Structure formation and characterization of injectable drug loaded biodegradable devices: In situ implants versus in situ microparticles

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ABSTRACT

The objective of the study was to investigate key formulation variables affecting the release of bupivacaine hydrochloride, a local anesthetic, from different in situ forming biodegradable drug delivery devices. The formulations included ISM systems [in situ microparticles, a poly(lactide)–solvent phase dispersed into an external oil phase] and poly(lactide) solutions (in situ implant systems). The solubility of the biodegradable polymer poly(ε,δ-lactide) (PLA) in various organic solvents was determined using the Hansen multicomponent solubility parameter concept. The solvent release from ISM and polymer solutions into phosphate buffer which influences the polymer precipitation rate was investigated as a function of the type of solvent, polymer concentration and polymer:oil phase ratio by using a HPLC assay. Scanning electron microscopy (SEM) was performed in order to relate the drug release to the surface properties of the precipitated implants or microparticles. Suitable solvents for the preparation of the in situ forming drug delivery systems, such as N-methyl-2-pyrrolidone (NMP), dimethylsulfoxide (DMSO) and 2-pyrrolidone were found using the Hansen multicomponent solubility parameter concept. The injection of the polymer solutions (in situ implants) into the aqueous medium led to a rapid solvent/non-solvent exchange. The resulting in situ implants were porous, thus explaining the rapid initial drug release. Upon contact with the release medium, the internal polymer phase of the ISM system solidified and formed microparticles as shown by SEM measurements. Due to the presence of an external oil phase the solvent release into the buffer medium from ISM was significantly slower compared to the polymer solutions. The solvent release of the ISM systems into the phosphate buffer decreased with increasing polymer concentration and decreasing polymer:oil phase ratio. The type of solvent used also affected the solvent release. A slower solvent release into the aqueous medium resulted in less porous microparticles, thus explaining the reduced initial drug release from ISM systems compared to the polymer solutions.

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1. Introduction

For the past 30 years, controlled release technology has emerged as an important field in the development of pharmaceutical dosage forms. Various intramuscular or subcutaneous controlled drug delivery systems (DDS) in the form of implants or microparticles have been developed. These systems are mainly based on biodegradable polymers such as poly(ε,δ-lactide) (PLA). The objective of the study was to investigate key formulation variables affecting the release of bupivacaine hydrochloride, a local anesthetic, from different in situ forming biodegradable drug delivery devices.
as poly(\(L, L\)-lactide) (PLA) and poly(\(D, L\)-lactide-co-glycolide) (PLGA) (Brannon-Peppas, 1995). The degradation of the polymer can be controlled by the monomer ratio and the molecular weight and can be varied between weeks to months (Lewis, 1990).

Various microencapsulation or extrusion techniques are available to form drug–polymer composites in the form of microcapsules or implants (Benoit et al., 1996; Rothen-Weinhold et al., 1998). Implants are formed by processes like melt extrusion, melt compression or injection molding. Problems of implants include elevated process temperatures, poor content uniformity, possibly irreproducible drug release patterns (as they are single-unit dosage forms), and the requirement of surgery. Biodegradable microparticles (multiple unit dosage forms) have been developed to overcome problems associated with implants. They are prepared by solvent evaporation, organic phase separation, spray-drying or supercritical fluid technology (Benoit et al., 1996). Drawback of these devices is that the preparation is based on complicated, multiple step processes with many process and formulation parameters to be controlled (Jail and Nixon, 1990).

As an alternative to solid implant or microparticle formulations in situ implants have been developed (Dunn et al., 1990; Graham et al., 1999; Hatefi and Amsden, 2002; Wang et al., 2003; Packhaueuser et al., 2004). For these systems PLA or PLGA are dissolved in water miscible solvents, such as NMP or DMSO. Upon injection of the drug containing polymer solution into an aqueous medium or the body tissue, the non-water soluble polymer precipitates due to solvent/non-solvent exchange. Disadvantages of these in situ implant systems (polymer solutions) are the initial rapid release prior to solidification of the polymer, difficult injectability of the highly viscous polymer solution and questions regarding the myotoxicity of the organic solvent used (Kranz et al., 2001). To decrease the high initial drug release in situ implants based on hydrophobic solvents (PLGA in triacetin or ethyl benzoate) have been proposed (Brodbeck et al., 1999). However these systems are still single-unit drug delivery devices based on high amounts of organic solvents.

As an alternative to microparticles or in situ implants systems, a novel in situ forming microparticle system (ISM) has been developed (Kranz and Bodmeier, 2007; Kranz et al., 2008). These ISM systems consist of an internal, drug containing polymer–solvent phase (polymer phase) emulsified into an external phase (for example an oil phase). Upon injection of this emulsion, the internal polymer phase precipitates and forms microparticles. Solvents for the polymers are for example NMP, DMSO or 2-pyrrolidone. Peanut oil can be chosen as a biocompatible external oil phase. The ISM systems have a significantly reduced myotoxicity compared to the in situ forming implants (Kranz et al., 2001). In addition, the preparation process for ISM is simple, when compared with classical techniques for the preparation of microparticles.

Feasibility of the ISM technique was demonstrated in vitro on structurally diverse molecules such as diltiazem hydrochloride and buserelin acetate (Kranz and Bodmeier, 2007). For both molecules the initial drug release from ISM was significantly reduced when compared to in situ implants. In vitro release profiles of diltiazem hydrochloride and buserelin acetate from ISM were comparable to drug release profiles from microparticles prepared by the solvent evaporation technique. In contrast to the low molecular weight diltiazem hydrochloride, the peptide release (buserelin acetate) was strongly dependent on the polymer degradation.

In vivo studies with in situ microparticles were carried out in rats (Kranz et al., 2008). Again, an advantage of the ISM system compared to in situ implants was the significantly reduced burst effect. This resulted in pharmacokinetic drug release profiles that were comparable to microparticles prepared by conventional methods. Furthermore, with the ISM system the pharmacodynamic effect of a local anesthetic was prolonged compared to the injection of a drug solution or in situ implant as shown in a hot plate model.

The objective of this study was to characterize possible biocompatible solvents for the in situ technology by using the Hansen multicomponent solubility parameter concept, to relate the drug release profiles of polymer solutions and ISM to the precipitation rate of the polymers and to the viscosities of the polymer phases. The polymer precipitation rate was studied by investigating the solvent release from polymer solutions and ISM using a HPLC-assay. Capillary viscosity and rheometer stress measurements were performed on diluted as well as on concentrated polymer solutions. Scanning electron microscopy (SEM) was performed in order to relate the drug release to the surface properties of the precipitated implants or microparticles.

2. Experimental

2.1. Materials

The following chemicals were obtained from commercial suppliers and used as received: poly(\(D, L\)-lactide) (PLA, R 203, MW 25,700, Boehringer Ingelheim, Ingelheim, Germany), dimethylsulfoxide (DMSO), acetonitrile (Merck, Darmstadt, Germany), 2-pyrrolidone (Soluphor®), Fluorochem F 68 (BASF AG, Ludwigshafen, Germany), peanut oil (Henry Lamotte GmbH, Bremen, Germany), aluminum-monostearate (Fluka Chemie AG, Buchs, Swiss), bupivacaine hydrochloride, \(N\)-methyl-2-pyrrolidone (NMP, Sigma Aldrich Company, St. Louis, USA). All chemicals were at least reagent grade.

2.2. Methods

2.2.1. Preparation of the in situ forming drug delivery systems

In situ implants (polymer solutions) were prepared by mixing PLA with the solvents (2-pyrrolidone, NMP or DMSO) (polymer phase) into a peanut oil phase (oil phase) at a polymer to oil phase ratio of 1:1, 0.5:1, 0.25:1 and 0.1:1 by probe sonication (Bandelin Sonopuls HD 200, Bandelin electronic, Berlin, Germany). The polymer concentration was
varied between 0 and 40% PLA (w/w, based on amount of solvent and polymer). Pluronic F 68 (1% w/w, based on the amount of the total formulation) was dissolved in the polymer phase and aluminum monostearate (2% w/w, based on the peanut oil) in the oil phase to increase the stability of the emulsions. For the preparation of the bupivacaine hydrochlo-
ride containing ISM systems, 10% drug (based on the weight of the polymer) was dissolved in the polymer phase.

2.2.2. Polymer solubility
The solubility of the polymer in various organic solvents was determined at room temperature. Dried polymer (200 mg) was added to glass vials containing 5 ml of the solvent. The vials were sealed with aluminum foil and agitated for at least 48 h at room temperature in a horizontal shaker. Solubility of the polymer in a particular solvent was indicated by clear solutions. All samples were weighed before and after each experiment to check for any potential solvent loss. Samples exceeding a 1% solvent loss were repeated.

Multicomponent solubility parameters have been intro-
duced by Hansen (1967) to predict the solubility of polymers. He divided the total solubility parameter (δ) into contributions from dispersion forces (δd), polar interactions (δp) and hydrogen bonding (δh):

\[ \delta = \delta_d + \delta_p + \delta_h \]

A triangular plotting technique of the Hansen parameters was developed by Teas (1968). The following equation was used to calculate the fractional solubility parameters:

\[ F_i = \frac{100\bar{h}_i}{(\delta_d + \delta_p + \delta_h)} \text{ where } i = d, p \text{ or } h \]

The fractional parameters \( F_i \) represents the coordinates of a triangular chart. The solubility envelope was constructed graphically from solvents dissolving the polymer. The boundary regions of the solubility envelope were established by increasing the non-solvent concentration in a binary mixture until formation of a cloudy solution. The values of the solubility parameter and Hansen's partial solubility parameter were taken from the literature (Barton, 1975).

2.2.3. Drug and solvent release studies
ISM systems and in situ implants were placed into dialysis bags (Medicell International Ltd., London, England; MW cut-off 12–14,000 Da) \( n = 3 \). The bags were placed into 50 ml phosphate buffer (pH 7.4 for the solvent release studies and pH 4.0 for the drug release studies) containing 0.05% (w/v) sodium azide as a preservative. The release studies were performed at 37 °C in a horizontal shaker (GFL 3033, Gesellschaft für Labortechnik, Burgwedel, Germany). At predetermined time intervals, 2 ml samples (which were replaced with fresh medium) were withdrawn and assayed. The bupivacaine hydrochloride content was measured with a microcomputer connected Shimadzu-HPLC system (SCL-10 A System Controller, LC-10 A pump, DGU-3 A degasser, SIL-10 A auto injector, SDS-10AV UV-detector, Class-LC 10 software, Shimadzu, Kyoto, Japan). A 40 µl volume was injected onto a LiChrospher-100 RP 18.5 µm vertex column (Knauer GmbH, Berlin, Germany) using as the mobile phase a mixture of 60 ml acetonitrile and 40 ml of phosphate buffer pH 4.0; flow rate: 1.0 ml/min; 40 °C; UV-detection at 220 nm. Bupivacaine hydrochloride solutions of known concentration (0.5–100 µg/ml) were used to generate calibration curves. The method was checked with respect to linearity \( r > 0.99 \), sensitivity (0.25 µg/ml), precision (1.2% R.S.D.) and accuracy (approx. 4.6% R.S.D.). The solvents (DMSO, NMP or 2-pyrrolidone) were measured with the computer connected Shimadzu-HPLC system at 220 nm and room temperature. A 40 µl volume was injected onto a LiChrospher-100 RP 18.5 µm vertex column (Knauer GmbH, Berlin, Germany) using as the mobile phase an acetonitrile/phosphate buffer mixture (10:90, v/v) at a flow rate of 1.0 ml/min. Solutions of DMSO, NMP or 2-pyrrolidone in phosphate buffer pH 7.4 of known concentrations were used to generate calibration curves. The method was checked with respect to linearity \( r > 0.99 \), sensitivity (2 µg/ml), precision (<1.3% R.S.D.) and accuracy (<5% R.S.D.).

2.2.4. Solubility of the drugs
Excess amount of bupivacaine hydrochloride was placed in 1 ml pH 4.0 buffer medium \( n = 3 \). The samples were shaken for 48 h at 37 °C using a horizontal shaker (GFL 3033, Gesellschaft für Labortechnik, Burgwedel, Germany). The saturated drug solution was filtered and then assayed after appropriate dilution as described above. The final pH of the saturated solutions in the phosphate buffer was adjusted to 4.0.

2.2.5. Miscibility studies
Two milliliters peanut oil were saturated with excess amount of the solvents (DMSO, 2-pyrrolidone, NMP) by shaking at room temperature for 48 h using a horizontal shaker (HS 501 Digital, IKA-Labortechnik, Staufen, Germany) \( n = 3 \). The peanut oil phase (1.5 ml) was withdrawn, 30 ml phosphate buffer pH 7.4 were added and shaken for 48 h. The amount of solvent in the buffer medium was determined after appropriate sample dilution by using the HPLC assay as described above. The recovery of the solvents from the peanut oil phase in the phosphate buffer was 98.6% in the range of 10–250 mg solvent/ml phosphate buffer.

2.2.6. Viscosity measurements
The viscosities of diluted polymer solutions were obtained by using capillary viscometers (size 0C and IA, Schott-Gerate GmbH, Hofheim, Germany) at 25 ± 0.5 °C. Viscosities in the concentration range of 0.100–4.000 g/dl of PLA in NMP, DMSO and 2-pyrrolidone were measured after shaking the solutions for 48 h. The relative viscosity was calculated as \( \eta_r = \eta/\eta_0 = t_{corrected}/t_{0corrected} \) where \( \eta \) and \( \eta_0 \) are the viscosities of the polymer solutions and the solvents and \( t \) and \( t_0 \) the corrected flow times of the polymer solutions and the solvents. Corrections of the flow times were performed according to the Ubbelohde Viscometer operating instructions for a given capillary. Flow times exceeded 240 s in all cases. A computer connected rheometer (Rheostress RS 100, Haake Meß-Technik GmbH, Karlsruhe, Germany) was used in order to obtain the viscosities of concentrated polymer solutions. PLA solutions (10, 20, 30 and 40% (w/w), based on the solvent and polymer) in 2-pyrrolidone, DMSO and NMP were analyzed in the controlled stress modulus being a sensitive tool for characterizing test samples at extremely low strains and shear rates. The samples were analyzed at 37 ± 0.5 °C using
the plate/cone (60 mm diameter, 1° angle) equipment. All measurements were performed in triplicate.

2.2.7. Scanning electron microscopy (SEC)
The dried in situ forming implants or microparticles were coated for 70 s under an argon atmosphere with gold–palladium (SCD 040, Balzers Union, Lichtenstein) and then observed with a scanning electron microscope (PW 6703/SEM 515, Philips, Eindhoven, Netherlands).

3. Results and discussion

In order to achieve high drug entrapment and suitable release profiles the solvents used for the in situ technologies have to form concentrated polymer solutions (Lambert and Peck, 1995). The Hildebrand or one-component solubility parameter (δ) has been described to be a useful tool for the prediction of the solubility of PLA and PLGA in different organic solvents (Lambert and Peck, 1995; Shively et al., 1995). Maximal PLA and PLGA solubility was found for solvents having a one-component solubility parameter (δ) between 9 and 11 or 9 and 12.3 (cal/cm³)¹/². Therefore, methylene chloride, acetone, ethyl acetate, NMP and DMSO were suitable solvents for dissolving PLA or PLGA (Table 1). In contrast, 2-pyrrolidone should not be able to dissolve these polymers. However, it was still possible to dissolve more than 40% (w/w), based on the solvent and polymer) of lower molecular weight PLA (R 203) in 2-pyrrolidone. An explanation for the observed discrepancy could be that the one-component solubility parameter concept does not consider molecular polarity or specific interactions.

To explain and describe the selection of the solvents that were used for the preparation of the in situ forming drug delivery systems, the three-dimensional solubility parameter concept of Hansen (1967) was used. The solubility of PLA in different solvents characterized by their partial solubility parameters was determined. Various graphical techniques are available to express the solubility of polymers in terms of the solubility parameters. A triangular plotting technique developed by Teas (1968) was found to be useful. The solubility envelope of PLA (R 203) (Fig. 1) indicates that DMSO and 2-pyrrolidone have similar properties, thus explaining the high solvating power for the PLA even when using 2-pyrrolidone. It has to be pointed out that the good PLA solubility in DMSO, NMP or 2-pyrrolidone did not necessarily indicate the ability for microparticle formation. The key parameters for the formation of microparticles will be discussed together with the solvent release studies.

The polymer/solvent interaction and thus the solvating power of the solvent can be explained by differences in the solubility parameter (Δδ) of the polymer (δₐp) and of the solvent (δₛ):

\[ Δδ = Σ(δ_{a_p} - δ_{d}) \]

The solvating power of the solvents increases with a decrease in Δδ. In general, the higher the solvating power of the solvent, the slower the rate of precipitation and the more non-solvent required for polymer precipitation. The solvent power increases towards the center of the solubility envelope. According to this rule, NMP was a better solvent for PLA than 2-pyrrolidone and DMSO, which are located close to the solubility boundary. Therefore a solution of PLA in NMP was expected to be longer in the non-precipitated or liquid state compared to a solution of PLA in DMSO or 2-pyrrolidone. A slower polymer precipitation was described to lead to a less porous implant surface, thus decreasing the initial drug release (Graham et al., 1999). This might explain slower bupivacaine hydrochloride release from in situ implants prepared with 40% PLA in NMP compared to implants prepared with DMSO (Fig. 2). However, it cannot explain the slow drug release from ISM systems prepared with 2-pyrrolidone. Bupivacaine hydrochloride was chosen as a candidate for the development of a long-acting formulation for the treatment of acute and

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**Table 1 – Solubility parameters of solvents**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>δ (cal/cm³)¹/²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylene chloride</td>
<td>9.7</td>
</tr>
<tr>
<td>Acetone</td>
<td>9.9</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>9.1</td>
</tr>
<tr>
<td>N-Methyl-2-pyrrolidone</td>
<td>11.3</td>
</tr>
<tr>
<td>Dimethylsulfoxide</td>
<td>12.0</td>
</tr>
<tr>
<td>2-Pyrrolidone</td>
<td>14.7</td>
</tr>
<tr>
<td>Ethanol</td>
<td>12.7</td>
</tr>
<tr>
<td>Water</td>
<td>23.4</td>
</tr>
</tbody>
</table>

δ: total solubility parameter thermodynamically determined; δₛ: total solubility parameter empirically determined; δₐ: partial solubility parameter for dispersion bonding; δₜ: partial solubility parameter for polar bonding; δₚ: partial solubility parameter for hydrogen bonding.

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**Fig. 1 – Solubility envelope for poly(L-Lactide) (PLA)—non-solvent: 1, water and solvents: 2, dimethylsulfoxide (DMSO); 3, 2-pyrrolidone; 4, N-methyl-2-pyrrolidone (NMP); 5, methylene chloride.**
chronic pain. In order to maintain sink conditions, the drug release was investigated in phosphate buffer pH 4.0 (bupivacaine solubility at pH 4.0: 37.53 mg/ml).

To further explain the in vitro drug release rates viscosity measurements were carried out on diluted and concentrated polymer solutions. Polymer molecules show different chain conformations depending on the quality of the solvent. In diluted polymer solutions viscosity increases with increasing solvent power due to higher polymer/solvent interactions. In contrast, at higher polymer concentration the viscosity is higher in poor solvents than in good solvents (Kaufman and Falcetta, 1977). This could be verified by capillary and controlled stress rheometer measurements. At low polymer concentration the viscosity of PLA in NMP was higher compared to PLA in DMSO and 2-pyrrolidone (Fig. 3A). Opposite phenomena were observed at high polymer concentration (Fig. 3B) where the viscosity of PLA in NMP was significantly lower compared to PLA in 2-pyrrolidone or DMSO. This confirms the location of the solvents in the solubility envelope, where NMP is a better solvent for PLA than DMSO or 2-pyrrolidone. In addition, the higher viscosity of the concentrated PLA solution in 2-pyrrolidone might explain the slow bupivacaine hydrochloride release from in situ implants prepared with 2-pyrrolidone as shown in Fig. 2. It is well known that with increasing viscosity the drug diffusion rate decreases.

ISM systems were shown to reduce the initial drug release compared to in situ implants (Kranz and Bodmeier, 2007). For the ISM systems the initial drug release decreased with decreasing polymer:oil phase ratio and increasing polymer concentration. In this study the drug release from ISM was investigated as a function of the type of solvent (polymer phase: 40% PLA in DMSO, NMP or 2-pyrrolidone; polymer:oil phase ratio 0.25:1) (Fig. 4). The bupivacaine hydrochloride release decreased in the rank order DMSO > NMP > 2-pyrrolidone, which is in agreement to the in situ implants (Fig. 2). It indicates that similar considerations, which have been discussed for the in situ implants can be hold for the in situ microparticles. However, after 48 h bupivacaine hydrochloride release from ISM systems prepared with DMSO, NMP and 2-pyrrolidone was 1.59, 2.03 and 2.01 times lower than from the in situ implants, respectively.

Four major diffusional motions have to be considered for the formation of in situ implants or in situ microparticles: solvent out, non-solvent in, drug out and probably low molecular weight fractions of PLA out. The solvent loss from in situ implants or ISM results in an increase in the concentration of the polymer. Once the limiting concentration for polymer precipitation is reached, phase separation will occur. In order to explain the reduced initial drug release from in situ microparticles compared to in situ implants the polymer precipitation rate was investigated next. It is well known that different polymer precipitation rates lead to different surface properties which affect the drug release rates (Graham et al., 1999). The polymer precipitation rate was investigated by studying the solvent release rate from in situ implants and
microparticles. Solvent release was investigated as a function of the polymer to oil phase ratio, polymer concentration and type of solvent by using a HPLC assay (Figs. 5–7).

After immersion of the polymer solutions or ISM systems into the buffer medium diffusion of the organic solvent into the aqueous medium was observed (Fig. 5). In contrast to the microparticle formation by the solvent evaporation method no decreasing solvent concentration in the aqueous medium was observed. This could be explained with the high boiling points of the organic solvents used for the in situ technologies (DMSO: 189 °C; NMP: 202 °C; 2-pyrrolidone: 245 °C). In contrast to the volatile methylene chloride or ethyl acetate that are used in the solvent evaporation method these solvents do not evaporate. For the in situ forming implants (40% PLA in 2-pyrrolidone, denoted 1:0) 85.5% of the solvent was released into the buffer medium after 15 min. With the in situ forming microparticles (internal polymer phase: 40% PLA in 2-pyrrolidone) the initial solvent diffusion rate decreased with decreasing polymer:oil phase ratio. After 15 min 18.6% or 15.2% of 2-pyrrolidone was released into the buffer medium from ISM systems with a polymer to oil phase ratio of 0.25:1 or 0.1:1. The solvent diffusion profiles during implant and microparticle formation can be explained as follows: when the polymer solution (in situ implant) was poured into the aqueous phase the water miscible 2-pyrrolidone rapidly leached into the aqueous medium, leading to fast phase separation and polymer precipitation. Similar results were obtained for in situ implants prepared with NMP or DMSO (data not shown). For the ISM systems the external oil phase acts as a barrier for the solvent. Hence, reduced solvent diffusion rates were observed. Due to the increased amount of external oil phase the initial solvent diffusion rate decreased with decreasing
polymer:oil phase ratio. For the polymer solution 98.7% of the 2-pyrrolidone was released into the aqueous medium after 8 h, indicating a poor solvent–polymer affinity. This leads to a low amount of residual solvent within the implants. As 2-pyrrolidone is partially miscible in the external peanut oil phase (92.08 mg/ml), the solvent release from ISM after 8 h was significantly reduced compared to in situ implants.

The effect of the polymer concentration on the solvent release into the buffer medium was investigated for ISM formulations at a constant polymer to oil phase ratio of 0.25:1 (Fig. 6). As expected the diffusion rate of 2-pyrrolidone into the aqueous medium decreased with increasing polymer concentration. After 15 min 43.6% or 18.6% of 2-pyrrolidone was released from ISM-emulsions prepared without polymer or 40% PLA. The decreased solvent diffusion rate can be attributed to the increased viscosity of the polymer phase as shown by the viscosity measurements. Interestingly, even after 8 h only 84.1% of the solvent was released from the solvent/oil mixtures without polymer, indicating the partial affinity of the 2-pyrrolidone to the external oil phase.

The effect of the type of solvent on the release of 2-pyrrolidone, NMP or DMSO into the buffer medium was investigated from ISM formulations containing 40% PLA at a polymer:oil phase ratio of 0.25:1 (Fig. 7). Solvent release rates decreased in the rank order DMSO > NMP ≫ 2-pyrrolidone. The solubility of DMSO or 2-pyrrolidone in peanut oil was 44.93 mg/ml or 92.08 mg/ml. NMP was completely miscible in peanut oil. Hence, miscibility in peanut oil did not confirm the rank order of solvent release. One possible explanation for the significantly decreased solvent release from ISM prepared with 2-pyrrolidone can be the higher viscosity of the polymer phases prepared with 2-pyrrolidone. For the solvent release studies it can be summarized that the initial solvent release from ISM systems was slower compared to the in situ implants. It decreased with decreasing polymer:oil phase ratio and increasing polymer concentration. The type of solvent used also affected the solvent release rate. Slower solvent release rates correlated in all cases with a decreased initial bupivacaine hydrochloride release.

To investigate the effect of the solvent release rates on the particle surface, scanning electron micrographs from in situ implants and microparticles were prepared as a function of the type of solvent and the polymer:oil phase ratio. Injection of 40% PLA solutions in DMSO, NMP or 2-pyrrolidone resulted in porous implants irrespectively of the type of solvent (Fig. 8). The structure of the in situ microparticles was strongly depended on the polymer:oil phase ratio and to a lesser extent on the type of solvent (Fig. 9) (photographs for ISM systems prepared with NMP and DMSO not shown). Increasing the amount of the external oil phase resulted in the formation of microparticles with less porous particle surface. This is in good agreement to the drug release studies (decreasing drug release rates with increasing amount of external oil phase). The implant or microparticle formation is a phase separation process. For the formation of a dense/non-porous implant surface a slow PLA precipitation rate was described to be the key parameter (Graham et al., 1999). Injection of a solution of PLA in water miscible solvents such as 2-pyrrolidone into an aqueous medium resulted in a fast solvent release causing rapid polymer precipitation thus explaining the irregular porous surface and high initial drug release. These findings are in good agreement to dark ground imaging analysis of the phase inversion process (Brodbeck et al., 1999). Due to rapid solvent/non-solvent exchange of PLA solutions in NMP highly concentrated polymer matrices with interconnecting polymer-lean phases were formed during phase inversion. After polymer hardening these polymer-lean phases were responsible for the formation of a porous net-
work. Alternatively, depots with low solvent/water affinity (PLGA in triacetin or ethyl benzoate) have been suggested for the in situ implant technology.

The key parameter for the preparation of microparticles by the solvent extraction/evaporation method is the partial water miscibility of the organic solvents (Bodmeier and McGinity, 1988). Partial water miscible solvents are able to form emulsion droplets within the continuous aqueous phase. Subsequently the polymer precipitates at the droplet surface due to the extraction/evaporation of the solvent. An increasing solvent removal rate results in porous microparticles. This can be explained by partial solidification of the polymer at the droplet surface and entrapment of organic solvents. The high toxicity of the standard solvent methylene chloride prohibits its use for the in situ technologies. In order to slow down solvent/non-

Fig. 9 – Scanning electron micrographs of ISM systems containing 40% poly(ε-caprolactone) (PLA) in 2-pyrrolidone as the inner polymer phase at different polymer:oil phase ratios (A) 1:1, (B) 0.25:1 and (C) 0.1:1 upon injection into phosphate buffer medium.

In conclusion, suitable solvents for the preparation of in situ implants and microparticles were evaluated using the multicomponent solubility parameters concept of Hansen. The particle surface was highly influenced by the rate of polymer precipitation as measured by solvent release into the aqueous medium. Due to the external oil phase the polymer precipitation from ISM-emulsions was significantly slower compared to in situ implants. This resulted in less porous microparticles with reduced initial drug release.
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