporation was verified by comparing the relation with a systematic data set of methylene chloride evaporation (i.e., as a function of rotational speed, temperature, impeller diameter, and bath volume), as shown in Fig. 4.

By contrast, in solvent extraction methods the rate of solvent removal from the o-phase is controlled by the volume of the continuous phase and the solubility of the solvent in the w-phase. It can be altered, e.g., by a stepwise replacement (Tice and Lewis, 1983) or dilution of the external phase (Jeyanthi et al., 1996), or by employing an alcohol/water mixture to extract the o-phase solvent (Gupta et al., 1992). The loss of hydrophobic agents from microparticles pre-

pared by extraction methods might be undesirably high due to the typically larger volumes of the w-phase and the extended presence of the solvent in the hardening bath. Applying reduced pressure or increasing the temperature helps to expedite the removal of solvents with a lower volatility. However, elevated temperatures of the hardening bath have been shown to result in an increased porosity and lower loading of microparticles, because the more rapid flux of solvent across the o/w interface stresses the precipitated polymer film and can cause small fractures (Bodmeier and McGinity, 1987b; Choi et al., 2002).

#### 4.1.4. Solvent toxicity and regulatory considerations

The toxicity of the solvent is important for the (i) operator, (ii) patient, and (iii) regulatory approval of the microparticle product. As can be seen from Table 2, solvents are categorized in different classes by the USP and likewise by the Ph. Eur. and the maximum residual solvent levels strongly depend on the toxicity of the respective solvent. This fact might be the reason why chloroform (class 2 solvent, limit as low as 60 ppm; ICH, 2003), sometimes employed by the pioneers of drug encapsulation (Beck et al., 1983a,b), is less often used in the present literature. However, most protocols include a final lyophilization or elevated temperature drying of the microparticles, which should help to reduce the amount of residual solvent to an acceptable value.



**Fig. 2.** Challenges of microencapsulation techniques used for the incorporation of hydrophobic drugs into biodegradable microparticles (MP). \*Vegetable oils, such as peanut oil, may cause allergic reactions.

#### 4.2. Cosolvents

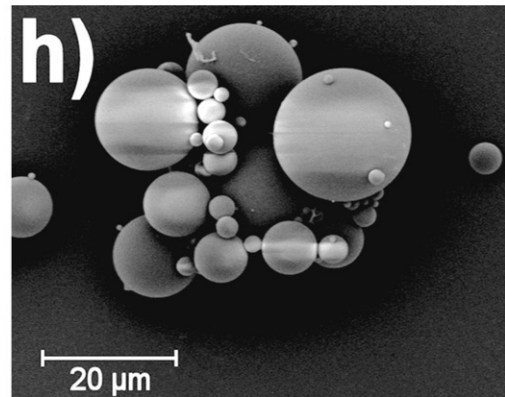
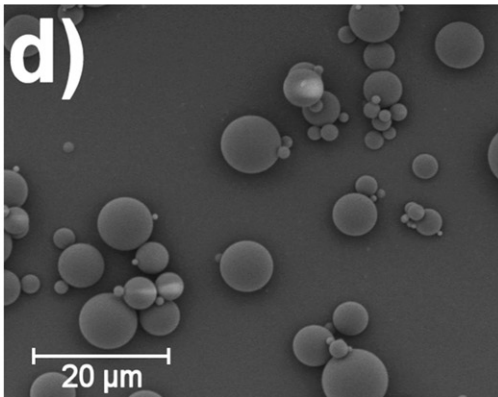
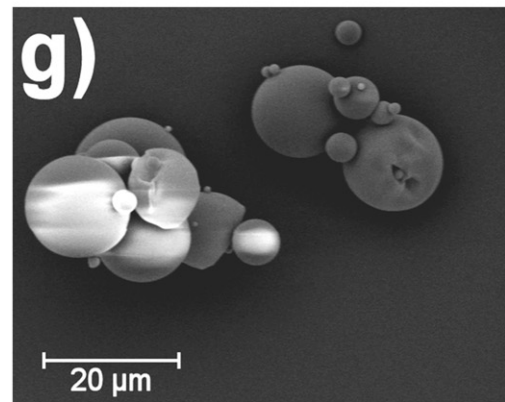
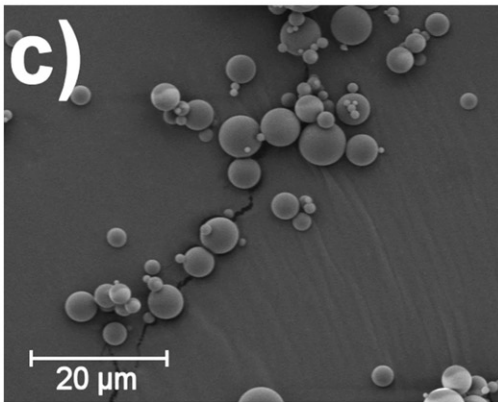
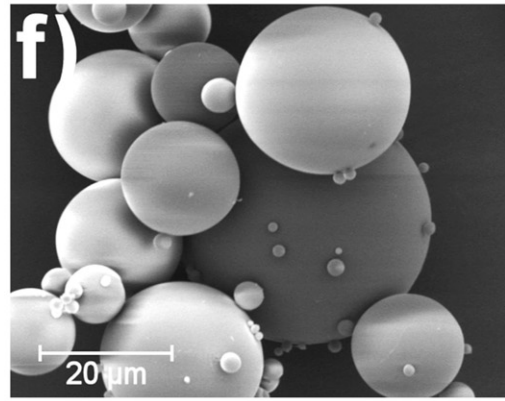
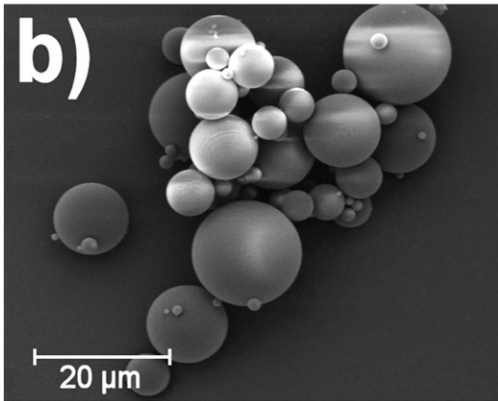
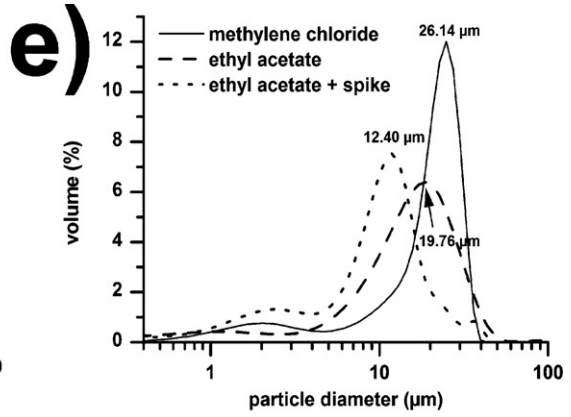
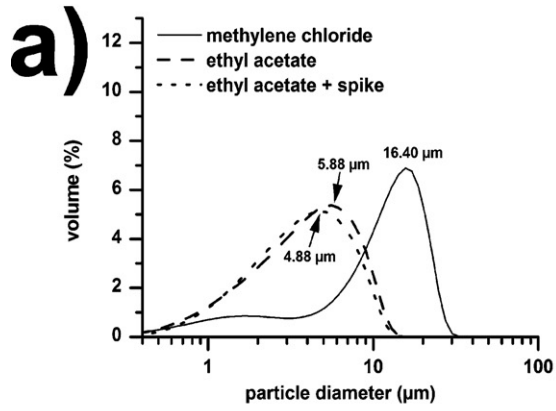
As already mentioned, the lack of an appropriate solubility of the drug in the organic phase can be overcome by the use of an *o/w* cosolvent method. Such cosolvents are commonly water-miscible and exhibit a high volatility, e.g., methanol (Birnbau et al., 2000; Tuncay et al., 2000; Panyam et al., 2004; Thote et al., 2005) or acetone (Yang et al., 1999; Ruan and Feng, 2003), but also dimethylformamide (Shenderova et al., 1997), various alcohols, dimethyl sulfoxide (DMSO), acetonitrile, tetrahydrofuran, and ethers (Albayrak, 2005; Graves et al., 2006). Although the presence of the cosolvents is required to allow the use of an *o/w* technique for certain drugs, it is also known to make the whole system more complex and potentially reduce the encapsulation efficiency and increase the amount of surface-associated drug crystals (Shenderova et al., 1997; Birnbaum et al., 2000).

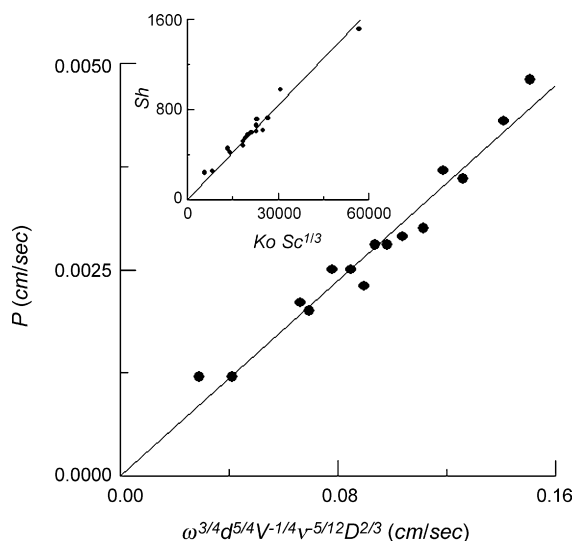
The presence of a water-miscible cosolvent in mixture with methylene chloride results in the initial extraction of a large amount of the cosolvent and some of the methylene chloride during particle formation. If the cosolvent is a non-solvent for PLGA, the drug/cosolvent and the PLGA/methylene chloride are expected to form polymer-poor and polymer-rich regions inside the *o*-phase, respectively. The pathways of water entering the organic phase

are either through one or both of these phases, with the former being limited by the concentration of the remaining cosolvent and the latter by the solubility of water in PLGA/methylene chloride. Therefore, a higher amount of cosolvent present in the nascent microparticles after the first extraction implicates a higher porosity of the microparticles (Li et al., 1999). Also the method of the subsequent removal of the solvent/cosolvent (Jeyanthi et al., 1996) and the ratio of dispersed to continuous phase can affect the porosity of the particles.

In order to successfully encapsulate a hydrophobic drug by a cosolvent method the amount of cosolvent should be kept at a minimum level, since a certain portion of the hydrophobic drug will follow the cosolvent and be lost to the continuous phase. Also, a fast solidification of the periphery of the droplet from the sol to a gel and further on to a glassy state can be advantageous and achieved by a higher polymer concentration or hydrophobicity (i.e., a higher molecular weight, a higher content of lactic acid, or a more hydrophobic end-group capping). Additionally, faster hardening can be observed when the volume ratio of continuous to dispersed phase is increased, which was successful in some cases to increase encapsulation efficiency (Bodmeier and McGinity, 1987b), but might in principle for other formulations result in a higher loss of drug to the continuous phase.







**Fig. 4.** Mass transfer coefficient ( $P$ ) of solvent evaporation is linearly related to 5-dimensional variables: impeller diameter,  $d$ ; rotational speed,  $\omega$ ; diffusion coefficient of the organic solvent in water,  $D$ ; kinematic viscosity,  $\nu$ ; and volume,  $V$ , of the water phase. Inset: degrees of freedom are reduced for scaling as the nondimensional  $P$  (i.e., the Sherwood number,  $Sh$ ) is similarly related to the product of the corrected Kolmogorov number,  $Ko$ , and the Schmidt number,  $Sc^{1/3}$ . See Wang and Schwendeman (1999) for details. Reproduced with permission from *J. Pharm. Sci.* (Wang and Schwendeman, 1999), Copyright © (1999) John Wiley & Sons, Inc.

## 5. Polymer types

### 5.1. Criteria for polymer selection

In the very early papers of drug microencapsulation into polyesters, the authors typically used hydrophobic polylactic acid (PLA) (Boswell and Scribner, 1973; Nuwayser et al., 1977; Gardner et al., 1977; Beck et al., 1979, 1980, 1981; Conti et al., 1992). Within the last two decades, poly(lactic-co-glycolic) acid (PLGA) has become the most commonly used biodegradable polymer for experimental and commercial drug encapsulation. This might be due to the typically slow degradation and drug release rate for PLA that can deliver drugs over months (e.g., Trenantone<sup>®</sup>), whereas PLGA degrades faster and can meet 2–4 week release criteria (e.g., Enantone<sup>®</sup>) that are often desired for initially developed LAR<sup>®</sup> formulations of previously daily administered drugs.

The selection of a polymer for pilot microencapsulation trials might be simplified by finding answers to the following questions: (i) Which route of administration seems to be best for the specific drug and what mass of polymer microparticles can be administered per unit dose?, (ii) At which rate should the drug be released from the microparticles daily in order to meet a therapeutic concentration?, (iii) Presuming a 20% drug loading and knowing the amount of particles per dose and the required release rates, how long will one dose last?, and (iv) Is the total required dose larger than can be fit into the maximal allowable injected polymer prohibiting the controlled release strategy? By answering these questions the maximum release period can be determined. Although the drug release

should be faster than the complete degradation of the respective polymer, the degradation times (Lactel<sup>®</sup> product information) might help to choose a polymer for the preliminary trial. Reasonable release times from PLGA microparticles are 1 week to 4 months based on products used or tested commercially.

### 5.2. Polymer degradation behavior

As mentioned above, PLGA is the most studied matrix of biodegradable microparticles. The polymer properties, e.g., the amount of water-uptake and the degradation time, can be adjusted by the selected molecular weight, the polymer end-group, the lactide/glycolide ratio, and, for PLA, the crystallinity of the polymer (Wu, 1995a; Witschi and Doelker, 1998; Li, 1999). Other impact factors on polymer degradation include the size of the microparticles, the pH, and the temperature of the medium (Chu, 1981; Dunne et al., 2000). Other reasons for the intensive use of PLA/PLGA are its approval in numerous biomedical and pharmaceutical products in the US and Europe for use in humans, its commercial availability (e.g., Resomer<sup>®</sup>, Lactel<sup>®</sup>), and its good solubility in numerous organic solvents.

Commonly, as long as 4–6 week release can be achieved by a 50:50 PLGA with a low to medium molecular weight (e.g., Resomer<sup>®</sup> RG 502 or 503), and if a slower release is required one might consider a polymer with a higher lactide/glycolide ratio. Also, the type of the polymer end-group will impact the water-uptake and degradation rate of the particles (Tracy et al., 1999; Wiggins et al., 2006). Free carboxyl groups lead to much more swelling of the matrix compared to the capped polymer bearing an aliphatic group at the very end of the polyester chain (e.g., Resomer<sup>®</sup> RG 503H vs. Resomer<sup>®</sup> RG 503), with methyl, ethyl, and lauryl alcohols being very common end-groups for capped polymers. The access of additional water and the catalytic activity of instantly present free carboxyl groups are described to cause the faster degradation of free-acid end-group PLGA.

In vivo polymer degradation was shown to be faster compared to in vitro assays in buffer solution, which was attributed to a plasticizing effect of biological substances such as lipids (Menei et al., 1993), or possibly even the immunological response (which could trigger local release of harmful substances like radicals) (Ali et al., 1994). Also, aggregation of microparticles in the tissue might increase the retention of acidic products in the aggregate and thus, accelerate autocatalytic chain scission (Sansdrap and Moës, 1997).

It is well-known that acidic products from the hydrolytic degradation of PLGA (Li et al., 1990; Park, 1995; van Apeldoorn et al., 2004; Ding and Schwendeman, 2004) accumulate inside the microparticles, as determined by EPR spectroscopy (Mäder et al., 1998), confocal imaging of pH-sensitive dyes (Shenderova et al., 1999; Fu et al., 2000; Li and Schwendeman, 2005), and liquid chromatographic analysis or titration of acidic degradation products (Ding and Schwendeman, 2004). The acidic microclimate pH in PLGA was successfully predicted based on the water-soluble content of acidic monomers/oligomers in the polymer matrix and the corresponding polymer-water partition coefficients of the same acids (Ding et al., 2006). Although considered to be disadvantageous for proteins and drugs that are sensitive to hydrolysis, the

**Fig. 3.** Effect of o-phase solvent and polymer concentration on the size distribution (determined by laser diffraction) and microstructure of microparticles prepared by an o/w solvent evaporation technique. Polymer (Resomer<sup>®</sup> RG 503H) concentration in the o-phase was 100 mg/ml (a–d) or 200 mg/ml (e–h), and the o-phase solvent was methylene chloride (b and f), ethyl acetate (c and g), and ethyl acetate with ethyl acetate saturated water phase (d and h). Lower polymer concentration resulted in smaller particles due to the decreased viscosity of the o-phase and thus an easier separation into droplets, but also due to later particle solidification that allows more shrinkage. Some deformed particles can be seen with ethyl acetate in (g) because of a too fast polymer precipitation that could be avoided by pre-saturating the w-phase with the solvent (h) and slowing down the solidification process. By contrast, spiking with ethyl acetate did not result in further particle size reduction in (a), because the solidification of the droplet surface is delayed at lower polymer concentration [100–200 mg Resomer<sup>®</sup> RG 503H/1 ml solvent; pre-emulsification in 2 ml 3% PVA (25 kDa) at 5,000 rpm with a rotor–stator homogenizer for 60 s; poured into a 20 ml hardening bath (1% PVA); mixed by magnetic stirring for 3 h].

acidic microclimate has been shown to stabilize certain hydrophobic drugs with maximum stability below pH 4 (Shenderova et al., 1999).

### 5.3. Polymer mixtures and alternative PLGA copolymers

Occasionally the release of hydrophobic drugs might be too slow from 50:50 PLGA copolymers. The use of a PLA-PEG-PLA block polymer instead of 50:50 PLGA was shown to result in a higher porosity and a faster release for the hydrophobic drug paclitaxel (Ruan and Feng, 2003). PLGA-glucose star-shaped polymer, with glucose being the initiator molecule for polymerization (Kissel et al., 1991), has successfully entered the market for octreotide delivery (Sandostatin LAR<sup>®</sup> Depot). Blends of high molecular weight (Mw) polymer with a small portion of a low Mw polymer, or use of low Mw polymer altogether, were among the first methods identified to insure continuous release of peptides (Hutchinson, 1986) and hydrophobic drugs (Bodmeier et al., 1989) by minimizing the lag phase to polymer mass loss and release. Such lag phases may appear after the initial burst release of drug from surface-near domains in medium to high molecular weight PLA/PLGA, when diffusion controlled release through the dense matrix is limited and the generation of matrix microporosity by hydrolytic polymer degradation takes time depending on polymer type, Mw, and matrix geometry (Hutchinson and Furr, 1985). The advantageous effect of polymer blends is often rationalized by the fact that low Mw PLGA degrades faster to the critical Mw that ultimately allows their removal from the matrix, forms pores, and thus more transport paths are available for water to access the particle core and drug being released. Also, during polymer degradation the increasing number of hydrophilic carboxyl group enhances the matrix hydrophilicity and water-uptake for medium and high Mw PLA/PLGA and, accordingly, the instant presence of more of such groups for blends with uncapped, low Mw PLGA will accelerate water-uptake (Hutchinson and Furr, 1989; Witschi and Doelker, 1998), being the precondition for matrix degradation and drug release.

### 5.4. Impact of drug properties and preparation procedure on polymer characteristics

The polymer and microparticle properties are typically strongly influenced by the preparation procedure, and in certain cases by interactions between the drug and polymer. On the one hand this may result in faster polymer degradation. For certain drugs, e.g., thioridazine, an amine-catalyzed hydrolysis of the polymer matrix during the particle preparation and a faster release was observed, which was reduced by performing the o/w emulsification at lower temperatures or erasing the drugs nucleophilicity by the formation of a salt (Maulding et al., 1986). In another study with solid solutions of different amine drugs in PLA, it was shown that an accelerated release correlated well with the ability of the respective drug to catalyze the polymer degradation, but not with the  $T_g$  reduction, the drugs  $pK_a$ , or the drugs octanol-water partition coefficient (Cha and Pitt, 1988, 1989). By contrast, the increased catalytic degradation of 50:50 PLGA was associated with a loading-dependent plasticization ( $T_g$  reduction) for microparticles containing a water-soluble acetylated amino acid, *N*-acetyl cysteine (Desai et al., 2008). Other drugs, namely haloperidol, changed the type of matrix erosion from bulk to surface-erosion (Siegel et al., 2006).

Ultrasound treatment for emulsification can lead to a reduction of polymer molecular weight and therefore, a faster degradation upon exposure to the release medium. This phenomenon was slightly increased in the presence of suspended solids (s/o-dispersion), which act as cavitation nuclei, but was dramatic in

the presence of dissolved quinine, again due to amine-catalyzed random chain cleavage of the polymer's ester bond (Reich, 1998). Gamma sterilization of estradiol-loaded microparticles resulted in a loss of polymer molecular weight by random chain scission, a faster release, and the formation of drug degradation products (Mohr et al., 1999).

On the other hand, the interaction of hydrophobic drug molecules with the polymer matrix via hydrophobic binding forces or of amine-groups with the polymer carboxyl groups by ionic bonds might cause a trapping of the drug inside the particles and therefore, a slower release. An inverse relationship between the release rate and the solid-state solubility of the drug in the polymer has been described for dexamethasone, where an increasing solid-state solubility can be observed with end-group capped polymers, higher lactide content, and lower molecular weight of the end-capped polymer (Panyam et al., 2004). By contrast, another drug, ketoprofen, dissolves up to 20% in PLGA, forms hydrogen bonds with PLGA, and acts as a plasticizer, which was hypothesized to reduce polymer chain-chain interactions and thus accelerate its own release (Blasi et al., 2007).

However, beside the polymer molecular weight, degradation characteristics, and drug-polymer interactions, there are other significant factors, like the polymer microstructure, that play a key role in the properties of microparticles. For instance the release of progesterone was found to be faster rather than slower from PLA compared to 85/15 PLGA microparticles. This was attributed to a rougher surface and higher porosity of the PLA particles, because PLA precipitated faster and the microparticles were not able to shrink and form the common smooth surface in this specific case (Yang and Owusu-Ababio, 2000).

## 6. Controlling the polymer microparticle size

### 6.1. Emulsification procedure

A large variety of o/w emulsification methods have been described ranging from simple set-ups with a beaker and stirrer to, for instance, methods based on static micromixers, where the particle size can be controlled by the flow rates of the o- and w-phase in the micromixer (Schalper et al., 2005; Wischke et al., 2006), or surface liquid spraying, where the o-phase is sprayed on the surface of the stirred water phase (Tang et al., 2007). Also, a "jet excitation method" has been described to achieve size-uniform microparticles by feeding the drug/polymer solution through a glass nozzle, which is equipped with an ultrasonic transducer, into a stream of aqueous carrier, followed by solvent removal in a standard evaporation procedure from the o/w emulsion (Berkland et al., 2001, 2002). Droplet formation by Rayleigh-Plateau instability (Lord Rayleigh, 1879; Eggers, 2006), a process driven by interfacial tension that results in axisymmetric undulations of the liquid jet (perturbations with wavelengths larger than the jet radius) and the break-up of the cylindrical fluid thread into droplets, is the basic principle of numerous micromixers. This classic mechanism of droplet formation is superimposed by ultrasonic high-frequency oscillation of the nozzle in the "jet excitation method", which allows prediction and control of the droplet size by the applied frequency and flow rate.

In order to obtain injectable microparticles for long-acting depot applications, a polydisperse particle size range of 20–100  $\mu\text{m}$  is usually desired. Smaller particles, <5–10  $\mu\text{m}$  are necessary, if the whole microparticles are passively targeted to phagocytic cells (Jeffery et al., 1991; Johanson et al., 2000; O'Hagan and Singh, 2004). Ultrasound (Muranishi et al., 1991) and high pressure homogenization provide a high energy density in the emulsification zone and are expected to produce a high fraction of nanoparticles rather

than microparticles. However, high-speed rotor–stator homogenizers such as Ultra-Turrax® can produce submicron droplets only at a high continuous phase viscosity, which is not the case in common microencapsulation procedures (Schuchmann and Danner, 2004). Therefore, rotor–stator homogenizers at low stirring speed, vortexers, or overhead or magnetical stirrers with the o-phase being poured or injected into the stirred continuous phase are frequently used to meet the 20–100 µm goal.

A simple formulation that requires a minimum of instrumental equipment and might act as a starting point employs (i) the preparation of the drug containing o-phase (e.g., 15–25% (w/v) Resomer® RG503 in methylene chloride), (ii) emulsifying one part of the o-phase in 1–3 parts of a 1–5% PVA (Mw 20–40 kDa, degree of hydrolysis 88%) solution with a vortexer, (iii) pouring the o/w emulsion in 25–100 parts of a hardening bath of a lower PVA concentration, (iv) stirring the nascent particles for about 3 h to allow solvent evaporation, (v) collecting the particles on a sieve or by centrifugation and washing them with water, and (vi) final drying the particle by lyophilisation or under vacuum (desiccator, vacuum oven).

## 6.2. Formulation parameters

A large number of formulation parameters may impact the particle size and ultrastructure, e.g., the o-phase volume and solvent, the concentration and type of polymer, the volume of the continuous phase and the type and concentration of stabilizer, the temperature, the stirring speed, and the stirrer type and geometry among others (Wu, 1995b; Jain et al., 1998). Discussion of all these variables is beyond the scope of this paper; however, some of the more important parameters will be briefly described.

In order to separate the o-phase into individual droplets, shear forces are commonly applied to the system. By increasing the intensity of these forces, e.g., by increasing the stirring speed of a rotor–stator homogenizer, the particle size can be reduced. However, the size distribution often increases simultaneously (Jalil and Nixon, 1990a,b,c). Due to the increasing interfacial energy for smaller dispersed droplets, i.e., for larger droplet surfaces, no kinetically stable reduction in the droplet size will be obtained without the presence of emulsifiers that reduce the high polymer phase/water interfacial tension. In a scenario with dissolved stabilizer molecules, which is typically the case during microencapsulation, the speed of the emulsifier diffusion through the continuous phase to the droplet surface, its adsorption, and finally its spreading on the o-phase droplets (Gibbs–Marangoni effect) will also impact the success of the emulsification. Once the emulsion is formed, its tendency towards coalescence will depend on efficiency of stabilizing mechanisms such as (i) static stabilization, i.e., electrostatic forces due to the droplets surface charge (by adsorbed ions, ionic surfactants, or polymer carboxylate groups introduced in the water phase) as described in the DLVO (Derjaguin, Landau, Verwey, and Overbeek) theory and/or steric repulsion due to adsorbed surface-active polymers (e.g., PVA) or solid particles that theoretically form a mechanic barrier prohibiting droplet approaching, (ii) dynamic stabilization, i.e., thermodynamic stabilization (loss of configurational entropy of adsorbed steric stabilizers when overlapping occurs for approaching droplets) and the Gibbs–Marangoni effect (especially relevant for mobile, small-molecule emulsifiers; when approaching droplets force liquid and some stabilizer molecules out of the gap between them, formation of surface tension gradient at the interface, stabilizer spreading, subsequent inward flux of medium in gap; voids from random stabilizer desorption immediately filled), and (iii) other aspects of interfacial rheology such as the viscoelasticity of the stabilizer film (for details see Heusch, 1983; Tadros, 2005).

Polyvinyl alcohol (PVA), which is a copolymer of vinyl acetate and vinyl alcohol, and is prepared by the controlled hydrolysis of polyvinyl acetate side groups (Herrmann and Haehnel, 1927), is the most commonly used emulsifier for the preparation of PLGA particles (Beck et al., 1979; Okada et al., 1987) because of its excellent interaction with PLGA surfaces (Boury et al., 1995). Increasing the PVA concentration or its molecular weight is known to result in smaller microparticles (Jobmann and Rafler, 1998). Although the faster droplet surface saturation due to the reduced average diffusion length of the stabilizer from the continuous phase to the, so far, uncovered droplet surface at higher PVA concentration might contribute to these findings, the main factor is expected to reside in the elevated viscosity of the continuous phase that hinders droplet collisions and coalescence. However, depending on the type of mixer and its efficiency at elevated viscosities of the continuous phase, an overly high PVA concentration can impede the complete separation of nascent droplets and result in a larger number of aggregated microparticles during solidification (Yang and Owusu-Ababio, 2000). An alternative explanation might be a bridging effect at overly high PVA concentration that causes particle aggregation. Also, one should consider that PVA can mediate solubility of certain drugs in the w-phase and thus, a higher PVA concentration can favor drug loss to the continuous phase (Yang and Owusu-Ababio, 2000).

Besides the viscosity of the external water phase, the viscosity of the disperse polymer phase needs to be considered. In a turbulent flow, which is present in most homogenizers, the droplet break-up is controlled by inertial forces which are dependent on the viscosity of the polymer phase. A higher o-phase viscosity requires a higher power density, i.e., stirring speed and/or exposure time to obtain the same droplet size (Schuchmann and Danner, 2004). Higher viscosities of the o-phase are present at increasing concentrations of the dissolved matrix polymer or polymer molecular weight and can, depending on the respective emulsification procedure, result in increased particle sizes (Jeffery et al., 1991; Jobmann and Rafler, 1998; Choi et al., 2002; Mao et al., 2008). When w/o/w double emulsion or s/o/w techniques are used, the viscosity of the polymer phase may also be influenced by the presence of the inner water phase or the amount, crystal size, and shape of dispersed drug, respectively. Also, the type and concentration of polymer along with the type of solvent and the potential presence of cosolvents will define the solidification speed and thus the particle size as already mentioned.

Performing the emulsification in an ice-bath or a cooled jacket beaker, which is required for certain thermo-sensitive drugs and was suggested to reduce amine-drug catalyzed polymer degradation (Maulding et al., 1986), will change the viscosity of both the continuous and the disperse phase and therefore presumably the particle size. However, the solvent diffusion out of the droplets, the hardening speed, and the solubility of the drug in the different phases is each certain to be altered as well.

## 6.3. Particle size analysis

In order to determine the particle size the operator can choose from the following widely established methods: (i) microscopy including light and electron microscopy, (ii) the Coulter principle, where particles are passed through an orifice (electrical sensing zone) by vacuum from one electrolyte chamber to another and cause an increased impedance in an electric field corresponding to the particle volume by blocking a certain part of the aperture, (iii) laser diffraction, where the light of a laser is diffracted at the surface of suspended microparticles resulting in a size specific diffraction pattern, (iv) dynamic light scattering (photon correlation spectroscopy) for submicron particles that correlates the fluctuation



of the scattering intensity with the particle's diffusion coefficient, which can be used to calculate their hydrodynamic diameter by the Stokes–Einstein equation or equivalent, (v) wet sieving of the particles through a column of sieves and balancing the amount on each sieve after drying.

The sieving method is an inexpensive and powerful tool not only to determine the particle size, but also to ensure that the same particle size of different formulations is compared in release studies, and that differences in release are not influenced by differing surface area and diffusion pathlength. The laser diffraction technology requires a mathematical model to calculate the particle size from the diffraction pattern. The widely used Fraunhofer model is only applicable for particles larger than  $\sim 10\ \mu\text{m}$  (Müller and Schuhmann, 1996). In order to calculate the size of smaller particles by the Mie theory refraction indices of the respective particles are required. By contrast the Coulter principle is unaffected by the particle color, refraction index, or light absorption and can provides information about the number, volume, and mass weighted particle distribution. Disadvantageous might be the limitation of a specific orifice tube to a certain particle size range, so that it might need to be changed between the measurements of very different samples, and difficulties arise if the microparticles are conductive (e.g., when highly porous).

## 7. Encapsulation efficiency

### 7.1. Methods to determine the encapsulation efficiency

For determining the encapsulation efficiency, i.e., the ratio of final (or actual) and theoretical drug loading, the microparticles are commonly first dissolved in an appropriate solvent. Such solvents might be typical o-phase solvents like methylene chloride (Wada et al., 1988), but also acetonitrile, acetone, tetrahydrofuran (Sah and Lee, 2006), DMSO (Shenderova et al., 1997), or 1,4-dioxane (Yang and Owusu-Ababio, 2000). If applicable, this solution can then be analyzed for drug content by UV/VIS or fluorescence spectroscopy. Lower drug loadings may require HPLC analysis, where an additional polymer precipitation step will be beneficial in order to protect the HPLC column. For this purpose a solvent is required that dissolves the drug but not the polymer, e.g., alcohols (Sah and Lee, 2006; Buntner et al., 1998; Giunchedi et al., 1998). Alternatively, others reported a polymer non-solvent, methanol, to be able to extract a hydrophobic substance from PLA microparticles within 24 h without dissolving the polymer (Bodmeier and McGinity, 1988). For hydrophobic drugs with comparatively high water solubility, the determination of the lost drug in the continuous phase and the washing medium (Arica and Lamprecht, 2005), or in select cases, the digestion of the microparticles with NaOH, may also be applicable (Jaraswekin et al., 2007). However, electron microscopy can help to understand whether the determined encapsulation efficiency includes non-encapsulated drug, e.g., free drug crystals of a similar size-scale as the microparticles or drug crystals present at the particle surface (Birnbaum et al., 2000).

### 7.2. Increasing the encapsulation efficiency

#### 7.2.1. Drug particle size

Low encapsulation efficiency may have different causes and strategies to overcome this issue will depend on the microencapsulation technique used. For s/o/w methods, the size of the solid drug particles for encapsulation should be significantly smaller than the final polymer microparticles, and if the solid drug powder is flocculated, appropriate solid dispersion in the o-phase may become necessary. A low polymer concentration may result in polymer

microparticles with drug crystals penetrating the polymer shell. In this case, an increase in the viscosity of the polymer solution, e.g., by increasing polymer concentration, molecular weight, and/or lactide content may be advantageous. Similarly, the viscosity of polymer solutions can be strongly increased by decreasing the temperature, as has been used to minimize loss of water-soluble peptides during the w/o/w emulsion method (Ogawa et al., 1988a; Yamamoto et al., 1994).

#### 7.2.2. Emulsifiers and drug solubility in the continuous phase

For the o/w technique, solubility most critically impacts the encapsulation efficiency. An increased partitioning of the drug to the external water phase might be caused by its solubility in the detergent solution (Yang and Owusu-Ababio, 2000). Hydrocortisone, a hydrophobic drug with a relatively high water solubility of 0.28 mg/ml was described to exhibit a strong increase in its solubility (1.38 mg/ml) in the presence of 1.5% PVA (Giunchedi et al., 1998). If possible, it is advisable to reduce the stabilizer concentration and/or the volume of the w-phase in this situation, or to switch to another detergent with a lower solubilizing effect on the drug. Several emulsion stabilizers have been employed in the literature as alternatives to common PVA, including gelatin (Wakiyama et al., 1982b; Wang et al., 1997), methylcellulose (Cavalier et al., 1986; Rosilio et al., 1991), hydroxypropylmethyl cellulose (Ertl et al., 1999), polyoxyethylene sorbitan monooleate (Tween® 80) (Bodmeier and McGinity, 1988), glycerine (Albayrak, 2005), dextran derivatives (Rouzes et al., 2003), salts of fatty acids like potassium oleate (Fong, 1981, 1983), and other surface-active molecules (Thies, 1991; Mogi et al., 2000). Also, PLA oligomers have interestingly been shown to have similar surface tension reducing effects as SDS (Schwendeman et al., 1995), and similarly, the incorporation of oligomeric PLA (2.4 kDa) in the o-phase has been shown to be an effective stabilizer, which introduces carboxylate groups at the oil/water interphase to provide emulsion stabilization by electrostatic repulsion (Vert et al., 1993; Carrio et al., 1995). However, a further reduction in the Mw of the matrix-like surfactant to 0.6 kDa results in low microparticle yields because of the loss of the stabilizer due to its solubility in the water phase. This is in agreement with the findings of another study that reported partitioning of 1/3 of their 1 kDa matrix polymer (PLA) to the continuous phase (Liggins and Burt, 2001).

Besides switching to other stabilizers, the particle hardening procedure can be changed, e.g., by using an interrupted solvent evaporation method (Benita et al., 1984; Benoit et al., 1986b; Rosilio et al., 1991), where the PVA solution is replaced by water after a certain time, which was not specified by the authors and may depend on different formulation parameters. For drugs with a pH-dependent solubility it is often wise to adjust the pH of the continuous phase to a value of low drug solubility (Wakiyama et al., 1982a,b; Mao et al., 2008), keeping in mind that extreme pH values can affect the shape of the particles (Bodmeier and McGinity, 1987c) and the integrity of the polymer and the drug. By changing the pH-value at different time points during the evaporation, it was shown that the loss of a pH-dependently charged model drug occurred within the first minutes of emulsification (Bodmeier and McGinity, 1987b). Thus, it may not be necessary to maintain an acidic or basic pH for the entire evaporation time and adjusting it back to neutral value after a couple minutes may help protect the polymer. In the case of easily accessible drugs and the need of a high loading, a pre-saturation of the water phase with the respective drug might be considered as was described for hydrophilic substances (Splenhauer et al., 1986). Because of the often somewhat low water-solubility of hydrophobic drugs, and thus reduced drug mass to saturate the hardening bath, this option becomes even more feasible from a cost stand point. Likewise, the incorporation of

compounds with pH-dependent solubility that were encapsulated at neutral pH in order to avoid polymer alteration (disadvantageously high solubility of the respective drug in the continuous phase) was beneficial with the pre-saturation concept (Bodmeier and McGinity, 1987b).

### 7.2.3. Mass transfer of *o*-phase solvents and microparticle hardening

As mentioned in Section 4.1.1, the flux of organic solvent to the water phase will facilitate leaching of dissolved drug from the polymer particle matrix. This effect may become strongly pronounced when water-miscible cosolvents are employed, which partition to a great extent into the water phase especially during the emulsification procedure, and before hardening of the nascent particles takes place. Therefore, the amount of cosolvent in the solvent mixture should be kept at the minimum level that is required to dissolve the drug.

A fast precipitation of the polymer is typically considered to be advantageous to achieve high encapsulation efficiencies, because the solid polymer film acts as a diffusion barrier for the drug. That is, a high polymer concentration, hydrophobicity, or molecular weight should result in a fast transition of the droplet surface from the sol to the gel state. Additionally, for drugs with a low solubility in the detergent solution, the formulation may benefit from a larger volume of the continuous phase with a faster polymer precipitation, due to a larger portion of the non-water-miscible polymer solvent being initially extracted by the *w*-phase (Bodmeier and McGinity, 1987b).

The hardening speed of PLGA microparticles was shown to increase by the addition of urea or a salt such as NaCl to the continuous phase. The faster precipitation of the polymer resulted in a much lower porosity of the microparticles prepared with a cosolvent method and higher encapsulation efficiency for hydrophobic drugs (Thote et al., 2005) and for highly water-soluble drug salts (Jaraswekin et al., 2007). However, occasionally  $w_1/o/w_2$  microparticles with larger  $w_1$ -volumes collapsed in the presence of NaCl due to fast hardening (Chen et al., 2004). In another *o/w* microencapsulation study with a partially water-soluble drug salt, quinidine sulfate, the encapsulation efficiency was increased due to the formation of new, less soluble drug salts in the *o*-phase, when counter ions, not common for this drug (e.g.,  $\text{ClO}_4^-$ ), were introduced in the continuous phase (Al-Maaieh and Flanagan, 2005).

As will be discussed in detail in Section 8.1, the diffusion rate of a hydrophobic drug out of the polymer particles can be controlled by its escape from the unstirred boundary layer. Therefore, higher encapsulation efficiency has been suggested by reducing diffusion velocity in the water phase, e.g., by addition of glycerine (Albayrak, 2005).

## 8. Drug release from the microparticles

### 8.1. *In vitro* assays—rationale for using sink conditions

While the experimental set-up for release studies is specified in the pharmacopoeiae (USP, Ph. Eur.) for conventional dosage forms, there is no such specification for long-acting release microparticles. However, one of the fundamentals of release studies is maintaining sink conditions and in the literature of particulate sustained release formulations only a few knowingly break this rule, e.g., by using very small volumes (5 ml) (Mittal et al., 2007). Others compared the release of a total 2.3 mg nifedipine from microparticles (solubility: 2.3 mg in 230 ml water) under non-sink conditions (shaking flask; 250 ml medium) with sink conditions (flow-through cell; 2500 ml circulating medium) and explained the absence of differences in the 14 day release profiles by the drug diffusion through the polymer

rather than its removal from the boundary layer as being the rate-limiting step (Sansdrap and Moës, 1993). However, the solubility of nifedipine in the employed phosphate buffer might be higher at 37 °C than in water, which would at least, if still not fulfilling sink conditions in the flask assay, increase the concentration gradient at the particle surface.

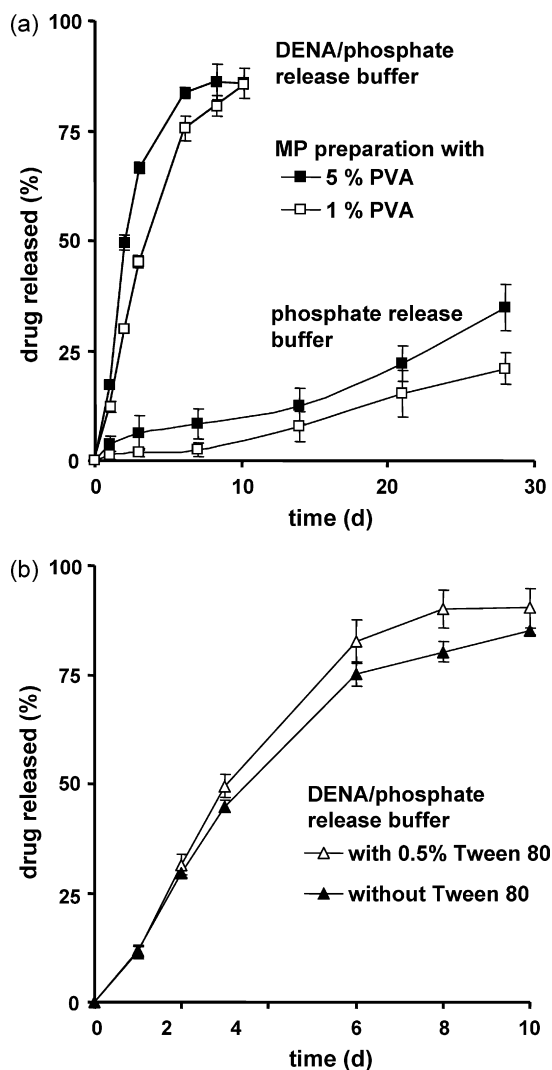
In addition, theoretical comparison of the release under sink vs. non-sink conditions has shown that deviations become relevant especially for slowly releasing formulations (Guy and Hadgraft, 1981). *In vivo*, at certain injection sites with low perfusion, the exposure to body fluids may not provide perfect sink conditions at all times, especially in the case of very hydrophobic drugs that are not easily removed from the microparticles' unstirred boundary layers. However, it is well-known that the polymer often degrades faster *in vivo* than under sink conditions *in vitro* due to the presence of certain endogenous substances (see Section 5). Therefore, it is important to verify that sink conditions are followed during release assays for hydrophobic drugs from particulate sustained release formulations.

### 8.2. *In vitro* assays—media to maintain sink conditions

For protein-loaded microparticles release studies are often performed in small volume microcentrifuge tubes and the release is determined from the supernatant after briefly centrifuging the microspheres under mild centrifugal forces. For hydrophobic drugs with low water solubility, larger volumes of release medium are required in order to maintain sink conditions. To reduce the volume of the aqueous media, the use of adsorbents (Wurster and Polli, 1961) or organic solvent reservoirs (Gibaldi and Feldman, 1967), has been suggested for release studies from conventional solid dosage forms, although in the latter solubility of organics in the water phase will likely influence the polymer properties (e.g., particle softening and fusion, solid-state diffusion, and polymer degradation rate) which may not be preferred for PLGA microparticles. In certain cases the solubility of the drug may fall below the detection limit of standard HPLC methods and its determination from the supernatant requires a time-consuming and potentially artefact-prone drug concentrating step to be in the useful concentration range for analysis. Therefore, the determination of the remaining drug in the microparticles can provide more precise release data and additional information concerning the drug stability inside the polymer matrix.

Another fundamental question concerns the correlation of the results from a release study to what can be expected *in vivo*. It would be beneficial both from an ethical and financial point of view to reduce animal studies by establishing *in vitro* assays with good predictive character for the *in vivo* release of different formulations. For conventional dosage forms the major pharmacopoeiae specify methods that do not display the conditions *in vivo* (e.g., a tablet will rarely encounter 900 ml of phosphate buffer in the stomach), but allow a fast prediction of the properties of a formulation for quality and *in-process* control.

As there is the issue of large volumes and time periods required to obtain a release profile for a hydrophobic drug from sustained release formulations, some authors followed the concept of speeding up the release in order to obtain a fast result that otherwise might take months. Such release media typically contain alcohols, e.g., methanol (Parikh et al., 1993), 27.5% ethanol (Beck et al., 1980), or 25% *n*-propanol (Yang and Owusu-Ababio, 2000) and exhibit a higher dissolving power for the drug while also plasticizing the polymer. However, when 50% methanol was applied by some authors, the maximum release was observed after 24 h and no differentiation was possible between different batches (Zaghoul et al., 2005, 2006). Another approach to accelerate the release for



**Fig. 5.** Speeding up the release by additives in the release medium. Release of paclitaxel from PLGA microparticles depending on the presence of (a) 2 M diethylnicotinamide (DENA) in the release medium (20% loading; particles were either prepared by emulsification in 1% PVA [larger particles] or 5% PVA [smaller particles]), and (b) 0.5% Tween 80 in a DENA containing buffer (1% PVA samples; 20% loading). Adapted figures reprinted from *Int. J. Pharm.* (Elkharraz et al., 2006), Copyright ©(2006), with permission from Elsevier.

analytical purposes might be a variation of the pH of the medium to instigate a faster hydrolysis of the polymer (Cowsar et al., 1985), performing the study at elevated temperatures (Shameem et al., 1999), adding high concentrations of the hydrotropic (for the drug paclitaxel) and catalytic (for PLGA degradation) agent, diethylnicotinamide (DENA) to the release medium (Baek et al., 2004; Elkharraz et al., 2006) (Fig. 5a), or employing certain surface-active molecules in aqueous media, e.g., Aerosol OT (Leelarasamee et al., 1986) or Tween® 80 (Fig. 5b) (Berkland et al., 2002; Elkharraz et al., 2006). The dramatic effect of DENA on the release profile of paclitaxel was attributed to increased polymer degradation (Elkharraz et al., 2006).

Surface-active agents are thought to improve the wetting of microparticles, reduce microparticle aggregation, and/or possibly alter the drug solubility (Leelarasamee et al., 1986; Berkland et al., 2002; Burgess and Hickey, 2005; Elkharraz et al., 2006). Tween® 80 aqueous solutions, at concentrations above the CMC (~0.015 mg/ml in water (25 °C); Wan and Lee, 1974), have shown a linear relationship between the dissolving power and the surfactant

concentration for some hydrophobic drugs (Barreiro-Iglesias et al., 2003), an effect that can be predicted from the drug octanol–water partition coefficient (Alvarez-Nunez and Yalkowsky, 2000). Moreover, some may not have considered that substances such as Tween® 80 might partition their polyethylene chain into the PLGA matrix and act as an alternative plasticizer for the polymer. The comparatively small impact of Tween® 80 in Fig. 5b might be attributed to the overwhelming effect of DENA in the respective study. However, from the well-known effect of Tween® surfactant on the solubility of poorly soluble drugs and potential plasticization of the polymer, one can reasonably expect that the presence of common non-ionic surfactants in the release media could drastically alter release kinetics of susceptible drug molecules.

### 8.3. *In vitro* assays—experimental set-up

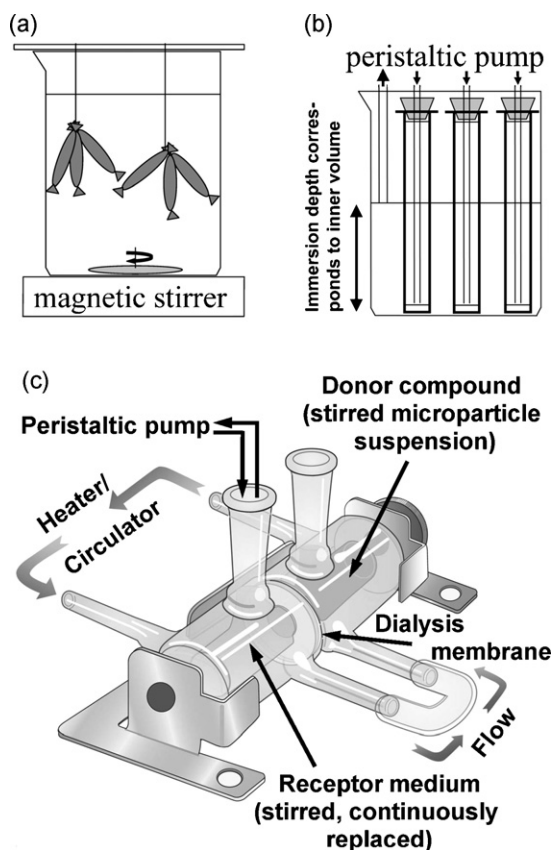
A key question is how to put into practice these concepts: maintenance of sink conditions, handling of large volumes, and changing of the release medium? If the study is expected to give an idea of what might happen in vivo, the release medium should be adapted to the conditions at the site of application and the volume of medium in direct contact with the particles should be reasonable. Additionally, the use of PBST (phosphate buffered saline + low concentration of Tween®) rather than PBS as a release medium is a very simple method that takes into account the large number of surface-active molecules in the body and will also help to overcome wetting issues that sometimes come up after drying microparticles.

There are numerous successfully employed *in vitro* methods, which, however, were not always designed closely to reflect the conditions in vivo, for example, an assay using 50 ml rotating tubes (Bodmeier et al., 1989) or the USP paddle apparatus with 900 ml medium (Yang and Owusu-Ababio, 2000). Any attempt that uses a low local volume around the particles but maintains sink condition will be based on diffusion or a flow-through concept. Schemes of some appropriate experimental set-ups are shown in Fig. 6 and will now be discussed further.

During drug release from the microparticles, an elevated drug concentration can be expected in the boundary layers of medium around the microparticles. For hydrophobic drugs with a low aqueous solubility these boundary layers and release media may become saturated with drug and hinder the release of any further drug molecules. This phenomenon has been mathematically modelled extensively (Zhou and Wu, 2003). Agitation can be used for the removal of the drug from the boundary layer, e.g., stirring, horizontal or rotating shakers, rocking platforms, etc. Finally, only a comparison of the profiles from (eventually different) *in vitro* release assays with the *in vivo* data of the same formulations of a specific drug at a specific site of application will allow conclusions on the *in vitro*–*in vivo* correlation (Sansdrap et al., 1999).

The method depicted in Fig. 6a includes dialysis bags to keep the microparticles in small compartments (mimicking the low volume of fluid at the injection site) and to allow an easy change of the whole external medium at the required time points or, even more comfortable for long-term studies, by continuous replacement with fresh medium using a peristaltic pump. It is obvious that in this way a large external volume can be realized and that this procedure is handier than removing some liters of medium by centrifugation, where particles might get lost with the supernatant and the drug might be artificially released by high centrifugal forces (Yang and Cleland, 1997). Standard dialysis tubing with a 12–14 kDa molecular weight cut-off (MWCO) will fit for most applications, but there are also membranes available with a MWCO ranging from 0.1 to 500 kDa. Regenerated cellulose might be the preferred tubing material, since it is more hydrophilic than for instance cellulose ester, and is expected to show less absorption of hydrophobic





**Fig. 6.** Various experimental configurations for release evaluation of hydrophobic drugs from biodegradable microparticles (MP) as discussed in detail in the text: (a) MP are placed into several dialysis bags and the external medium is replaced to maintain sink conditions; (b) MP are located on the bottom of filter bags and fresh medium is provided by a peristaltic pump—immersion depth can be changed to control the inner volume in the bags; and (c) side-by-side diffusion cells with the MP suspension in the donor compartment which is separated by a dialysis membrane from continuously replaced release medium in the receptor cell (scheme of side-by-side cells kindly provided by PermeGear Inc., Bethlehem, PA, USA).

drugs. However, when selecting a MWCO for a specific drug one should also consider the principle of solubilization of the respective compound in the release medium. For highly hydrophobic drugs, micellar solubilization might be the main mechanism for detergent containing media like PBST. Such Tween® surfactant micelles will rarely pass through the pores of standard membranes as they are known to have sizes up to 5–20 nm (Türk and Lietzow, 2004), which corresponds to about 40–300 kDa. In addition, the micelles can grow in size when loaded with drug due to the incorporation of both the drug and extra surface-active molecules (Attwood et al., 1989). In this case the dialysis bag rather than the microparticles will control the release and the determination of dissolved drug inside the bag will show values above sink conditions.

Due to the lack of dialysis bags with larger pore size, one might switch to bags for liquid filtration for industrial applications, which can be purchased with ratings down to 1  $\mu\text{m}$ . Using these instead of dialysis bags for microparticles  $>20 \mu\text{m}$  in the set-up of Fig. 6a will create a system with easy micellar drug diffusion, while retaining the microparticles. At predefined time points, triplicates of the dialysis or the filter bags can be removed, dried, extracted, and assayed for the remaining drug. The temperature can be controlled by performing this study in an incubator. In order to obtain a highly permeable compartment for microparticles, others have used multiple layers of tea bags in release studies (Wang et al., 2005), however, such bags often come with pores up to 500  $\mu\text{m}$  and particle loss might always be an issue with this attempt.

In Fig. 6b, it is demonstrated how filter bags can be used in a flow-through configuration. Fresh release medium is continuously delivered by a multichannel peristaltic pump to the microparticle samples sitting at the bottom of the filter bag. The inner volume can be controlled by the immersion depth of the bags, the minimization of the boundary layer by the flow rate, and the temperature by placing the system into a water bath. In order to mimic the conditions in vivo, e.g., site-specific delivery to the brain, some have invented a flow-through system with very low flow rates (Aubert-Pouëssel et al., 2002).

A more intensive agitation of the release medium surrounding the microparticles can be achieved by mini stir bars inside of side-by-side cells (Fig. 6c). Originally designed to study diffusion of drugs through barriers such as the skin or polymer membranes, these glass cells allow the separation of microparticles in the donor compartment from the continuously replaced release medium through an appropriate membrane that must not hinder drug diffusion. A special bench assembly of magnetic stirrers is available for mounting cells and providing the opportunity to split up the water flow from an extra heater to the jackets of a couple of such glass cells for temperature control.

By changing the stirring speed or flow rate in the side-by-side cells or flow-through method, respectively, different release profiles might be obtained, in the event of unstirred boundary layer diffusion control, which later can be evaluated for potential correlation with in vivo data. However, both the flow-through configuration and the side-by-side cells may limit the number of parallel studies due to the required special equipment.

#### 8.4. Controlling drug release

##### 8.4.1. Typical release pattern from PLGA microparticles

The release of encapsulated drugs from polymeric matrices is required to meet the therapeutic goal of released drug/time in order to allow efficient treatment of the specific disease (Tzafirri et al., 2005). The release can in principle be controlled by diffusion, erosion, osmotic-mediated events, or combinations of these mechanisms (for details see Schwendeman et al., 1997). For small molecular weight drugs diffusion through the polymer can be expected to contribute significantly to the release if not being the main release mechanism, although some steroid drugs were found to exhibit very low diffusion rates through certain unplasticized PLA films (Pitt et al., 1979).

As PLGA matrices are subjected to bulk erosion at acidic and neutral pH values (von Burkersroda et al., 2002) it can take some time until the polymer is degraded down to a critical chain length and a lag phase will appear in the release profile for erosion-controlled formulations. Finally, a disproportionately fast drug release within the first couple hours to couple days, i.e., the so-called burst release, has been often attributed to surface-associated drug (see below for more recent alternative view). Hence, a triphasic release profile is commonly observed, consisting of initial burst, lag time depending on the Mw and end-capping of the polymer with a slow or absent diffusion-controlled release, and finally erosion-accelerated release (Makino et al., 2000). As stated in Section 5, blending the high molecular weight polymer with a small portion of a low molecular weight polymer, or using entirely low molecular weight polymer, can help to overcome such lag phases (Hutchinson, 1986; Ogawa et al., 1988b; Bodmeier et al., 1989).

##### 8.4.2. Burst release

The presence of drug crystals at the particle surface for o/w methods (Jalil and Nixon, 1989) has been attributed to the solvent flux out of the o-phase during the solvent evaporation that is able to transport drug to the particle surface. Once the solvent



partitions into the water phase its dissolving power disappears and the drug precipitates at the particle surface or in the suspension medium. Similarly, drug appearing steadily at the particle surface from diffusion through the polymer-rich phase may also be expected to provide a source of particle surface drug crystallization. Some attempts to reduce the drug loss to the continuous phase have been discussed before (Section 7.2). As drug crystals often cannot be removed from the particle surface during the regular washing steps with water an extra washing step with 75% ethanol has been suggested for progesterone-loaded microparticles (Bodmeier and McGinity, 1987a) in order to reduce the burst release. An alternative might be the coating of such particles as described in Sections 3.2 and 3.7.

Additional influences of elevated burst release from microparticles have been shown to include (a) initial surface pores in the polymer, (b) pores created during water entry and polymer swelling, and (c) spontaneous pore closing, shutting off further release to begin the lag phase (Wang et al., 2002, 2004; Kang and Schwendeman, 2007). However, it must be stressed that the osmotic forces present in highly water-soluble peptide formulations as studied by these authors will be much lower for poorly water-soluble drugs.

#### 8.4.3. Effect of particle size and porosity on the release rate

Keeping in mind that the removal of drug out of the boundary layer can be a key process for hydrophobic drugs, an increase in the surface area:volume ratio, i.e., a decrease in the particles size, often results in higher release for this drug class (Zhifang et al., 1993; Elkharraz et al., 2006; Berklund et al., 2002) (Fig. 7a). Formulation parameters that impact the particles size have been discussed in Section 6.2.

Also, a larger inner surface, induced by a higher porosity of the particles, can potentially increase the uptake of the release medium into the particles and accelerate the drug pore-diffusion and release. As described in Sections 4.1 and 4.2 the porosity of microparticles depends, in part, on the entrance of water into the nascent microparticles, which will, in the case of a cosolvent method, correspond to the amount of residual cosolvent forming a polymer pure phase inside the o-phase.

Beside classical cosolvents like alcohols, other substances are used as extractable porogens such as the nonvolatile solubilizer isopropyl myristate (Wang et al., 1996, 1997), or water-soluble polymers such as PVP (Lalla and Sapna, 1993) and Pluronic® F127 (Kim et al., 2006). Others incorporated fatty acids or esters of fatty acids to increase release rates from hydrophobic matrices (Juni et al., 1986). If no water-miscible cosolvents are employed, the amount of water that is able to enter the nascent particles will be affected by, e.g., the solubility of water in the o-phase solvent (see Table 2) and the type and concentration of polymer including the speed of its solidification, presuming limited water-solubility of the drug and any other excipients. When cosolvents are not desired and the drug release is too slow the formulation might benefit from the potential of the  $w_1/o/w_2$  technique. By adding different amounts of salt to the inner water phase the porosity of the resulting particles can be controlled (Fig. 8) by increasing the osmotic gradient and the flux of water from  $w_2$  into the  $w_1$ /polymer phase. Suspensions of sugars (Leelarasamee et al., 1986) or salts in the o-phase are expected to act in the same way, and a major increase in water-uptake, e.g., by incorporation of suspended NaCl, has been proven with PLGA films (Zhang et al., 1997).

#### 8.4.4. Drug diffusion through the particle polymer matrix and effect of drug loading on release

For diffusion-controlled release the dissolved drug needs to pass to the particle surface either through the polymer matrix or through

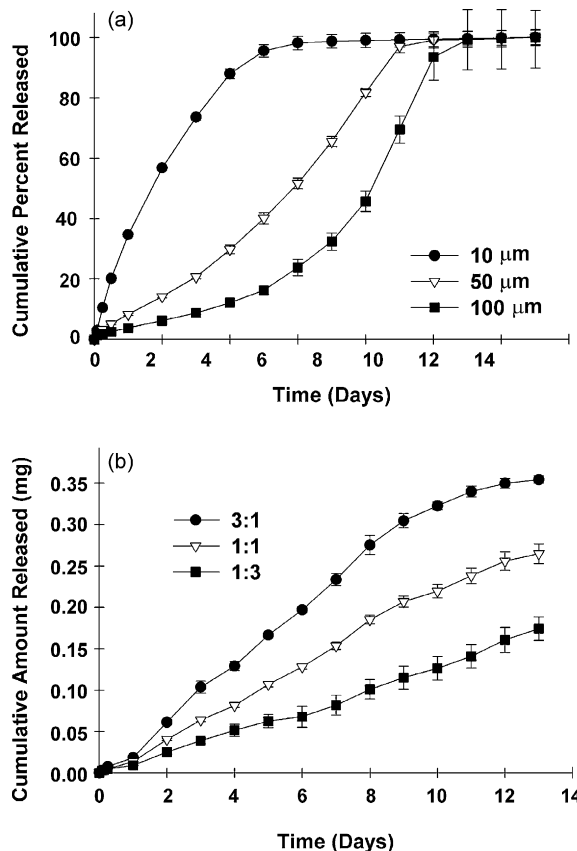
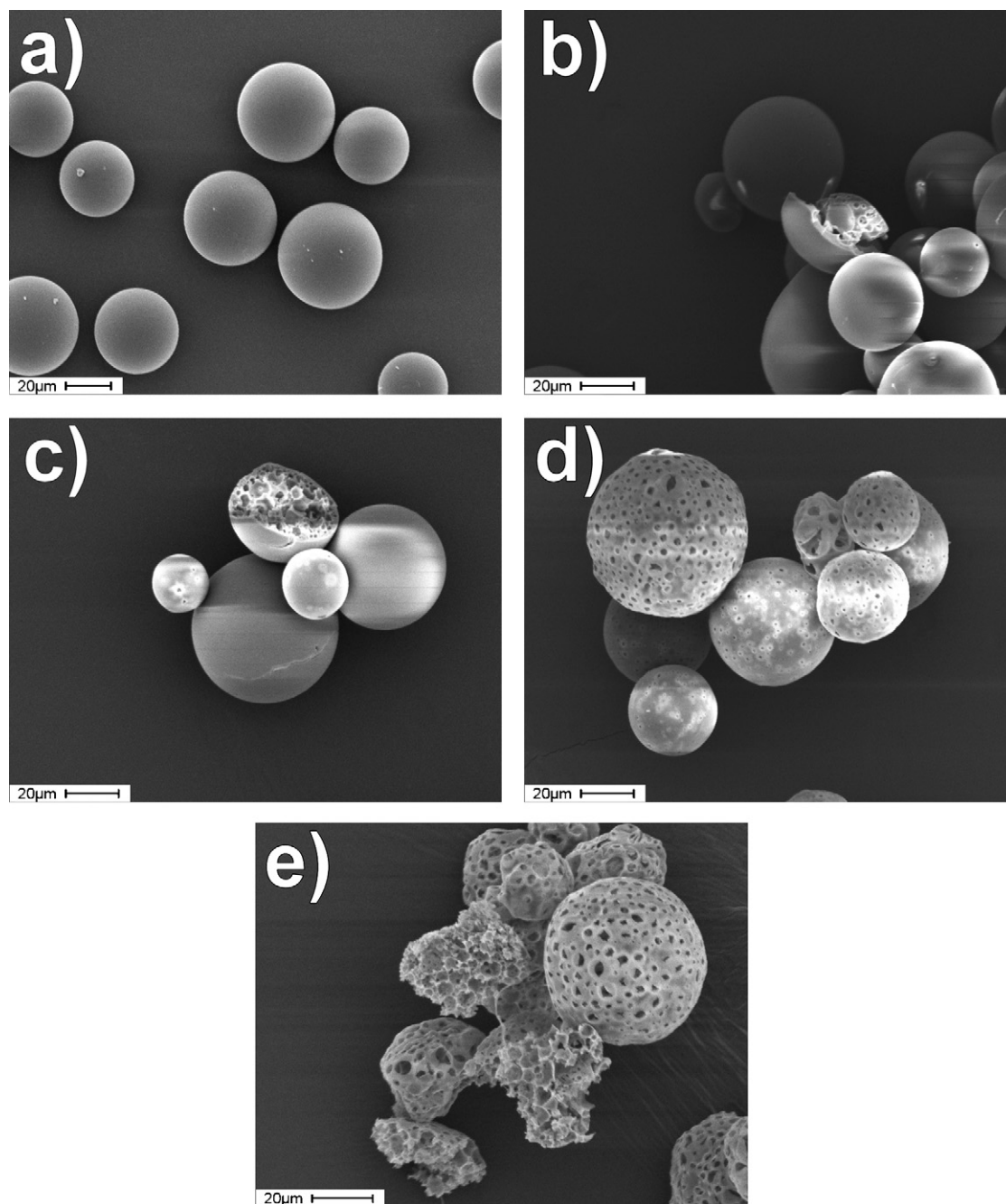


Fig. 7. Smoothing the release profile by mixing slow and fast releasing piroxicam loaded PLGA microparticles. (a) Release in % from different microparticles sizes (10, 50, and 100  $\mu\text{m}$ ). (b) Release in mg from mixtures of 10  $\mu\text{m}$  and 50  $\mu\text{m}$  microparticles shown in Fig. 6a. Release studies were performed in 0.5% Tween® 20 in PBS. Adapted figures reprinted from *J. Controlled Release* (Berklund et al., 2002), Copyright ©(2002), with permission from Elsevier.

water-filled pores. The specific properties of the network of polymer chains, e.g., the chain length, their flexibility and mobility, their water-uptake and swelling behavior, extent of plasticization, or potential interactions between polymer and drug will all potentially affect the diffusion rates in the polymer matrix, and therefore, the drug release rate. In the case of s/o/w microparticles the dissolution of the hydrophobic active compound can also in theory be the rate-limiting process.

Since PLA and PLGA are linear polymers, the overall mobility of their chains will increase with decreasing Mw and therefore a low Mw polymer might allow the drug to diffuse faster through the particle matrix (Wakiyama et al., 1982a; Liggins and Burt, 2001). Higher glycolide contents, e.g., PLGA 50/50 vs. 85/15, or polymers with a free carboxyl end-group are considered to be more hydrophilic and thus water molecules might enter the particle matrix to a greater extent and hydrate the polymer. However, it should be emphasized that many other parameters, which impact the release, may be altered when switching to a different Mw or an uncapped version of the respective polymer. Such parameters include the microparticle surface structure, porosity, particle size, and drug loading. As already mentioned in Section 5, the use of block copolymers with a hydrophilic PEG block can result in an easier diffusion and a faster release of some drugs (Ruan and Feng, 2003).

In the case of high drug loading (>10–20%), increasing loading often results in faster release of the drug (Wakiyama et al., 1981; Leelarasamee et al., 1986; Choi et al., 2002) (Fig. 9). This can be attributed to the smaller amount of polymer on a percentage basis

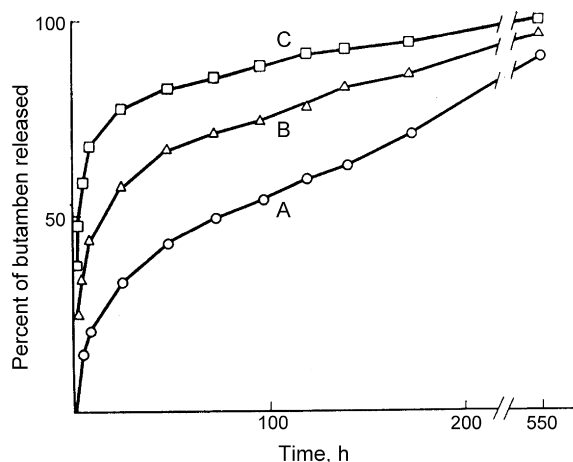


**Fig. 8.** Impact of salt concentration in the  $w_1$ -phase ( $w_1/o/w_2$  technique) on the microstructure of PLGA (Resomer® RG 502H) microparticles (MP). SEM micrographs of MP: (a) no inner water phase, (b) 1× PBS, (c) 2× PBS, (d) 5× PBS, and (e) 10× PBS.

that acts as a diffusion barrier as well as the larger number of voids available for water entrance after the release of the same portion of the drug. Moreover, high volume fraction of drug particles (related to drug loading) may approach a lower percolation threshold, i.e., the lowest volume fraction of drug particles that creates physically interconnecting drug particles from the polymer surface to deep within the polymer (i.e., percolating clusters). By contrast, the opposite result can be observed if the hydrophobic drug is dissolved in PLGA at low concentrations, precipitates and crystallizes at higher loading, and thus shows a reduced release rate due to the necessity of first being dissolved from the crystalline state (Mogi et al., 2000; Mao et al., 2008).

The glass transition temperature ( $T_g$ ) of a polymer describes the change of a polymer from the glassy to the rubbery state. In the rub-

bery state, the diffusion of the drug out of the microparticle matrix is easier due to the high mobility of the polymer chains. The value of the  $T_g$  depends on the type of polymer and the molecular weight, but water (Blasi et al., 2005) as well as drug–polymer interactions are known to plasticize the polymer and reduce its  $T_g$ . As common 50:50 PLGA polymers exhibit  $T_g$ 's in the range of 40–50 °C, their  $T_g$  are usually exceeded under release conditions at 37 °C in the presence of water, especially if drug–polymer interactions further lower the  $T_g$ . However, the ability of a drug to interact with the polymer depends on its solubility in the polymer and increasing the loading, without inducing drug crystallization, can be expected to lower the  $T_g$  further (Rosilio et al., 1991). Changing the polymer composition, e.g., increasing the content of the more hydrophilic glycolide in the copolymer up to 50%, might alter the solubility of a drug in



**Fig. 9.** Release of butamben from o/w poly(D,L-lactic acid) microparticles depending on the initial drug loading: (A) 8.1%; (B) 15.3%, and (C) 25.5%. Reproduced with permission from *Chemical & Pharmaceutical Bulletin*, vol. 29, No. 11 (Wakiyama et al., 1981). Copyright ©(1981) Pharmaceutical Society of Japan.

the polymer and therefore its effect on the  $T_g$ . However, it needs to be mentioned that due to the polymer degradation the  $T_g$  will be exceeded anyway at some point during the release study.

#### 8.4.5. Onset of erosion-controlled release and attempts to obtain zero-order profiles

Erosion-controlled drug release is expected to begin after a lag time when the polymer Mw falls below a critical value (In the future, our group will disclose data in support of an alternative hypothesis to this commonly held view). Different polymer types are known to require different times for complete degradation (Lactel® product information), with larger molecular weight and particularly higher lactide content, and, in the case of L- or D-PLA, crystalline instead of amorphous structures, resulting in a slower degradation and an expected slower release. However, there have been reports showing that higher lactide contents (Yang and Owusu-Ababio, 2000) or higher crystallinity (Izumikawa et al., 1991) resulted in faster rather than slower drug release because of alterations in the microparticle surface structure. These reports highlight the importance of considering other parameters (e.g., those that may influence degradation and release mechanisms) in addition to lactide/glycolide ratio as determinants of release kinetics and duration.

Overall, zero-order release kinetics (i.e., release rate is essentially constant) is desired for most applications. However, for PLGA such profiles are not always observed in the literature. Therefore, the combination of fast and slow releasing particles might be beneficial to obtain the desired release rates (Cha and Pitt, 1988; Berklund et al., 2002) (Fig. 7b) or copolymers with PEG could be evaluated. In other cases with a one-time-only application, a limited burst release might be advantageous to obtain a fast saturation of the target structure, e.g., receptors. However, this can also be obtained by co-injection of a bolus of soluble drug (loading dose) and zero-order releasing microparticles (maintenance dose).

## 9. Conclusions and future outlook

This review focuses on the microencapsulation of hydrophobic drugs, describes a variety of techniques for their preparation and analytics, and provides some guiding principles to begin early stage encapsulation studies by using methods that are easy to perform, feasible for drugs of limited availability, and expected to provide material for pilot in vivo experiments in a reasonable time frame.

Moreover, it has been pointed out how formulation parameters can be used to control the microparticle characteristics and, based on a mechanistic approach, how undesired microparticle properties can be overcome.

Easily injectable, biodegradable formulations of hydrophobic drugs provide a unique and powerful method to treat chronic diseases. Both patients and health care professionals benefit, as slow-release microparticles require a lower administration frequency and thus, generally increase compliance of drug therapy. Moreover, such sustained release formulations might enable a class of drugs with good therapeutic and safety profile but poor solubility and oral bioavailability to provide their medicinal benefit to the patients. Therefore, the consideration of PLGA biodegradable microparticles – the most commonly studied biodegradable carrier for controlled release – for long-term delivery of their drug candidate might help companies to decide about the fate of new chemical entities with disadvantageous physicochemical properties.

Overall, as companies need to concentrate their resources, an early selection of the most promising candidates from the increasing group of active agents with problematic bioavailability features among the discovered drugs is necessary. Thus, it will be of increasing importance to consider formulation aspects, including alternative drug delivery concepts such as biodegradable microparticles, in the preclinical stages of pharmaceutical development.

The cessation of marketing for Nutropin Depot, the first and only protein-loaded PLGA microparticle formulation on the market, in 2004 for economic reasons raises the common concern as to whether the difficulties and high costs associated with development and commercial preparation of protein-loaded microparticles will be recovered easily by the demand and profit of such products. We believe that this question must be carefully evaluated on a case-by-case basis. However, this concern combined with the increasing number of poor orally bioavailable drug candidates, which, compared to proteins, are generally less expensive to obtain, more stable, easier to handle, characterize, encapsulate, and store, could translate into an increase in long-acting release formulations of hydrophobic drugs entering development in the near future.

## Acknowledgments

Financial support was generously provided by Merck and Co. The authors wish to thank Andreas Schendler and Sam Reinhold for their support in different parts of this work. The Hitachi microscope used to obtain some of the shown electron micrographs was acquired under grant #EAR-96-28196 from the National Science Foundation.

## References

- Adamson, A.W., 1999. *Physical Chemistry of Surfaces*, 5th Ed. Wiley-Interscience, New York, p. 40.
- AGL9909 study, 2000. A six-month, open label, fixed dose study to evaluate the safety, tolerance, pharmacokinetics and endocrine efficacy of two doses of LA-2550 22.5 mg in patients with advanced prostate cancer. Eligard® product information, <http://products.sanofi-aventis.us/eligard/eligard.225.html>, downloaded on January 23, 2008.
- Albayrak, C., 2005. Induced phase transition method for the production of microparticles containing hydrophobic active agents. US Patent 6,899,898 (31 May).
- Ali, S.A.M., Doherty, P.J., Williams, D.F., 1994. Molecular biointeractions of biomedical polymers with extracellular exudate and inflammatory cells and their effects on the biocompatibility, in vivo. *Biomaterials* 10, 779–785.
- Al-Maaieh, A., Flanagan, D.R., 2005. New drug salt formation in biodegradable microspheres. *Int. J. Pharm.* 303, 153–159.
- Alsante, K.M., Ando, A., Brown, R., Ensing, J., Hatajik, T.D., Kong, W., Tsuda, Y., 2007. The role of degradant profiling in active pharmaceutical ingredients and drug products. *Adv. Drug Del. Rev.* 59, 29–37.
- Alsensz, J., Kansy, M., 2007. High throughput solubility measurement in drug discovery and development. *Adv. Drug Del. Rev.* 59, 546–567.

- Alvarez-Nunez, F.A., Yalkowsky, S.H., 2000. Relationship between Polysorbat 80 solubilization descriptors and octanol–water partition coefficients of drugs. *Int. J. Pharm.* 200, 217–222.
- Anderson, L.C., Wise, D.L., Howes, J.F., 1976. An injectable sustained release fertility control system. *Contraception* 13, 375–384.
- Anderson, J.M., Shive, M.S., 1997. Biodegradation and biocompatibility of PLA and PLGA microspheres. *Adv. Drug Del. Rev.* 28, 5–24.
- Arica, B., Lamprecht, R., 2005. In vitro evaluation of Betamethasone-loaded nanoparticles. *Drug Dev. Ind. Pharm.* 31, 19–24.
- Attwood, A., Ktistis, G., McMormick, Y., Story, M.J., 1989. Solubilization of indometacin by polysorbat 80 in mixed water-sorbitol solvents. *J. Pharm. Sci.* 41, 83–86.
- Aubert-Pouëssel, A., Bibby, D.C., Venier-Julienne, M.-C., Hindré, F., Benoît, J.P., 2002. A novel in vitro delivery system for assessing the biological integrity of protein upon release from PLGA microspheres. *Pharm. Res.* 19, 1046–1051.
- Avdeef, A., 2007. Solubility of sparingly-soluble drugs. *Adv. Drug Del. Rev.* 59, 568–590.
- Baek, N., Lee, J., Park, K., 2004. Aqueous *N,N*-diethylnicotinamid (DNA) solution as a medium for accelerated release study of paclitaxel. *J. Biomater. Sci. Polym. Edn.* 15, 527–542.
- Barreiro-Iglesias, R., Alvarez-Lorenzo, C., Concheiro, A., 2003. Controlled release of estradiol solubilised in carbopol/surfactant aggregates. *J. Controlled Release* 93, 319–330.
- Basak, A.K., Raw, A.S., Yu, L.X. (Eds.), 2007. *Pharmaceutical impurities: analytical, toxicological, and regulatory aspects*. *Adv. Drug Del. Rev.* 59 (Theme issue).
- Beck, L.R., Cowsar, D.R., Lewis, D.H., Gibson, J.W., Flowers, C.E., 1979. New long-acting injectable microcapsule contraceptive system. *Am. J. Obstet. Gynecol.* 135, 419–426.
- Beck, L.R., Pope, V.Z., Cowsar, F.R., Lewis, D.H., Tice, T.R., 1980. Evaluation of a new three-month injectable contraceptive microsphere system in primates (baboon). *Contracept. Del. Syst.* 1, 79–86.
- Beck, L.R., Ramos, R.A., Flowers, C.E., Lopez, G.Z., Lewis, D.H., Cowsar, D.R., 1981. Clinical evaluation of injectable biodegradable contraceptive system. *Am. J. Obstet. Gynecol.* 140, 799–806.
- Beck, L.R., Flowers, C.E., Pope, V.Z., Wilborn, W.H., Tice, T.R., 1983a. Clinical evaluation of an improved injectable microcapsule contraceptive system. *Am. J. Obstet. Gynecol.* 147, 815–821.
- Beck, L.R., Pope, V.Z., Flowers, C.E., Cowsar, D.R., Tice, T.R., Lewis, D.H., Dunn, L.R., Moore, A.B., Gilley, R.M., 1983b. Poly(D,L-lactide-co-glycolide)/norethisterone microcapsules: an injectable biodegradable contraceptive. *Biol. Reprod.* 28, 186–195.
- Beck, L.R., Pope, V.Z., Tice, T.R., Gilley, R.M., 1985. Long-acting injectable microsphere formulation for the parenteral administration of levonorgestrel. *Adv. Contracept.* 1, 119–129.
- Benelli, P., Conti, B., Genta, I., Costantini, M., Montanari, L., 1998. Clonazepam microencapsulation in poly-D,L-lactide-co-glycolide microspheres. *J. Microencapsul.* 15, 431–443.
- Benita, S., Benoît, J.P., Puisieux, F., Thies, C., 1984. Characterization of drug-loaded poly(D,L-lactide) microspheres. *J. Pharm. Sci.* 73, 1721–1724.
- Benoît, J.P., Benita, S., Puisieux, F., Thies, C., 1984. Stability and release kinetics of drugs incorporated within microspheres. In: Davis, S.S., Illum, L., McVie, J.G., Tomlinson, E. (Eds.), *Microspheres and Drug Therapy*. Pharmaceutical, Immunological and Medical Aspects. Elsevier, Amsterdam, pp. 91–102.
- Benoît, J.P., Rosilio, V., Deyme, M., Thies, C., Mademont, G., 1986a. A physico-chemical study of the morphology of progesterone-loaded 85:15 poly(D,L-lactide-co-glycolide) microspheres. *Proc. Int. Symp. Contr. Rel. Bioact. Mater.* 13, 171–172.
- Benoît, J.P., Courteille, F., Thies, C., 1986b. A physicochemical study of the morphology of progesterone-loaded poly (D,L-lactide) microspheres. *Int. J. Pharm.* 29, 95–102.
- Benoît, J.P., 1996. Microsphere morphology. In: Benita, S. (Ed.), *Microencapsulation. Methods and Industrial Application*. Marcel Dekker, New York, pp. 133–154.
- Berkland, C., Kim, K., Pack, D.W., 2001. Fabrication of PLG microspheres with precisely controlled and monodisperse size distributions. *J. Controlled Release* 73, 59–74.
- Berkland, C., King, M., Cox, A., Kim, K., Park, D.W., 2002. Precise control of PLG microsphere size provides enhanced control of drug release rate. *J. Controlled Release* 82, 137–147.
- Bernard, H., Mondoulet, L., Drumare, M.F., Pathy, E., Scheinmann, P., Thai, R., Wal, J.M., 2007. Identification of a new natural Ara h 6 isoform and of its proteolytic product as major allergens in peanut. *J. Agric. Food Chem.* 55, 9663–9669.
- Birnbaum, D.T., Kosmala, J.D., Henthorn, D.B., Brannon-Peppas, L., 2000. Controlled release of  $\beta$ -estradiol from PLGA microparticles: the effect of organic phase solvent on encapsulation and release. *J. Controlled Release* 65, 375–387.
- Birnbaum, D.T., Brannon-Peppas, L., 2003. Microparticle drug delivery systems. In: Braun, A.M. (Ed.), *Drug Delivery Systems in Cancer Therapy*. Humana Press, Totowa, pp. 117–135.
- Bissery, M.-C., Valeriote, F., Thies, C., 1984. In vitro and in vivo evaluation of CCNU-loaded microspheres prepared from poly( $\pm$ )-lactide and poly( $\beta$ -hydroxybutyrate). In: Davis, S.S., Illum, L., McVie, J.G., Tomlinson, E. (Eds.), *Microspheres and Drug Therapy*. Pharmaceutical, Immunological and Medical Aspects. Elsevier, Amsterdam, pp. 217–227.
- Blagden, N., de Matas, M., Gavan, P.T., York, P., 2007. Crystal engineering of active pharmaceutical ingredients to improve solubility and dissolution rates. *Adv. Drug. Del. Rev.* 59, 617–630.
- Blasi, P., D'Souza, S.S., Selmin, F., DeLuca, P.P., 2005. Plasticizing effect of water on poly(lactide-co-glycolide). *J. Controlled Release* 108, 1–9.
- Blasi, P., Schoubben, A., Giovagnoli, S., Peroli, L., Ricci, M., Rossi, C., 2007. Ketoprofen poly(lactide-co-glycolide) physical interaction. *AAPS PharmSciTech* 8 (article 37) <http://www.aapspharmstech.org>.
- Bleich, J., Kleinbudde, P., Müller, B.W., 1994. Influence of gas density and pressure on microparticles produced with the ASES process. *Int. J. Pharm.* 106, 77–84.
- Bleich, J., Müller, B.W., 1996. Production of drug loaded microparticles by the use of supercritical gases with the aerosol solvent extraction system (ASES) process. *J. Microencapsul.* 13, 131–139.
- Bodmeier, R., McGinity, J.W., 1987a. The preparation and evaluation of drug-containing poly(D,L-lactide) microspheres formed by the solvent evaporation method. *Pharm. Res.* 4, 465–471.
- Bodmeier, R., McGinity, J.W., 1987b. Poly(lactic acid) microspheres containing quinidine base and quinidine sulphate prepared by solvent evaporation technique. II. Some process parameters influencing the properties of microspheres. *J. Microencapsul.* 4, 289–297.
- Bodmeier, R., McGinity, J.W., 1987c. Poly(lactic acid) microspheres containing quinidine base and quinidine sulphate prepared by solvent evaporation technique. I. Methods and Morphology. *J. Microencapsul.* 4, 279–288.
- Bodmeier, R., Chen, H., 1988. Preparation of biodegradable poly( $\pm$ )-lactide microparticles using a spray-drying technique. *J. Pharm. Pharmacol.* 40, 754–757.
- Bodmeier, R., McGinity, J.W., 1988. Solvent selection in the preparation of poly(D,L-lactide) microspheres prepared by the solvent evaporation method. *Int. J. Pharm. Sci.* 43, 179–186.
- Bodmeier, R., Oh, K.H., Chen, H., 1989. The effect of the addition of low molecular weight poly(D,L-lactide) on drug release from biodegradable poly(D,L-lactide) drug delivery systems. *Int. J. Pharm.* 51, 1–8.
- Bodmeier, R., 1998a. Verfahren zur in-situ Herstellung von Partikeln. German Patent Application Publication DE 197 24 784 (10 December).
- Bodmeier, R., 1998b. Multiphase system. International Publication WO 98/55100 (10 December).
- Bodmeier, R., 2002. Sustained release particle dispersion. International Publication WO 02/19988 (14 March).
- Bodmer, D., Fong, J.W., Kissel, T., Maulding, H.V., Nagele, O., Pearson, J.E., 1996a. Octreotidepamoat und dessen Verwendung zur Herstellung von pharmazeutischen Formulierungen. Swiss Patent CH 686 252 (15 February).
- Bodmer, D., Fong, J.W., Kissel, T., Maulding, H.V., Nagele, O., Pearson, J.E., 1996b. Sustained release formulations of water soluble peptides. US Patent 5,538,739 (23 July).
- Bodmer, D., Fong, J.W., Kissel, T., Maulding, H.V., Nagele, O., Pearson, J.E., 1997. Sustained release formulations of water soluble peptides. US Patent 5,639,480 (17 June).
- Boswell, G.A., Scribner, R.M., 1973. Polylactide–drug mixtures. US Patent 3,773,919 (20 November).
- Boury, F., Ivanova, T., Panaiotov, I., Proust, J.E., Bois, A., Richou, J., 1995. Dynamic properties of poly(D,L-lactide) and polyvinyl alcohol monolayers at the air/water and dichloromethane/water interfaces. *J. Colloids Interface Sci.* 169, 380–392.
- BP (British Pharmacopoeia), 2001. General Notices–Solubility. British Pharmacopoeia Commission, distributed by The Stationery Office Bookshop, p. 11.
- Brady, J.M., Cutright, D.E., Miller, R.A., Battistone, G.C., 1973. Resorption rate, route of elimination, and ultrastructure of implant site of polylactic acid in the abdominal wall of the rat. *J. Biomed. Mater. Res.* 7, 155–166.
- Brittain, H.A., Dickason, D.A., Hotz, J., Lyons, S.L., Ramstack, M.J., Wright, S.G., 2004. Polymorphic forms of naltrexone. International Publication WO 2004/108084 (16 December).
- Brodbeck, K.J., Gaynor-Duarte, A.T., Shen, T.T., 2000. Gel compositions and methods. US Patent 6,130,200 (10 October).
- Buntner, B., Novak, M., Ryba, J., Grieb, P., Walski, M., Dobrzynski, P., Bero, M., 1998. The application of microspheres from the copolymers of lactide and  $\epsilon$ -caprolactone to the controlled release of steroids. *J. Controlled Release* 56, 159–167.
- Burgess, D.J., Hickey, A.J., 2005. Microsphere: design and manufacturing. In: Burgess, D.J. (Ed.), *Injectable Dispersed Systems. Formulation, Processing, and Performance*. Taylor & Francis, Boca Raton, pp. 305–353.
- Burks, A.W., Williams, L.W., Thresher, W., Connaughton, C., Cockrell, G., Helm, R.M., 1992. Allergenicity of peanut and soybean extracts altered by chemical or thermal denaturation in patients with atopic dermatitis and positive food challenge. *J. Allergy Clin. Immunol.* 90, 889–897.
- Burns, P.J., Gibson, J.W., Tipton, A.J., 2000. Compositions suitable for controlled release of the hormone GNRH and its analogs. US Patent 6,051,558 (18 April).
- Burt, H.M., Jackson, J.K., Bains, S.K., Liggins, R.T., Oktaba, A.M.C., Arsenault, A.L., Hunter, W.L., 1995. Controlled delivery of taxol from microspheres composed of a blend of ethylene-vinyl acetate copolymer and poly(D,L-lactic acid). *Cancer Lett.* 88, 73–79.
- Butler, S.M., Tracy, M.A., Tilton, R.D., 1999. Adsorption of serum albumin to thin films of poly(lactide-co-glycolide). *J. Controlled Release* 58, 335–347.
- Cao, X., Yu, L., Sun, D., 2008. Drug absorption principles. In: Krishna, R., Yu, L. (Eds.), *Biopharmaceutics Application in Drug Development*. Springer, New York, pp. 75–100.
- Carrio, A., Schwach, G., Coudane, J., Vert, M., 1995. Preparation and degradation of surfactant-free PLGA microspheres. *J. Controlled Release* 37, 113–121.
- Cavalier, M., Benoît, J.P., Thies, C., 1986. The formation and characterization of hydrocortisone-loaded poly( $\pm$ )-lactide microspheres. *J. Pharm. Pharmacol.* 38, 249–253.
- Cha, Y., Pitt, C.G., 1988. A one-week subdermal delivery system for l-methadone based on biodegradable microcapsules. *J. Controlled Release* 8, 69–78.



- Cha, Y., Pitt, C.G., 1989. The acceleration of degradation controlled drug delivery from polyester microspheres. *J. Controlled Release* 8, 259–265.
- Chang, T.M.S., 1976. Biodegradable semipermeable microcapsules containing enzymes, hormones, vaccines, and other biologicals. *J. Bioeng.* 1, 25–32.
- Chattopadhyay, P., Huff, R., Shekunov, B.Y., 2006. Drug encapsulation using supercritical fluid extraction of emulsions. *J. Pharm. Sci.* 95, 667–679.
- Chen, J.-L., Yeh, M.-K., Chiang, C.-H., 2004. The mechanism of surface-indentation protein-loaded PLGA microcapsule formation: the effect of salt (NaCl) on the solidification process. *J. Microencapsul.* 21, 877–888.
- Chenite, A., Chaput, C., Selmani, A., 2002. Method for preparation of polymer microparticles free of organic solvent traces. European Patent EP 934 353 (03 July).
- Chiang, C.N., Hollister, L.E., Kishimoto, A., Barnett, A., 1984. Kinetics of a naltrexone sustained release formulation. *Clin. Pharmacol. Ther.* 36, 704–708.
- Choi, H.S., Seo, S.-A., Khang, G., Rhee, J.M., Lee, H.B., 2002. Preparation and characterization of fentanyl-loaded microspheres: in vitro release profiles. *Int. J. Pharm.* 234, 195–203.
- Chu, C.C., 1981. The in-vitro degradation of poly(glycolic acid) sutures—effect of pH. *J. Biomed. Mater. Res.* 15, 795–804.
- Cong, H., Beck, L.R., 1991. Preparation and pharmacokinetic evaluation of a modified long-acting injectable norethisterone microsphere. *Adv. Contracept.* 7, 251–256.
- Conti, B., Pavanetto, F., Genta, I., 1992. Use of polylactic acid for the preparation of microparticulate drug delivery systems. *J. Microencapsul.* 9, 153–166.
- Cowsar, D.R., Tice, T.R., Gilley, R.M., English, J.P., 1985. Poly(lactide-co-glycolide) microcapsules for controlled release of steroids. *Methods Enzymol.* 112, 101–116.
- Cutright, D.E., Beasley, J.D., Perez, B., 1971. Histologic comparison of polylactic and polyglycolic acid sutures. *Oral Surg.* 32, 165–173.
- Davies, O.R., Lewis, A.L., Whitaker, M.J., Tai, H., Shakesheff, K.M., Howdle, S.M., 2008. Applications of supercritical CO<sub>2</sub> in the fabrication of polymer systems for drug delivery and tissue engineering. *Adv. Drug Del. Rev.* 60, 373–387.
- Dean, R.-L., 2005. The preclinical development of Medisorb® Naltrexone, a once a month long-acting injection, for the treatment of alcohol dependence. *Front. Biosci.* 10, 643–655.
- Demetrick, J.S., Liggins, R.T., Machan, L., Davis, N.L., Burt, H.M., Hunter, W.L., 1997. The development of a novel intraperitoneal tumor-seeding prophylactic. *A. J. Surg.* 173, 403–406.
- Desai, K.G.H., Mallery, S.R., Schwendeman, S.P., 2008. Formulation and characterization of injectable poly(DL-lactide-co-glycolide) implants loaded with N-acetylcysteine, a MMP inhibitor. *Pharm. Res.* 25, 586–597.
- Dhanaraju, M.D., Vema, K., Jayakumar, R., Vamsadhara, C., 2003. Preparation and characterization of injectable microspheres of contraceptive hormones. *Int. J. Pharm.* 268, 23–29.
- Dhanaraju, M.D., Jayakumar, R., Vamsadhara, C., 2004. Influence of manufacturing parameters on development of contraceptive steroid loaded injectable microspheres. *Chem. Pharm. Bull.* 52, 976–979.
- Dhanaraju, M.D., RayKannan, R., Selvaraj, D., Jayakumar, R., Vamsadhara, C., 2006. Biodegradation and biocompatibility of contraceptive-steroid loaded poly(DL-lactide-co-glycolide) injectable microspheres: in vitro and in vivo study. *Contraception* 74, 148–156.
- Didziapetris, R., Jaspertas, P., Avdeef, A., Petrauskas, A., 2003. Classification analysis of P-glycoprotein substrate specificity. *J. Drug Target* 11, 391–406.
- Ding, A.G., Schwendeman, S.P., 2004. Determination of water-soluble acid distribution in poly(lactide-co-glycolide). *J. Pharm. Sci.* 93, 322–331.
- Ding, A.G., Shenderova, A., Schwendeman, S.P., 2006. Prediction of microclimate pH in poly(lactide-co-glycolic acid) films. *J. Am. Chem. Soc.* 128, 5384–5390.
- Discoll, D.F., 2006. Lipid injectable emulsions: 2006. *Nutr. Clin. Pract.* 21, 381–386.
- Dittgen, M., Fricke, S., Gerecke, H., Möller, I.-P., Völkel, C., 1998. Injizierbares Implantat. German Patent DE 197 01 912 (14 May).
- Doolittle, A.K., 1954. The technology of solvents and plasticizers. In: *Properties of Individual Solvents*. Wiley, New York, pp. 492–742 (Chapter 12).
- Drugbank, 2008. <http://redpoll.pharmacy.ualberta.ca/drugbank/index.html>.
- Dubernet, C., 1995. Thermoanalysis of microspheres. *Thermochim. Acta* 248, 259–269.
- Dunn, L.R., English, J.P., Cowsar, D.R., Vanderbilt, D.P., 1990. Biodegradable in-situ forming implants and methods of producing the same. US Patent 4,938,763 (3 July).
- Dunn, L.R., English, J.P., Cowsar, D.R., Vanderbilt, D.P., 1994. Biodegradable in-situ forming implants and methods of producing the same. US Patent 5,278,201 (11 January).
- Dunn, L.R., Garrett, J.S., Ravivarapu, H., Chandrashekar, B.L., 2003. Polymeric delivery formulations of leuprolide with improved efficacy. US Patent 6,565,874 (20 May).
- Dunne, M., Corrigan, O.I., Ramtoola, Z., 2000. Influence of particle size and dissolution conditions on the degradation properties of polylactide-co-glycolide particles. *Biomaterials* 21, 1659–1668.
- Eggers, J., 2006. A brief history of droplet formation. In: Alart, P., Maisonneuve, O., Rockafellar, R.T. (Eds.), *Nonsmooth Mechanics and Analysis*. Advances in Mechanics and Mathematics, vol. 12. Springer, New York, pp. 163–172.
- Elkharraz, K., Faisant, N., Guse, C., Siepmann, F., Arica-Yegin, B., Oger, J.M., Gust, R., Goepferich, A., Benoit, J.P., Siepmann, J., 2006. Paclitaxel-loaded microparticles and implants for the treatment of brain cancer: preparation and physicochemical characterization. *Int. J. Pharm.* 314, 127–136.
- EMEA (European Medicines Agency), 2004. Final position paper on the allergenic potency of herbal medicinal products containing soya or peanut proteins. Downloaded from <http://www.emea.europa.eu/pdfs/human/hmpc/003704en.pdf> on January 28th, 2008.
- Ertl, B., Platzer, P., Wirth, M., Gabor, F., 1999. Poly(D,L-lactide-co-glycolic acid) microspheres for sustained delivery and stabilization of camptothecin. *J. Controlled Release* 61, 305–317.
- Falk, R., Randolph, T.W., Meyer, J.D., Kelly, R.M., Manning, M.C., 1997. Controlled release of ionic compounds from poly(L-lactide) microspheres produced by precipitation with a compressed antisolvent. *J. Controlled Release* 44, 77–85.
- Faller, B., Ertl, P., 2007. Computational approaches to predict solubility. *Adv. Drug Del. Rev.* 59, 522–545.
- Ferrante, M.G., Ruby, B.C., 1977. Betamethasone dipropionate. In: Florey, K. (Ed.), *Analytical Profiles of Drug Substances*, vol. 6. Academic Press, New York, pp. 43–60.
- Florey, K., 1972a. Triamcinolone. In: Florey, K. (Ed.), *Analytical Profiles of Drug Substances*, vol. 1. Academic Press, New York, pp. 367–396.
- Florey, K., 1972b. Triamcinolone diacetate. In: Florey, K. (Ed.), *Analytical Profiles of Drug Substances*, vol. 1. Academic Press, New York, pp. 423–442.
- Florey, K., 1972c. Triamcinolone acetonide. In: Florey, K. (Ed.), *Analytical Profiles of Drug Substances*, vol. 1. Academic Press, New York, pp. 397–421.
- Fong, J.W., 1979. Processes for preparation of microspheres. US Patent 4,166,800 (4 September).
- Fong, J.W., 1981. Making microspheres using carboxylic acid salts as emulsifiers. UK Patent Application Publication GB 2 077 693A (23 December).
- Fong, J.W., 1983. Processes for preparation of microspheres. US Patent 4,384,975 (24 May).
- Fong, J.W., Nazareno, J.P., Pearson, J.E., Maulding, H.V., 1986. Evaluation of biodegradable microspheres prepared by a solvent evaporation process using sodium oleate as emulsifier. *J. Controlled Release* 3, 119–130.
- Frank, A., Rath, S.K., Venkatraman, S.S., 2005. Controlled release from bioerodible polymers: effect of drug type and polymer composition. *J. Controlled Release* 102, 333–344.
- Fu, K., Pack, D.W., Klibanov, A.M., Langer, R., 2000. Visual evidence of acidic environment within degrading poly(lactide-co-glycolic acid) (PLGA) microspheres. *Pharm. Res.* 17, 100–106.
- Gander, B., Merkle, H.-P., 1997. Process for the production of microcapsules. US Patent 5,648,096 (15 July).
- Gangur, V., Kelly, C., Navuluri, L., 2005. Sesame allergy: a growing food allergy of global proportions? *Ann. Allergy Asthma Immunol.* 95, 4–11.
- Gardner, D.L., Patanus, A.J., Fink, D.J., 1977. Steroid release from microcapsules. In: Gabelnick, H.L. (Ed.), *Drug Delivery Systems*. US Department of Health, Education, and Welfare, Washington, DC, pp. 265–278 (USDHEW Pub. No. (NIH) 77-1238).
- Ghaderi, R., Artursson, P., Carlfors, J., 2000. A new method for preparing biodegradable microparticles and entrapment of hydrocortisone in DL-PLG microparticles using supercritical fluids. *Eur. J. Pharm. Sci.* 10, 1–9.
- Gibaldi, M., Feldman, S., 1967. Establishment of sink conditions in dissolution rate determinations: theoretical consideration and application to disintegrated dosage forms. *J. Pharm. Sci.* 56, 1238–1242.
- Ginty, P.J., Whitaker, M.J., Shakesheff, K.M., Howdle, S.M., 2005. Drug delivery goes supercritical. *Mater. Today* 8, 42–48.
- Giunchedi, P., Alpar, H.O., Conte, U., 1998. PDLLA microspheres containing steroids: spray drying, o/w and w/o/w emulsification as preparation methods. *J. Microencapsul.* 15, 185–195.
- Gombotz, W.R., Healy, M.S., Brown, L.R., 1991. Very low temperature casting of controlled release microspheres. US Patent 5,019,400 (28 May).
- Graves, R.A., Freeman, T., Pamajula, S., Praetorius, N., Moiseyev, R., Mansal, T.K., 2006. Effect of co-solvents on the characteristics of enkephalin microcapsules. *J. Biomater. Sci. Polym. Edn.* 17, 709–720.
- Green, T.D., LaBelle, V.S., Steele, P.H., Kim, E.H., Lee, L.A., Mankad, V.S., Williams, L.W., Anstrom, K.J., Burks, A.W., 2007. Clinic characteristics of peanut-allergic children: recent changes. *Pediatrics* 120, 1304–1310.
- Gresser, J.D., Wise, D.L., Beck, L.R., Howes, J.F., 1978. Larger animal testing of an injectable sustained release fertility control system. *Contraception* 17, 253–266.
- Gu, Z.-W., Ye, W.-P., Li, J.-Y., Chen, X.-L., Zhong, G.W., Feng, X.-D., 1992. Biodegradable block copolymers matrices for long-acting contraceptives with constant release. *J. Controlled Release* 22, 3–14.
- Gupta, P.K., Mehta, R.C., Douglas, R.H., DeLuca, P.P., 1992. In vivo evaluation of biodegradable microspheres in mares. *Pharm. Res.* 9, 1502–1506.
- Gupte, A., Ciftci, K., 2004. Formulation and characterization of Paclitaxel, 5-FU and Paclitaxel + 5-FU microspheres. *Int. J. Pharm.* 276, 93–106.
- Guy, R.H., Hadgraft, J., 1981. Theoretical comparison of release rates of drugs into sink and nonsink conditions. *J. Pharm. Sci.* 70, 1243–1245.
- Hatefi, A., Amsden, B., 2002. Biodegradable injectable in situ forming drug delivery systems. *J. Controlled Release* 80, 9–28.
- Hahn, D.W., McGuire, J.L., Cohn, R.M., Beck, L.R., Tice, T.R., Lewis, D.H., 1983. Development of microencapsulated norgestimate as a long-acting contraceptive. In: Zaptuchni, G.I., Goldsmith, A., Shelton, J.D., Sciarra, J.J. (Eds.), *Long Acting Contraceptive Delivery Systems*. Harper & Row, Philadelphia, pp. 96–112 (Chapter 8).
- Herrero-Vanell, R., Ramirez, L., Fernandez-Carballido, A., Refolo, M.F., 2000. Biodegradable PLGA microspheres loaded with ganciclovir for intraocular administration. Encapsulation technique, in vitro release profiles, and sterilization process. *Pharm. Res.* 17, 1323–1328.
- Herrmann, W.O., Haehnel, W., 1927. Über den Polyvinylalkohol. *Ber. Deut. Chem. Ges.* 60, 1658–1663.

- Herrmann, J., Bodmeier, R., 1995. Somatostatin containing biodegradable microspheres prepared by a modified solvent evaporation method based on w/o/w multiple emulsions. *Int. J. Pharm.* 126, 129–138.
- Herrmann, J., Bodmeier, R., 1998. Biodegradable, somatostatin acetate containing microspheres prepared by various aqueous and non-aqueous solvent evaporation methods. *Eur. J. Pharm. Biopharm.* 45, 72–85.
- Heusch, R., 1983. Elementary processes in the production and stabilization of emulsions. *Chem. Ing. Tech.* 55, 608–616.
- Hill, V.L., Paserini, N., Craig, D.Q.M., Vickers, M., Anwar, J., Feely, L.C., 1998. Investigation of progesterone loaded poly(D,L-lactide) microspheres using TMDCS, SEM and PXRD. *J. Therm. Anal.* 54, 673–685.
- Ho, R.H., Kim, R.B., 2005. Transporters and drug therapy: implications for drug disposition and disease. *Clin. Pharmacol. Ther.* 78, 260–277.
- Horvath, A.L., 1982. *Halogenated Hydrocarbons*. Marcel Dekker, New York, pp. 658–660.
- Hutchinson, F.G., Furr, B.J.A., 1985. Biodegradable polymers for the sustained release of peptides. *Biochem. Soc. Trans.* 13, 520–523.
- Hutchinson, F.G., 1986. Continuous release pharmaceutical compositions. European Patent EP 58 481 (27 January).
- Hutchinson, F.G., Furr, B.J.A., 1989. Biodegradable polymers for the controlled release of peptides and proteins. *Horiz. Biochem. Biophys.* 9, 111–129.
- ICH Q1B Guidance (International Conference on Harmonisation), 1996. Guidance for Industry. Q1B Photostability testing of new drug substances and products. Text available from FDA at <http://www.fda.gov/cder/Guidance/1318fnl.pdf>.
- ICH draft (International Conference on Harmonisation), 1997. Draft Guideline on Impurities, Residual Solvents. Published by FDA in: Fed. Regist., 62, pp. 24302–24309.
- ICH Q3C Guidance (International Conference on Harmonisation), 2003. Guidance for Industry. Q3C Impurities: Residual solvents. Text and tables available from FDA at <http://www.fda.gov/cber/gdlns/q3cresolvent.pdf> and <http://www.fda.gov/cber/gdlns/ichq3cstabl.pdf>, respectively.
- ICH Q3B Guidance (International Conference on Harmonisation), 2006. Guidance for Industry. Q3B(R2) Impurities in new drug products. Text available from FDA at <http://www.fda.gov/cder/guidance/7385fnl.pdf>.
- Ignatius, A.A., Cleas, L.E., 1996. In vitro biocompatibility of bioresorbable polymers: poly(L, DL-lactide) and poly(L-lactide-co-glycolide). *Biomaterials* 17, 831–839.
- Im-Emsap, W., 2002. In vitro and in vivo properties of injectable biodegradable in situ forming microparticle systems. Ph.D. Thesis. Freie Universität Berlin, Germany.
- Izumikawa, S., Yoshioka, S., Aso, Y., Takeda, Y., 1991. Preparation of poly(L-lactide) microspheres of different crystalline morphology and effect of crystalline morphology on drug release rate. *J. Controlled Release* 15, 133–140.
- Jain, R., Shah, N.H., Malick, A.W., Rhodes, C.T., 1998. Controlled drug delivery by biodegradable poly(ester) devices: different preparative approaches. *Drug Dev. Ind. Pharm.* 24, 703–727.
- Jain, R.A., Rhodes, C.T., Railkar, A.M., Malick, A.W., Shah, N.H., 2000a. Controlled release of drugs from injectable in situ formed biodegradable PLGA microspheres: effect of various formulation variables. *Eur. J. Pharm. Biopharm.* 50, 257–262.
- Jain, R.A., Rhodes, C.T., Railkar, A.M., Malick, A.W., Shah, N.H., 2000b. Controlled delivery of drugs from a novel injectable in situ formed biodegradable PLGA microsphere system. *J. Microencapsul.* 17, 343–362.
- Jain, R.A., Rhodes, C.T., Railkar, A.M., Malick, A.W., Shah, N.H., 2000c. Comparison of various injectable protein-loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices: in situ-formed implant versus in-situ-formed microspheres versus isolated microspheres. *Pharm. Dev. Technol.* 5, 201–207.
- Jain, R.A., 2000. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. *Biomaterials* 21, 2475–2490.
- Jalil, R., Nixon, J.R., 1989. Microencapsulation using poly(L-lactic acid) I: microcapsule properties affected by the preparation technique. *J. Microencapsul.* 6, 473–484.
- Jalil, R., Nixon, J.R., 1990a. Microencapsulation using poly(L-lactic acid) II: preparative variables affecting microcapsule properties. *J. Microencapsul.* 7, 25–39.
- Jalil, R., Nixon, J.R., 1990b. Microencapsulation using poly(L-lactic acid) III: effect of the polymer molecular weight on the microcapsule properties. *J. Microencapsul.* 7, 41–52.
- Jalil, R., Nixon, J.R., 1990c. Microencapsulation using poly(L-lactic acid) IV: release properties of microcapsule containing phenobarbitone. *J. Microencapsul.* 7, 53–66.
- Janoria, K.G., Mitra, A.K., 2007. Effect of lactide/glycolide ratio on the in vitro release of ganciclovir and its lipophilic prodrug (GCV-monobutyrate) from PLGA microspheres. *Int. J. Pharm.* 338, 133–141.
- Jaraswekin, S., Prakongpan, S., Bodmeier, R., 2007. Effect of poly(lactide-co-glycolide) molecular weight on the release of dexamethasone sodium phosphate from microspheres. *J. Microencapsul.* 24, 117–128.
- Jeyanthi, R., Thanoo, B.C., Metha, R.C., DeLuca, P.P., 1996. Effect of solvent removal technique on the matrix characteristics of poly(lactide/glycolide) microspheres for peptide delivery. *J. Controlled Release* 38, 235–244.
- Jeffery, H., Davis, S.S., O'Hagan, D.T., 1991. The preparation and characterisation of poly(lactide-co-glycolide) microspheres: I: oil-in-water emulsion solvent evaporation. *Int. J. Pharm.* 77, 169–175.
- Jeong, J.H., Lim, D.W., Han, D.K., Park, T.G., 2000. Synthesis, characterization and protein adsorption behaviors of PLGA:PEG di-block co-polymer blend films. *Colloids Surf. B* 18, 371–379.
- Jiang, W., Gupta, R.K., Deshpande, M.C., Schwendeman, S.P., 2005. Biodegradable poly(lactide-co-glycolic acid) microparticles for injectable delivery of vaccine antigens. *Adv. Drug Del. Rev.* 57, 301–410.
- Jobmann, M., Rafler, G., 1998. Controlled release systems of biodegradable polymers. 6th communication: control of particle size and size distribution of microparticles produced by the solvent evaporation technique. *Pharm. Ind.* 60, 979–982.
- Johanson, P., Men, Y., Merkle, H.P., Gander, B., 2000. Revisiting PLA/PLGA microspheres: an analysis of their potential in parenteral vaccination. *Eur. J. Pharm. Biopharm.* 50, 129–146.
- Johnson, O.L., Cleland, J.L., Lee, H.J., Charnis, M., Duenas, E., Jaworowicz, W., Shepard, D., Shahzamani, A., Jones, A.J.S., Putney, S.D., 1996. A month-long effect from a single injection of microencapsulated human growth hormone. *Nat. Med.* 2, 705–709.
- Johnson, O.L., Ganmukhi, M.M., Bernstein, H., Auer, H., Khan, M.A., 1997. Composition for sustained release of human growth hormone. US Patent 5,654,010 (5 August).
- Jung, J., Perrut, M., 2001. Particle design using supercritical fluids: literature and patent survey. *J. Supercrit. Fluids* 20, 179–219.
- Juni, K., Nakano, M., Kubota, M., 1986. Controlled release of aclarubicin, an anticancer antibiotic, from poly- $\beta$ -hydroxybutyric acid microspheres. *J. Controlled Release* 4, 25–32.
- Kabasakalian, P., Britt, E., Yudis, M.D., 1996. Solubility of some steroids in water. *J. Pharm. Sci.* 55, 642.
- Kang, J., Schwendeman, S.P., 2007. Pore closing and opening in biodegradable polymers and their effect on the controlled release of proteins. *Mol. Pharm.* 4, 104–118.
- Katragadda, S., Budda, B., Anand, B.S., Mitra, A.K., 2005. Role of efflux pumps and metabolizing enzymes in drug delivery. *Exp. Opin. Drug Deliv.* 2, 683–705.
- Kent, J.S., Sanders, L.M., Lewis, D.H., Tice, T.R., 1986. Microencapsulation of water soluble polypeptides. European Patent EP 52 510 (27 August).
- Kim, J.-H., Paxton, T.E., Tomasko, D.L., 1996. Microencapsulation of naproxen using rapid expansion of supercritical solutions. *Biotechnol. Prog.* 12, 650–661.
- Kim, H.K., Chung, H.J., Park, T.G., 2006. Biodegradable polymeric microparticles with “open/closed” pores for sustained release of human growth hormone. *J. Controlled Release* 112, 167–174.
- Kim, J., Hong, D., Chung, Y., Sah, H., 2007. Ammonolysis-induced solvent removal: a facile approach for solidifying emulsion droplets into PLGA microspheres. *Biomacromology* 8, 3900–3907.
- Kino, S., Osajima, T., Minuta, H., 1997. Sustained release microsphere preparation containing antipsychotic drug and production process thereof. US Patent 5,656,299 (12 August).
- Kissel, T., Brich, Z., Bantle, S., Lancranjan, I., Nimmerfall, F., Vit, P., 1991. Parenteral depot-system on the basis of biodegradable polyesters. *J. Controlled Release* 16, 27–42.
- Klippel, K.F., Winkler, C.J., Jocham, D., Rübber, H., Möser, B., Gulati, A., 1999. Wirksamkeit und Verträglichkeit von 2 Applikationsformen (s.c. und i.m.) von Decapeptyl® Depot bei Patienten mit fortgeschrittenem Prostatakarzinom. *Urologe A* 38, 270–275 (Article in German; mentions the switch to a new hardening agent, a fact that strongly indicates the use of a coacervation technique).
- Klose, D., Siepmann, F., Elkharraz, K., Siepmann, J., 2008. PLGA-based drug delivery systems: importance of the type of drug and device geometry. *Int. J. Pharm.* 354, 95–103.
- Kranz, H., Brazeau, G.A., Napaporn, J., Martin, R.L., Millard, W., Bodmeier, R., 2001. Myotoxicity studies of injectable biodegradable in-situ forming drug delivery systems. *Int. J. Pharm.* 212, 11–18.
- Kranz, H., Bodmeier, R., 2007. A novel in situ forming drug delivery system for controlled parenteral drug delivery. *Int. J. Pharm.* 332, 107–114.
- Kulkarni, R.K., Moore, E.G., Hegyelli, A.F., Leonard, F., 1971. Biodegradable poly(lactic acid) polymers. *J. Biomed. Mater. Res.* 5, 169–181.
- Lactel® product information. <http://www.absorbables.com/properties.htm>.
- Lalla, J.K., Sapna, K., 1993. Biodegradable microspheres of poly(DL-lactic acid) containing piroxicam as a model drug for controlled release via parenteral route. *J. Microencapsul.* 10, 449–460.
- Lambert, W.J., Peck, K.D., 1995. Development of an in situ forming biodegradable poly-lactide-co-glycolide system for the controlled release of proteins. *J. Controlled Release* 33, 189–195.
- Lambert, O., Ausborn, M., Petersen, H., Löffler, R., Bonny, J.-D., 2004. Pharmaceutical composition comprising microparticles. International Patent Application Publication WO 2004/045633 (3 June).
- Lapka, G.G., Mason, N.S., Thies, C., 1986. Process for preparation of microcapsules. US Patent 4,622,244 (11 November).
- Laurencin, C.T., Elgendy, H., 1994. The biocompatibility and toxicity of degradable polymeric materials: implications for drug delivery. In: Domb, A.J. (Ed.), *Polymeric Site-specific Pharmacotherapy*. Wiley, Chichester, pp. 27–46.
- Lawter, J.R., Lanzilotti, M.G., 1996. Phase separation-microencapsulated pharmaceuticals compositions useful for alleviating dental disease. US Patent 5,500,228 (19 March).
- Leach, W.T., Simpson, D.T., Val, T.N., Yu, Z., Lim, K.T., Park, E.J., Williams III, R.O., Johnson, K.P., 2005. Encapsulation of protein nanoparticles into uniform-sized microspheres formed in a spinning oil film. *AAPS PharmSciTech* 6 (article 75) <http://www.aapspharmscitech.org>.
- Lee, H.J., Riley, G., Johnson, O., Cleland, J.L., Kim, N., Charnis, M., Bailey, L., Duenas, E., Shahzamani, A., Marian, M., Jones, A.J.S., Putney, S.D., 1997. In vivo characterization of sustained-release formulations of human growth hormone. *J. Pharmacol. Exp. Ther.* 281, 1431–1439.

- Leelarasamee, N., Howard, S.A., Malanga, C.J., Luzzi, L.A., Hogan, T.F., Kandazari, S.J., Ma, J.K.H., 1986. Kinetics of drug release from polylactic acid-hydrocortisone microcapsules. *J. Microencapsul.* 3, 171–179.
- Leeson, P.D., Springthorpe, B., 2007. The influence of drug-like concepts on decision-making in medicinal chemistry. *Nat. Rev. Drug Discov.* 6, 881–890.
- Li, S.M., Garreau, H., Vert, M., 1990. Structure–property relationships in the case of the degradation of massive aliphatic poly( $\alpha$ -hydroxy acids) in aqueous media. Part 1: degradation of lactide-glycolide copolymers: PLA37.5GA25 and PLA75GA25. *J. Mater. Sci., Mater. Med.* 1, 131–139.
- Li, S., Girod-Holland, S., Vert, M., 1996. Hydrolytic degradation of poly(DL-lactic acid) in the presence of caffeine base. *J. Controlled Release* 40, 41–53.
- Li, W., Anderson, K.W., Mehta, R.C., DeLuca, P.P., 1999. Prediction of solvent removal profile and effect on properties for peptide-loaded PLGA microspheres prepared by solvent extraction/evaporation method. *J. Controlled Release* 37, 199–214.
- Li, S., 1999. Hydrolytic degradation characteristics of aliphatic polyesters derived from lactic and glycolic acids. *J. Biomed. Mater. Res. (Appl. Biomater.)* 48, 342–353.
- Li, L., Schwendeman, S.P., 2005. Mapping neutral microclimate pH in PLGA microspheres. *J. Controlled Release* 101, 163–173.
- Liggins, R.T., D'Amours, S., Demetrick, J.S., Machan, L.S., Burt, H.M., 2000. Paclitaxel loaded poly(L-lactic acid) microspheres for the prevention of intraperitoneal carcinomatosis after a surgical repair and tumor cell spill. *Biomaterials* 21, 1959–1969.
- Liggins, R.T., Burt, H.M., 2001. Paclitaxel loaded poly(L-lactic acid) microspheres: properties of microspheres made with low molecular weight polymers. *Int. J. Pharm.* 222, 19–33.
- Lill, N., Sandow, J., 1995. Langwirkende bioabbaubare Mikropartikel und ein Verfahren zur Herstellung. European Patent EP 505 966 (28 June).
- Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J., 1997. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Del. Rev.* 23, 2–25.
- Lipinski, C.A., 2000. Drug-like properties and the causes of poor solubility and poor permeability. *J. Pharm. Toxicol. Meth.* 44, 235–249.
- Lipinski, C.A., 2004. Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discov. Today Technol.* 1, 337–341.
- Lord Rayleigh, F.R.S., 1879. On the instability of jets. *Proc. Lond. Math. Soc.* 10, 4–13 (appeared in the volume of 1878).
- Luan, X., Bodmeier, R., 2006a. In situ forming microparticle systems for controlled delivery of leuprolide acetate: influence of the formulation and processing parameters. *Eur. J. Pharm. Sci.* 27, 143–149.
- Luan, X., Bodmeier, R., 2006b. Influence of the poly(lactide-co-glycolide) type on the leuprolide release from in situ forming microparticle systems. *J. Controlled Release* 110, 266–272.
- Luan, X., 2006. Biodegradable microparticle and in situ microparticle systems. Ph.D. Thesis. Freie Universität Berlin, Germany. Available at <http://www.diss.fu-berlin.de/2006/27/>.
- Lucke, A., Kiermaier, J., Göpferich, A., 2002. Peptide acylation by poly( $\alpha$ -hydroxy esters). *Pharm. Res.* 19, 175–181.
- Lunberg, B., 1979. Temperature effect on the water solubility and water-octanol partition of some steroids. *Acta Pharm. Suec.* 16, 151–159.
- Lyons, S.L., Wright, S.G., 2004. Apparatus and method for preparing microparticles. US Patent 6,713,090 (30 March).
- Lyons, S.L., Wright, S.G., 2005. Method and apparatus for preparing microparticles using in-line solvent extraction. US Patent 6,939,033 (6 September).
- Mäder, K., Bittner, B., Li, Y., Wohlauf, W., Kissel, T., 1998. Monitoring microviscosity and microacidity of the albumin environment inside degrading microparticles from poly(lactide-co-glycolide) (PLG) or ABA-triblock polymers containing hydrophobic poly(lactide-co-glycolide) A blocks and hydrophilic poly(ethyleneoxide) B blocks. *Pharm. Res.* 15, 787–793.
- Makino, K., Mogi, T., Ohtake, N., Yoshida, M., Ando, S., Nakajima, T., Ohshima, H., 2000. Pulsatile drug release from poly(lactide-co-glycolide) microspheres: how does the composition of the polymer matrices affect the time interval between the initial burst and the pulsatile release of drugs? *Colloids Surf. B* 19, 173–179.
- Mank, R., Gustafsson, J., Hörig, J., Köchling, W., Nerlich, B., 1996. Microencapsulation of water-soluble medicaments. US Patent 5,503,851 (2 April).
- Mao, S., Shi, Y., Li, L., Xu, J., Schaper, A., Kissel, T., 2008. Effects of process and formulation parameters on characteristics and internal morphology of poly(D,L-lactide-co-glycolide) microspheres formed by solvent evaporation method. *Eur. J. Pharm. Biopharm.* 68, 214–223.
- Martin, A., 1993. *Physical Pharmacy*. Lea & Febiger, Philadelphia, p. 213.
- Martin, T.M., Bandi, N., Shulz, R., Roberts, C.B., Kompella, U.B., 2002. Preparation of budesonide and budesonide-PLA microparticles using supercritical fluid precipitation technology. *AAPS PharmSciTech* 3 (article 18) <http://www.aapspharmstech.org>.
- Maulding, H.V., Tice, T.R., Cowar, D.R., Fong, J.W., Pearson, J.E., Nazareno, J.P., 1986. Biodegradable microcapsules: acceleration of polymeric excipient hydrolytic rate by incorporation of a basic medication. *J. Controlled Release* 3, 103–117.
- Menei, P., Daniel, V., Montero-Menei, C., Brouillard, M., Pouplard-Barthelais, A., Benoit, J.P., 1993. Biodegradation and brain tissue reaction to poly(D,L-lactide-co-glycolide) microspheres. *Biomaterials* 14, 470–478.
- Merck Index, 2006, 14th Edition. Merck Publishing, Rahway.
- Mesens, J., Rickey, M.E., Atkins, T.J., 2003. Microencapsulated 3-Piperidinyl-substituted 1,2-benzisoxazoles and 1,2-benzisothiazoles. US Patent 6,544,559 (8 April).
- Minkov, N.K., Zozikov, B.I., Yaneva, Z., Uldry, P.A., 2001. A phase II trial with new triptorelin sustained release formulations in prostatic carcinoma. *Int. Urol. Nephrol.* 33, 379–383.
- Mishima, K., 2008. Biodegradable particle formation for drug and gene delivery using supercritical fluid and dense gas. *Adv. Drug Del. Rev.* 60, 411–432.
- Mittal, G., Sahana, D.K., Bhardwaj, V., Kumar, M.N.V.R., 2007. Estradiol loaded PLGA nanoparticles for oral administration: effect of polymer molecular weight and copolymer composition on release behavior in vitro and in vivo. *J. Controlled Release* 119, 77–85.
- Miyajima, M., Koshika, A., Okada, J., Kusai, A., Ikeda, M., 1998. The effect of drug physicochemical properties on release from copoly(lactic/glycolic acid) matrix. *Int. J. Pharm.* 169, 255–263.
- Mogi, T., Ohtake, N., Yoshida, M., Chimura, R., Kamaga, Y., Ando, S., Tsukamoto, T., Nakajima, T., Uenodan, H., Otsuka, M., Matsuda, Y., Ohshima, H., Makino, K., 2000. Sustained release of 17 $\beta$ -estradiol from poly(lactide-co-glycolide) microspheres in vitro and in vivo. *Colloids Surf. B* 17, 153–165.
- Mohr, D., Wolff, M., Kissel, T., 1999. Gamma irradiation for terminal sterilization of 17 $\beta$ -estradiol loaded poly(D,L-lactide-co-glycolide) microparticles. *J. Controlled Release* 61, 203–217.
- Montini, M., Pedroncelli, A., Tengattini, F., Pagani, M.D., Gianola, D., Cortesi, L., Lancranjan, I., Pagani, G., 1993. Medical applications of intramuscularly administered bromocriptin microspheres. In: Rolland, A. (Ed.), *Pharmaceutical Particulate Carriers*. Marcel Dekker, New York, pp. 227–274.
- Mu, L., Feng, S.-S., 2001. Fabrication, characterization and in vitro release of paclitaxel (Taxol®) loaded poly(lactic-co-glycolic acid) microspheres prepared by spray drying technique with lipid/cholesterol emulsifiers. *J. Controlled Release* 76, 239–254.
- Mu, L., Teo, M.-M., Ning, H.-Z., Tan, C.-S., Feng, S.-S., 2005. Novel powder formulations for controlled delivery of poorly soluble anticancer drug: application and investigation of TPGS and PEG in spray-dried particulate system. *J. Controlled Release* 103, 565–575.
- Müller, R.H., Schuhmann, R., 1996. *Teilchengrößenmessung in der Laborpraxis*. Wissenschaftliche Verlagsgesellschaft, Stuttgart.
- Muranishi, S., Ikada, Y., Yoshikawa, H., Gen, S., 1991. Polylactic acid microspheres and process for producing the same. US Patent 4,994,281 (19 February).
- Myers-Davit, B., Conner, D.P., 2008. Food effects on drug bioavailability: implications for new and generic drug development. In: Krishna, R., Yu, L. (Eds.), *Biopharmaceutics Application in Drug Development*. Springer, New York, pp. 317–335.
- Na, D.H., Youn, Y.S., Lee, S.D., Son, M.-W., Kim, W.-B., DeLuca, P.P., Lee, K.C., 2003. Monitoring of peptide acylation inside degrading PLGA microspheres by capillary electrophoresis and MALDI-TOF mass spectrometry. *J. Controlled Release* 92, 291–299.
- Nakano, M., Wakiyama, N., Kojima, T., Juni, K., Iwaoku, R., Inoue, S., Yoshida, Y., 1984. Biodegradable microspheres for prolonged local anesthesia. In: Davis, S.S., Illum, L., McVie, J.G., Tomlinson, E. (Eds.), *Microspheres and Drug Therapy*. Pharmaceutical, Immunological and Medical Aspects. Elsevier, Amsterdam, pp. 327–335.
- Nandi, I., Bateson, M., Bari, M., Joshi, H.N., 2003. Synergistic effect of PEG-400 and Cyclodextrin to enhance solubility of progesterone. *AAPS PharmSciTech* 4 (article 1) <http://www.aapspharmstech.org>.
- Nerlich, B., Mank, R., Gustafsson, J., Hörig, J., Köchling, W., 1996. Mikroverkapselung wasserlöslicher Wirkstoffe. European Patent EP 579,347 (24 April).
- Nihant, N., Grandfils, C., Jerome, R., Teyssie, P., 1995. Microencapsulation by coacervation of poly(lactide-co-glycolide) IV. Effect of the processing parameters on coacervation and encapsulation. *J. Controlled Release* 35, 117–125.
- Nuwayser, E.S., Williams, D.L., Meier, P.M., Wu, T.C., Merchant, S.F., Kerrigan, J.H., 1977. A microcapsule system for drug delivery. In: Gabelnick, H.L. (Ed.), *Drug Delivery Systems*. US Department of Health, Education, and Welfare, Washington, DC, pp. 193–251 (USDHEW Pub. No. (NIH) 77-1238).
- Nykamp, G., Carstensen, U., Müller, B.W., 2002. Jet milling—a new technique for microparticle preparation. *Int. J. Pharm.* 242, 79–86.
- Ogawa, Y., Yakamoto, M., Okada, H., Yashiki, T., Shimamoto, T., 1988a. A new technique to efficiently entrap leuprolide acetate into microcapsules of polylactic acid or Copoly(lactic/glycolic) acid. *Chem. Pharm. Bull.* 36, 1095–1103.
- Ogawa, Y., Yakamoto, M., Takada, S., Okada, H., Shimamoto, T., 1988b. Controlled-release of leuprolide acetate from polylactic acid or copoly(lactic/glycolic) acid microcapsules: influence of molecular weight and copolymer ratio of polymer. *Chem. Pharm. Bull.* 36, 1502–1507.
- Ogawa, Y., Okada, H., Yakamoto, M., Shimamoto, T., 1988c. In vivo release profiles of leuprolide acetate from microcapsules prepared with polylactic acids or copoly(lactic/glycolic) acids and in vivo degradation of these polymers. *Chem. Pharm. Bull.* 36, 2576–2581.
- Ogawa, Y., Okada, H., Heya, T., Shimamoto, T., 1989. Controlled release of LHRH agonist, leuprolide acetate, from microcapsules: serum drug level profiles and pharmacological effects in animals. *J. Pharm. Pharmacol.* 41, 439–444.
- Ogawa, Y., 1992. Monthly microcapsule-depot form of LHRH agonist, leuprolide acetate (Enantone® Depot): formulation and pharmacokinetics in animals. *Eur. J. Hosp. Pharm.* 2, 120–127.
- O'Hagan, D.T., Singh, M., 2004. Microparticles as vaccine adjuvants and delivery systems. In: Kaufmann, S.H.E. (Ed.), *Novel Vaccination Strategies*. Wiley, Weinheim, pp. 147–172.
- Okada, H., Ogawa, Y., Yashiki, T., 1987. Prolonged release microcapsule and its production. US Patent 4,652,441 (24 March).



- Okada, H., Ionoue, Y., Ogawa, Y., Toguchi, H., 1992. Three-month release injectable microspheres of leuprolin acetate. *Proc. Intern. Symp. Contr. Rel. Bioact. Mater.* 19, 52–53.
- Okada, H., Yamamoto, M., Heya, T., Inoue, Y., Kamei, S., Ogawa, Y., Toguchi, H., 1994. Drug delivery using biodegradable microspheres. *J. Controlled Release* 28, 121–129.
- Okada, H., 1997. One- and three-month release injectable microspheres of the LH-RH superagonist leuprolide acetate. *Adv. Drug Del. Rev.* 28, 43–70.
- Orsolini, P., 1992. Method for preparing a pharmaceutical composition in the form of microparticles. US Patent 5,134,122 (28 July).
- Orsolini, P., 1993. Pharmaceutical composition in the form of microparticles. US Patent 5,225,205 (6 July).
- Packhaeuser, C.B., Schnieders, J., Oster, C.G., Kissel, T., 2004. In situ forming parenteral drug delivery systems: an overview. *Eur. J. Pharm. Biopharm.* 58, 445–455.
- Panyam, J., Williams, D., Dash, A., Leslie-Pelecky, D., Labhasetwar, V., 2004. Solid-state solubility influences encapsulation efficiency and release of hydrophobic drugs from PLGA/PLA nanoparticles. *J. Pharm. Sci.* 93, 1804–1814.
- Parikh, B.V., Upadrashta, S.M., Neau, S.H., Nuessle, N.O., 1993. Oestrone loaded poly(L-lactic acid microspheres: preparation, evaluation and in vitro release kinetics. *J. Microencapsul.* 10, 141–153.
- Park, T.G., 1995. Degradation of poly(lactic-co-glycolic acid) microspheres: effect of copolymer composition. *Biomaterials* 16, 1123–1130.
- Pavanetto, F., Genta, I., Giunchedi, P., Conti, B., 1993. Evaluation of spray-drying as a method for polylactide and poly(lactide-co-glycolide) microsphere preparation. *J. Microencapsul.* 10, 487–497.
- Pellet, M., Roume, C., 2001. Sustained-release composition and method for preparing the same. US Patent 6,217,893 (17 April).
- Pellet, M., Roume, C., 2002. Sustained release compositions and the process for their preparation. US Patent 6,475,507 (5 November).
- Perings, S.M., Hennersdorf, M., Koch, J.-A., Perings, C., Kelm, M., Heintzen, M.P., Strauer, B.E., 2001. Lipoid pneumonia following attempted suicide by intravenous injection of lamp oil. *Med. Klin.* 96, 685–688 (English abstract, article in German).
- Petersen, H., Ahlheimer, M., 2007. Sustained release formulation comprising octreotide and two or more polylactide-co-glycolide polymers. International Publication WO 2007/071395 (28 June).
- Ph. Eur. (Pharmacopoeia Europea) 4, 2002a. Parenteral Preparations. European Pharmacopoeia Commission, Strasbourg, pp. 548–550.
- Ph. Eur. (Pharmacopoeia Europea) 4, 2002b. Vegetable Fatty Oils. European Pharmacopoeia Commission, Strasbourg, pp. 530–531.
- Ph. Eur. (Pharmacopoeia Europea) 4, 2002c. Triglycerides, Medium-chain. European Pharmacopoeia Commission, Strasbourg, pp. 2071–2073.
- Pitt, C.G., Jeffcoat, A.R., Zweidinger, R.A., Schindler, A., 1979. Sustained drug delivery systems: I. The permeability of poly( $\epsilon$ -caprolactone), poly(DL-lactic acid), and their copolymers. *J. Biomed. Mater. Res.* 13, 497–507.
- Pohanish, R.P., Greene, S.A., 1996. Hazardous Material Handbook. ITP, New York, pp. 1089–1090.
- Pourmortazavi, S.M., Hajimirsadeghi, S.S., 2007. Supercritical fluid extraction in plant essential and volatile oil analysis. *J. Chromatogr. A* 1163, 2–24.
- Rafler, G., Jobmann, M., 1997. Controlled release systems of biodegradable polymers. 5th communication: microparticle preparation by a salting out process. *Pharm. Ind.* 59, 620–627.
- Ramstack, J.M., Riley, M.G., Zale, S.E., Hotz, J.M., Johnson, O.L., 2003. Preparation of injectable suspensions having improved injectability. US Patent 6,667,061 (23 December).
- Rathi, R., Zentner, G.M., Jeong, B., 2001. Biodegradable low molecular weight triblock poly(lactid-co-glycolid) poly-ethylene glycol copolymers having reverse thermal gelation properties. US Patent 6,201,072 (13 March).
- Redding, T.W., Schally, A.V., Tice, T.R., Meyers, W.E., 1984. Long-acting delivery systems for peptides: inhibition of rat prostate tumors by controlled release of [D-Trp6] luteinizing hormone releasing hormone from injectable microcapsules. *Proc. Natl. Acad. Sci. U.S.A.* 81, 5845–5848.
- Reich, G., 1998. Ultrasound induced degradation of PLA and PLGA during microsphere processing: influence of formulation variables. *Eur. J. Pharm. Biopharm.* 45, 165–171.
- Remington, G.J., Adams, M.E., 1995. Depot neuroleptic therapy: clinical considerations. *Can. J. Psychiat.* 40, S5–S11.
- Rickey, M.E., Ramstack, J.M., Lewis, D.H., Mesens, J., 1998. Preparation of extended shelf-life biodegradable, biocompatible microparticles containing a biologically active agent. US Patent 5,792,477 (11 August).
- Rosilio, V., Benoit, J.P., Deyme, M., Thies, C., Madelmont, G., 1991. A physicochemical study of the morphology of progesterone-loaded microspheres fabricated from poly(DL-lactide-co-glycolide). *J. Biomed. Mater. Res.* 25, 667–682.
- Roux, K.H., Teuber, S.S., Sathe, S.K., 2003. Tree nut allergens. *Int. Arch. Allergy Immunol.* 131, 234–244.
- Rouzes, C., Leonard, M., Durand, A., Dellacherie, E., 2003. Influence of polymeric surfactants on the properties of drug-loaded PLA nanospheres. *Colloids Surf. B* 32, 125–135.
- Royals, M.A., Fujita, S.M., Yewey, G.L., Rodriguez, J., Schultheiss, P.C., Dunn, R.L., 1999. Biocompatibility of a biodegradable in situ forming implant system in rhesus monkeys. *Biomed. Mater. Res.* 45, 231–239.
- Ruan, G., Feng, S.-S., 2003. Preparation and characterisation of poly(lactic)-poly(ethylene glycol)-poly(lactic acid) (PLA-PEG-PLA) microspheres for controlled release of paclitaxel. *Biomaterials* 24, 5037–5044.
- Ruiz, J.-M., 1992. Sustained release particles preparation. UK Patent Application Publication GB 2 246 514 (5 February).
- Rungseewijitprapa, W., Brazeau, G.A., Simkins, J.W., Bodmeier, R., 2008. Myotoxicity studies of O/W in situ-forming microparticle systems. *Eur. J. Pharm. Biopharm.* 69, 126–133.
- Sah, H., Smith, M.S., Chern, R.T., 1996. A novel method of preparing PLGA microcapsules utilizing methylethyl ketone. *Pharm. Res.* 13, 360–367.
- Sah, H., 1997. Microencapsulation techniques using ethyl acetate as a dispersed solvent: effect of its extraction rate on the characteristics of PLGA microspheres. *J. Controlled Release* 47, 233–245.
- Sah, H., 1999. Protein behaviour at the water methylene chloride interface. *J. Pharm. Sci.* 88, 1320–1325.
- Sah, H., 2000. Ethyl formate—alternative dispersed solvent useful in preparing PLGA microspheres. *Int. J. Pharm.* 195, 103–113.
- Sah, H., Lee, B.-J., 2006. Development of new microencapsulation techniques useful for the preparation of PLGA microspheres. *Macromol. Rapid Commun.* 27, 1845–1851.
- Salole, E.G., 1986. Estradiol. In: Florey, K. (Ed.), *Analytical Profiles of Drug Substances*, vol. 15. Academic Press, New York, pp. 283–318.
- Sanders, L.M., Kent, J.S., McRae, G.I., Vickery, B.H., Tice, T.R., Lewis, D.H., 1984. Controlled release of a luteinising hormone-releasing hormone analogue from poly(DL-lactide-co-glycolide) microspheres. *J. Pharm. Sci.* 73, 1294–1297.
- Sansdrap, P., Moës, A.J., 1993. Influence of manufacturing parameters on the size characteristics and the release profiles of nifedipine from poly(DL-lactide-co-glycolide) microspheres. *Int. J. Pharm.* 98, 157–164.
- Sansdrap, P., Moës, A.J., 1997. In vitro evaluation of the hydrolytic degradation of dispersed and aggregated poly(DL-lactide-co-glycolide) microspheres. *J. Controlled Release* 43, 47–58.
- Sansdrap, P., Fontaine, J., Moës, A.J., 1999. Nifedipine-loaded PLGA microspheres: in vitro/in vivo comparison of drug release and polymer degradation. *S. T. P. Pharm. Sci.* 9, 443–446.
- Savage, J.H., Limb, S.L., Brereton, N.H., Wood, R.A., 2007. The natural history of peanut allergy: extending our knowledge beyond childhood. *J. Allergy Clin. Immunol.* 120, 717–719.
- Schalper, K., Harnisch, S., Müller, R.H., Hildebrandt, G.E., 2005. Preparation of microparticles by micromixers—characterisation of o/w process and prediction of particle size. *Pharm. Res.* 22, 276–284.
- Schuchmann, H.P., Danner, T., 2004. Emulsification: more than just communication. *Chem. Ing. Tech.* 76, 364–375.
- Schwendeman, S.P., Gupta, R.K., Siber, G.R., Langer, R., 1995. Pathways of inactivation of tetanus toxoid in the presence of PLA 2000. Presentation at the AAPS meeting, Miami Beach, Florida, USA; abstract reprinted in *Pharm. Res.* 12, S-80.
- Schwendeman, S.P., Cardamone, M., Klibanov, A., Langer, R., Brandon, M.R., 1996. Stability of proteins and their delivery from biodegradable polymer microspheres. In: Cohen, S., Bernstein, H. (Eds.), *Microparticulate Systems for the Delivery of Proteins and Vaccines*. Marcel Dekker, New York, pp. 1–49.
- Schwendeman, S.P., Costantino, H.R., Gupta, R.K., Langer, R., 1997. Progress and challenges for peptide, protein, and vaccine delivery from implantable polymeric systems. In: Park, K. (Ed.), *Controlled Drug Delivery: Challenges and Strategies*. The American Chemical Society, Washington, DC, pp. 229–267.
- Shah, N.H., Raikar, A.S., Chen, F.C., Tarantino, R., Kumar, S., Murjani, M., Palmer, D., Infeld, M.H., Malick, A.W., 1993. A biodegradable injectable implant for delivering micro and macromolecules using poly (lactic-co-glycolic) acid (PLGA) copolymers. *J. Controlled Release* 27, 139–147.
- Shameem, M., Lee, H., DeLuca, P.P., 1999. A short-term (accelerated release) approach to evaluate peptide release from PLGA depot formulations. *AAPS PharmSci* 1 (article 7) <http://www.aapsj.org/>.
- Shareef, A., Angove, M.J., Wells, J.D., Johnson, B.B., 2006. Aqueous solubility of estrone, 17 $\beta$ -estradiol, 17 $\alpha$ -ethynylestradiol, and bisphenol A. *J. Chem. Eng. Data* 51, 879–881.
- Shenderova, A., Burke, T.G., Schwendeman, S.P., 1997. Stabilisation of 10-Hydroxycamptothecin in poly(lactide-co-glycolide) microsphere delivery vehicles. *Pharm. Res.* 14, 1406–1414.
- Shenderova, A., Burke, T.G., Schwendeman, S.P., 1999. The acidic microclimate in poly(lactide-co-glycolide) microspheres stabilizes camptothecins. *Pharm. Res.* 16, 241–248.
- Shimamoto, T., 1987. Pharmaceutical aspects: nasal and depot formulations of Leuprolide. *J. Androl.* 8, S14–16.
- Sicherer, S.H., Sampson, H.A., 2007. Peanut allergy: emerging concepts and approaches for an apparent epidemic. *J. Allergy Clin. Immunol.* 120, 491–503.
- Siegel, S.J., Kahn, J.B., Metzger, K., Winey, K.L., Werner, K., Dan, N., 2006. Effect of drug type on the degradation rate of PLGA matrices. *Eur. J. Pharm. Biopharm.* 64, 287–293.
- Simmons, A., Rouf, E., Whittle, J., 2007. Not your typical pneumonia: a case of exogenous lipid pneumonia. *J. Gen. Intern. Med.* 22, 1613–1616.
- Sinha, V.R., Trehan, A., 2003. Biodegradable microspheres for protein delivery. *J. Controlled Release* 90, 261–280.
- Smith, A., Hunnyball, I.M., 1986. Evaluation of poly(lactic acid) as a biodegradable drug delivery system. *Int. J. Pharm.* 30, 215–220.
- Soppimath, K.S., Aminabhavi, T.M., 2002. Ethyl acetate as a dispersing solvent in the production of poly(DL-lactide-co-glycolide) microspheres: effect of process parameters and polymer type. *J. Microencapsul.* 19, 281–292.
- Splenhauer, G., Veillard, M., Benoit, J.-P., 1986. Formation and characterization of cis-platin loaded poly(DL-lactide) microspheres for chemoembolization. *J. Pharm. Sci.* 75, 750–755.



- Straub, J.A., Chickering, D.E., Lovely, J.C., Zhang, H., Shah, B., Waud, W.R., Bernstein, H., 2005. Intravenous hydrophobic drug delivery: a porous particle formulation of paclitaxel (Al-850). *Pharm. Res.* 22, 347–355.
- Suzuki, K., Price, J.C., 1985. Microencapsulation and dissolution properties of a neuroleptic in a biodegradable polymer, poly(D,L-lactide). *J. Pharm. Sci.* 74, 21–24.
- Tadros, T.F., 2005. Applications of surfactants in emulsion formation and stabilisation. In: *Applied Surfactants: Principles and Applications*. Wiley-VCH, Weinheim, pp. 115–185 (Chapter 6).
- Takechi, N., Ohtani, S., Nagai, A., 2002. Production of microspheres. European Patent EP 779 072 (26 June).
- Tamber, H., Johansen, P., Merkle, H.P., Gander, B., 2005. Formulation aspects of biodegradable polymeric microspheres for antigen delivery. *Adv. Drug Del. Rev.* 57, 357–376.
- Tang, H., Xu, N., Meng, J., Wang, C., Nie, S.-F., Pan, W.-S., 2007. Application of a novel approach to prepare biodegradable poly(lactic-co-glycolic acid) microspheres: surface liquid spraying. *Yakugaku Zasshi* 127, 1851–1862.
- Taylor, H.F., 1938. Vitamin preparations and methods of making same. US Patent 2,183,053 (12 December).
- Taylor, S.L., Busse, W.W., Sachs, M.I., Parker, J.L., Yunginger, J.W., 1981. Peanut oil is not allergenic to peanut-sensitive individual. *J. Allergy Clin. Immunol.* 68, 372–375.
- Tewes, F., Boury, F., Benoit, J.-P., 2006. Biodegradable microspheres: advances in production technology. In: Benita, S. (Ed.), *Microencapsulation. Methods and Industrial Applications*, 2nd Ed. Taylor & Francis, New York, pp. 1–53.
- Thies, C., 1991. Formation of biodegradable drug-loaded microparticles by in-liquid drying processes. In: Donbrow, M. (Ed.), *Microcapsules and Nanoparticles in Medicine and Pharmacy*. CRC Press, Boca Raton, pp. 47–71.
- Thomasi, C., Merkle, H.P., Gander, B.A., 1997. Physico-chemical parameters governing protein microencapsulation into biodegradable polyesters by coacervation. *Int. J. Pharm.* 147, 173–186.
- Thomasi, C., Nam-Tran, H., Merkle, H.P., Gander, B., 1998a. Drug Microencapsulation by PLA/PLGA coacervation in the light of thermodynamics. 1. Overview and theoretical considerations. *J. Pharm. Sci.* 87, 259–268.
- Thomasi, C., Merkle, H.P., Gander, B., 1998b. Drug Microencapsulation by PLA/PLGA coacervation in the light of thermodynamics. 2. Parameters determining microsphere formation. *J. Pharm. Sci.* 87, 269–275.
- Thote, A.J., Chappell, J.T., Kumar, R., Gupta, R.B., 2005. Reduction in the initial burst release by surface crosslinking of PLGA microparticles containing hydrophilic or hydrophobic drugs. *Drug Dev. Ind. Pharm.* 31, 43–57.
- Thummel, K.E., Kunze, K.L., Shen, D.D., 1997. Enzyme-catalyzed processes of first-pass hepatic and intestinal drug. *Adv. Drug Del. Rev.* 27, 99–127.
- Tice, T.R., Lewis, D.H., 1983. Microencapsulation process. US Patent 4,389,330 (21 June).
- Tice, T.R., Gilley, R.M., 1985. Preparation of injectable controlled release microcapsules by a solvent evaporation process. *J. Controlled Release* 2, 343–352.
- Tipton, A.J., Fujita, S.M., 1991. A biodegradable, injectable delivery system for nonsteroidal anti-inflammatory drugs. *Pharm. Res.* 8, S-196.
- Tipton, A.J., 1999. High viscosity liquid controlled delivery system as a device. US Patent 5,968,542 (19 October).
- Tong, W.-Q., 2008. Molecular and biochemical aspects impacting oral absorption of drugs. In: Krishna, R., Yu, L. (Eds.), *Biopharmaceutics Application in Drug Development*. Springer, New York, pp. 317–335.
- Tracy, M.A., 1998. Development and scale-up of a microsphere protein delivery system. *Biotechnol. Prog.* 14, 108–115.
- Tracy, M.A., Ward, K.L., Firouzabadian, L., Wang, Y., Dong, N., Qian, R., Zhang, Y., 1999. Factors affecting the degradation rate of poly(lactide-co-glycolide) microspheres in vivo and in vitro. *Biomaterials* 20, 1057–1063.
- Tsakala, M., Gillard, J., Roland, M., Chabot, F., Vert, M., 1988. Pyrimethamine sustained release systems based on bioresorbable polyesters for chemoprophylaxis of rodent malaria. *J. Controlled Release* 5, 233–242.
- Türk, M., Lietzow, R., 2004. Stabilized nanoparticles of phytosterol by rapid expansion from supercritical solution into aqueous solution. *AAPS PharmSciTech* 5 (article 56) <http://www.aapspharmsci.org>.
- Tuncay, M., Calis, S., Kas, H.S., Ercan, M.T., Peksoy, I., Hincal, A.A., 2000. Diclofenac sodium incorporated PLGA (50:50) microspheres: formulation considerations and in vitro/in vivo evaluation. *Int. J. Pharm.* 195, 179–188.
- Tzafiriri, A.R., Lerner, E.L., Flashner-Narak, M., Hinchcliffe, M., Ratner, E., Parnas, H., 2005. Mathematical modeling and optimization of drug delivery from intratumorally injected microspheres. *Clin. Cancer Res.* 11, 826–834.
- USCG (United States Coast Guard), 1999. Chemical Hazards Response Information System. Methyl dichloroacetate datasheet. Downloaded from <http://www.chrismanual.com/findform.htm> on January 25, 2008.
- USP (United States Pharmacopoeia)—in-process revision, 2006. Lipid injectable formulations. *Pharmacopeial forum*, 32, pp. 350–352.
- USP (United States Pharmacopoeia) 30, 2007. (1) Injections. *US Pharmacopeial Convention*, Rockville, pp. 33–36.
- van Apeldoorn, A.A., van Manen, H.-J., Bezemer, J.M., de Bruijn, J.D., van Blitterswijk, A.C., Otto, C., 2004. Raman imaging of PLGA microsphere degradation inside macrophages. *J. Am. Chem. Soc.* 126, 13226–13227.
- Varma, M.V.S., Perumal, O.P., Panchagnula, R., 2006. Functional role of P-glycoprotein in limiting peroral drug absorption: optimizing drug delivery. *Curr. Opin. Chem. Biol.* 10, 367–373.
- Vatanara, A., Najafabadi, A.R., Khajeh, M., Yamini, Y., 2005. Solubility of some inhaled glucocorticoids in supercritical carbon dioxide. *J. Supercrit. Fluid.* 33, 21–25.
- Veber, D.F., Johnson, S.R., Cheng, H.-Y., Smith, B.R., Ward, K.W., Kopple, K.D., 2002. Molecular properties that influence the oral bioavailability of drug candidates. *J. Med. Chem.* 45, 2615–2625.
- Vert, M., Coudane, J., Ustariz, C., Schwach, G., 1993. Bioresorbable polymer microspheres free from surface active agents, their preparation and application as drug. International Publication WO 93/25191 (23 December).
- Voigt, V., 2006. Biodegradable in situ forming systems and sponge-like implants. Ph.D. Thesis. Freie Universität Berlin, Germany. Available at <http://www.diss.fu-berlin.de/2006/284/>.
- von Burkersroda, F., Schedl, L., Göpferich, A., 2002. Why degradable polymers undergo surface erosion or bulk erosion. *Biomaterials* 23, 4221–4231.
- Vrancken, M.N., Claeys, D.A., 1970a. Process for encapsulating water and compounds in aqueous phase by evaporation. US Patent 3,523,906 (11 August).
- Vrancken, M.N., Claeys, D.A., 1970b. Process for encapsulating water and compounds in aqueous phase by extraction. US Patent 3,523,907 (11 August).
- Wada, R., Hyon, S.-H., Ikada, Y., Nakao, Y., Yoshikawa, H., Muranishi, S., 1988. Lactic acid oligomer microspheres containing an anticancer agent for selective lymphatic delivery: I. In vitro studies. *J. Bioact. Compat. Polym.* 3, 126–136.
- Wada, R., Hyon, S.-H., Ikada, Y., 1990. Lactic acid oligomer microspheres containing hydrophilic drugs. *J. Pharm. Sci.* 79, 919–924.
- Wagenaar, B.W., Müller, B.W., 1994. Piroxicam release from spray-dried biodegradable microspheres. *Biomaterials* 15, 49–54.
- Wakiyama, N., Juni, K., Nakano, M., 1981. Preparation and Evaluation in vitro of poly(lactic acid) microspheres containing local anesthetics. *Chem. Pharm. Bull.* 29, 3363–3368.
- Wakiyama, N., Juni, K., Nakano, M., 1982a. Influence of physicochemical properties of poly(lactic acid) on the characteristics and in vitro release patterns of poly(lactic acid) microspheres containing local anesthetics. *Chem. Pharm. Bull.* 30, 2621–2626.
- Wakiyama, N., Juni, K., Nakano, M., 1982b. Preparation and evaluation of in vitro and in vivo of poly(lactic acid) microspheres containing dibucaine. *Chem. Pharm. Bull.* 30, 3719–3727.
- Wan, L.S.C., Lee, P.F.S., 1974. CMC of polysorbates. *J. Pharm. Sci.* 63, 136–137.
- Wang, Y.M., Sato, H., Adachi, I., Horikoshi, I., 1996. Preparation and characterization of poly(lactic-co-glycolic acid) microspheres for targeted delivery of a novel anticancer agent, taxol. *Chem. Pharm. Bull.* 44, 1935–1940.
- Wang, Y.M., Sato, H., Horikoshi, I., 1997. In vitro evaluation of taxol release from poly(lactic-co-glycolic acid) microspheres containing isopropyl myristate and degradation of microspheres. *J. Controlled Release* 49, 157–166.
- Wang, J., Schwendeman, S.P., 1999. Mechanisms of solvent evaporation encapsulation processes: prediction of solvent evaporation rate. *J. Pharm. Sci.* 88, 1090–1099.
- Wang, J., Wang, B.M., Schwendeman, S.P., 2002. Characterization of the initial burst release of a model peptide from poly(D,L-lactide-co-glycolide) microspheres. *J. Controlled Release* 82, 289–307.
- Wang, J., Wang, B.M., Schwendeman, S.P., 2004. Mechanistic evaluation of the glucose-induced reduction in initial burst release of octreotide acetate from poly(D,L-lactide-co-glycolide). *Biomaterials* 25, 1919–1927.
- Wang, S.H., Zhang, L.C., Lin, F., Sa, X.Y., Zuo, J.B., Shao, Q.X., Chen, G.S., Zeng, S., 2005. Controlled release of levonorgestrel from biodegradable poly(D,L-lactide-co-glycolide) microspheres: in vitro and in vivo studies. *Int. J. Pharm.* 31, 217–225.
- Whitaker, M.J., Hao, J., Davies, O.R., Serhatkulu, G., Stolnik-Trenkic, S., Howdle, S.M., Shakesheff, K.M., 2005. The production of protein-loaded microparticles by supercritical fluid enhanced mixing and spraying. *J. Controlled Release* 101, 85–92.
- Wichert, B., Rohdewald, P., 1990. A new method for the preparation of drug containing poly(lactic acid) microparticles without using organic solvents. *J. Controlled Release* 14, 269–282.
- Wiggins, J.S., Hassan, M.K., Mauritz, K.A., Storey, R.F., 2006. Hydrolytic degradation of poly(D,L-lactide) as a function of end group: carboxylic acid vs. hydroxyl. *Polymer* 47, 1960–1969.
- Williams, J.R., Clifford, A.A., Al-Saidi, S.H.R., 2002. Supercritical fluids and their applications in biotechnology and related areas. *Mol. Biotechnol.* 22, 263–286.
- Wischke, C., Lorenzen, D., Zimmermann, J., Borchert, H.-H., 2006. Preparation of protein loaded poly(D,L-lactide-co-glycolide) microparticles for the antigen delivery to dendritic cells using a static micromixer. *Eur. J. Pharm. Biopharm.* 62, 247–253.
- Witschi, C., Doelker, E., 1998. Influence of the microencapsulation method and peptide loading on poly(lactic acid) and poly(lactic-co-glycolic acid) degradation during in vitro testing. *J. Controlled Release* 51, 327–341.
- Wright, S.G., Rickley, M.E., Ramstack, J.M., Lyons, S.L., Hotz, J.M., 2001. Method for preparing microparticles having a selected polymer molecular weight. US Patent 6,264,987 (11 August).
- Wu, X.S., 1995a. Synthesis and properties of biodegradable lactic/glycolic acid polymers. In: Wise, D., Trantolo, D.J., Altobelli, D.E., Yaszems, M.J. (Eds.), *Encyclopedic Handbook of Biomaterials and Bioengineering: Materials/Applications*. Marcel Dekker, New York, pp. 1015–1054 (Chapter 29).
- Wu, X.S., 1995b. Preparation, characterization, and drug delivery application of microspheres based biodegradable lactic/glycolic acid polymers. In: Wise, D., Trantolo, D.J., Altobelli, D.E., Yaszems, M.J. (Eds.), *Encyclopedic Handbook of Biomaterials and Bioengineering: Materials/Applications*. Marcel Dekker, New York, pp. 1151–1200 (Chapter 32).
- Wurster, D.E., Polli, G.P., 1961. Investigation of drug release from solids IV: influence of adsorption on the dissolution rate. *J. Pharm. Sci.* 50, 403–406.

- Xie, M., Zhou, L., Hu, T., Yao, M., 2007. Intratumoral delivery of paclitaxel-loaded poly(lactic-co-glycolic acid) microspheres for Hep-2 laryngeal squamous cell carcinoma xenografts. *Anti-Cancer Drugs* 18, 459–466.
- Yamamoto, M., Takada, S., Ogawa, Y., 1994. Sustained release microcapsule. US Patent 5,330,767 (19 July).
- Yang, J., Cleland, J.L., 1997. Factors effecting the in vitro release of recombinant human interferon- $\gamma$  (rhIFN- $\gamma$ ) from PLGA microspheres. *J. Pharm. Sci.* 86, 914–980.
- Yang, Q., Reams, R., Owusu-Ababio, G., 1999. Effect of solvent composition during preparation on the characteristics of enoxacin microparticles. *J. Pharm. Pharmacol.* 51, 659–665.
- Yang, Q., Owusu-Ababio, G., 2000. Biodegradable progesterone microsphere delivery system for osteoporosis therapy. *Drug Dev. Ind. Pharm.* 26, 61–70.
- Yolles, S., Leafe, T.D., Woodland, J.H.R., Meyer, F.J., 1975. Long acting delivery systems for narcotic antagonists II: release rates of naltrexone from poly(lactic acid) composites. *J. Pharm. Sci.* 64, 348–349.
- Yolles, S., Sartori, M.F., 1980. Degradable polymers for sustained drug release. In: Juliano, R.L. (Ed.), *Drug Delivery Systems: Characteristics and Biomedical Applications*. Oxford University Press, Oxford, pp. 84–111.
- Yoshikawa, H., Nakao, Y., Takada, K., Muranishi, S., Wada, R., Tabata, Y., Hyon, S.-H., Ikada, Y., 1989. Targeted and sustained delivery of aclarubicin to lymphatics by lactic acid-oligomer microsphere in rat. *Chem. Pharm. Bull.* 37, 802–804.
- Young, J.L., Zonana, H.V., Shepler, L., 1984. Medication non-compliance in schizophrenia: codification and update. *Bull. Am. Acad. Psych. Law* 14, 105–122.
- Zaghloul, A.-A.A., Mustafa, F., Siddiqui, A., Khan, M., 2005. Biodegradable microparticulates of beta-estradiol: preparation and in vitro characterization. *Drug Dev. Ind. Pharm.* 31, 803–811.
- Zaghloul, A.-A.A., Mustafa, F., Siddiqui, A., Khan, M., 2006. Response surface methodology to obtain  $\beta$ -estradiol biodegradable microspheres for long-term therapy of osteoporosis. *Pharm. Dev. Technol.* 11, 377–387.
- Zdenek, B., Kissel, T., 1986. Polyolester, deren Herstellung und Verwendung. Swiss Patent CH 656 884 (31 July).
- Zdenek, B., Kissel, T., 1989. Polyolester, deren Herstellung und Verwendung. Swiss Patent CH 672 133 (31 March).
- Zentner, G.M., Rathi, R., Shih, C., McRea, J.C., Seo, M.H., Oh, H., Rhee, B.G., Mestecky, J., Moldoveanu, Z., Morgan, M., Weitman, S., 2001. Biodegradable block copolymers for delivery of proteins and water-insoluble drugs. *J. Controlled Release* 72, 203–215.
- Zhang, Y., Zale, S., Sawyer, L., Bernstein, H., 1997. Effect of metal salts on poly(DL-lactide-co-glycolide) polymer hydrolysis. *J. Biomed. Mater. Res.* 34, 531–538.
- Zhifang, Z., Mingxing, Z., Shenghao, W., Fang, L., Wenzhao, S., 1993. Preparation and evaluation in vitro and in vivo of copoly(lactic/glycolic) acid microspheres containing norethisterone. *Biomater. Art. Cells Immob. Biotech.* 21, 71–84.
- Zhou, T., Lewis, H., Foster, R.E., Schwendeman, S.P., 1998. Development of a multiple-drug delivery implant for intraocular management of proliferative vitreoretinopathy. *J. Controlled Release* 55, 281–295.
- Zhou, Y., Wu, X.Y., 2003. Modeling and analysis of dispersed-drug release into a finite medium from sphere ensembles with a boundary layer. *J. Controlled Release* 90, 23–36.