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Development of PLGA based injectable delivery systems for hydrophobic fenretinide

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Abstract

Purpose: Although efficient in vitro, fenretinide has not been successful clinically for either of the targeted indication—cancer prevention and dry age-related macular degeneration, because of various issues, such as low oral bioavailability. Therefore, controlled release carriers for parenteral delivery of fenretinide were developed.

Methods: After examining the solubility profile of fenretinide, the drug was encapsulated in poly(lactic-*co*-glycolic acid) (PLGA) microparticles at 20% drug loading by an s/o/w methodology as well as into *in situ*-forming PLGA implants. The carrier morphology and drug release kinetics in an elevated polysorbate 80-containing release medium were studied.

Results: Preformulation studies revealed elevated fenretinide solubility in various PLGA solvents including N-methylpyrrolidone (NMP) and 1:9 v/v methanol:methylene chloride. Co-solvent emulsion methods resulted in low encapsulation efficiency. With an s/o/w method, fenretinide release rates from injectable microparticles were adjusted by the o-phase concentration of end-capped PLGA, the drug particle size, and the particle porosity. *In situ* implants from non-capped PLGA in NMP exhibited a continuous release of ~70% drug over one month.

Conclusions: Injectable carriers for fenretinide were successfully prepared exhibiting excellent drug stability. Based on the different carriers, the preferred injection sites and release rates will be determined in future preclinical *in vivo* studies.

Keywords: fenretinide, hydrophobic drug, s/o/w PLGA microparticle, *in situ* implant, controlled release

1. Introduction

Poly(lactic-co-glycolic acid) [PLGA] is the most commonly used biodegradable polymer for parenteral controlled release formulations because of its biodegradability (1), biocompatibility (2), and ability to control the release of encapsulated substances over days to months depending on the composition of the copolymer, the type of drug, and the properties of the prepared drug loaded vehicle. After initially being studied for delivery of hydrophobic drugs, e.g., contraceptives in the late 1970s and early 1980s (3) (4), research has mostly focused on delivery of proteins and peptides in the last decade (5) (6).

However, as recently summarized (7), there is an increasing number of hydrophobic compounds that are discovered and evaluated as active pharmaceutical ingredient (API) by the pharmaceutical industry. A significant percentage of them are not only hydrophobic, but also exhibit low oral bioavailability. Some of these substances target diseases that require chronic administration. Such drugs may benefit from the advantages of injectable PLGA delivery systems, i.e., i) a higher bioavailability for BCS (8) class 3 or 4 compounds compared to oral administration, ii) a sustained drug release with constant plasma levels in the therapeutic window, iii) a reduced frequency of drug administration, which may increase compliance and therapeutic success, and, iv) if applicable, a local delivery at the site of application, e.g., to the brain (9) or to tumors by intratumoral injection (10).

Fenretinide is a hydrophobic drug, which is structurally derived from vitamin A, and therefore, categorized as a retinoid (11). Fenretinide has prominent anti-tumor effects including the induction of apoptosis in tumor cells (12). Due to its accumulation in fatty tissue such as the mammary gland (11) and its low elimination rates from this compartment (13), fenretinide was suggested for the treatment of breast cancer. Besides breast cancer, the chemopreventive and chemotherapeutic usage in other types of cancer has been addressed so far in more than 30 clinical phase 1 to phase 3 studies (14). Moreover, a less studied application of fenretinide is in

the treatment of dry age-related macular degeneration (AMD) and Stargardt disease, which presently is being evaluated in a phase 2 clinical study (14). Although caused by different mechanisms, both diseases phenomenologically lead to lipofuscin deposits containing toxic vitamin A byproducts and, finally, geographic atrophy of the retina (15). By interfering with vitamin A transport proteins, fenretinide reduces lipofuscin deposition (16).

Fenretinide shows very poor oral bioavailability due to low solubility and low permeability (likely BCS class IV). As a consequence, the high oral doses that are required have occasionally precluded dose escalation in experimental tumor therapy by the high number of capsules to be taken (17). Therefore, one approach suggests embedding the drug in a lipid matrix that forms chylomicron-like particles for increased intestinal absorption by the lymphatic pathway (18). Intravenous injections may be an alternative route of fenretinide administration, which, however, is problematic because of the low aqueous solubility of pure drug. Conjugates of fenretinide with water-soluble polymers like polyvinylalcohol (19) or polyglutamic acid (20) as well as drug encapsulation in micelles from block copolymers (21) have been studied to increase drug solubility, enable intravenous administration, and improve targeting of tumors other than breast cancer by the enhanced permeability and retention effect.

For both, applications in tumor therapy and ocular diseases like AMD, a controlled-release formulation would be advantageous, which can be administered as a parenteral depot and provide drug release over a longer period of time. Two formulation approaches based on biodegradable PLGA were developed in this study to accomplish this, namely, microparticles (7) and *in situ*-forming implants (22). In contrast to conventional pre-formed implant rods, *in situ* implants are solidified in the body after injection of the drug/polymer/solvent solution, due to polymer precipitation resulting from the extraction of the biocompatible solvent (23) into the surrounding tissue.

To the authors' knowledge, fenretinide controlled delivery systems based on polymeric matrix systems have not been reported to date. Therefore, to develop such controlled release systems in this study, an initial characterization of physicochemical properties of fenretinide relevant to microencapsulation processes and release experiments were first carried out. Adjustment of fenretinide release rates was achieved by controlling the morphology of two carrier systems, microparticles and *in situ* implants, both based on biodegradable PLGA. Particularly notable issues with this drug included a strong susceptibility to photo-oxidation (requiring special handling) and the almost complete absence of any significant water-solubility.

2. Materials and Methods

2.1 Materials

Fenretinide [N-(4-hydroxyphenyl)retinamide] was provided by Merck & Co., Inc. and was stringently handled under light protection in all steps of the entire study (Whitehouse Station, NJ, USA). Poly(lactic-co-glycolic acid) [PLGA] of medical grade quality (Resomer® RG 502H, inherent viscosity 0.20 dl/g; RG 503, i.v. 0.42 dl/g) was purchased from Boehringer Ingelheim Chemicals, Inc. (Petersburg, VA, USA). Detergents used in this study were polyvinyl alcohol (PVA, M_w 10kDa, 80% hydrolyzed) and Polysorbate 80 (SigmaUltra) both from Sigma-Aldrich (St. Louis, MO, USA). All solvents and other chemicals were HPLC or USP grade or higher.

2.2 Determination of fenretinide solubility

In order to determine the solubility of fenretinide, two different methods were employed depending on the solvent type and the expected extent of drug solubility. For organic solvents with a large dissolving power, practical solubility was measured by placing tightly locking test

tubes with a known amount of drug on an analytical balance (AG285, Mettler-Toledo, Inc., Columbus, OH, USA) and stepwise addition of the respective solvent until the drug was completely dissolved. For aqueous media with low drug solubility, an excess of drug was suspended in the medium in 15 ml plastic tubes with screw caps and incubated at 37 °C on a horizontal shaker for 3 days mimicking release conditions. Solubility studies in PVA solutions were performed at two extreme temperatures potentially relevant for solvent evaporation, i.e., 4 °C and 30 °C in glass bottles on a low speed magnetic stirrer. After filtering out undissolved drug (0.2 μm membrane filter), the filtered drug solutions were analyzed by HPLC (see section 2.7). The filters used for filtering the solubility samples were previously checked for absence of drug adsorption. If required, drug samples were concentrated by lyophilizing the filtrate and extraction with a small volume of acetonitrile prior to HPLC analysis.

2.3 Solubility parameters

The Hansen solubility parameters of fenretinide were estimated by the 'Yamamoto molecular breaking' group contribution method from its structure by the HSPiP software, 3rd edition (C. Hansen, S. Abbott) (24). From the experimental data of pure good solvents, the Hansen solubility parameters of fenretinide were additionally obtained by fitting.

2.4 Microparticle preparation

Different methods were evaluated for the microencapsulation of fenretinide while keeping the theoretical loading at 20% (w/w). For example, for the standard cosolvent method, 125 mg of drug were first dissolved in 2.5 ml of a mixture of methylene chloride and the cosolvent in a glass tube, followed by the addition of 500 mg Resomer® RG 503 to form the o-phase. For the

solid-in-oil-in-water technique (s/o/w), varying amounts of the polymer were dissolved in 2.5 ml methylene chloride and subsequently the drug was suspended at 10.000 rpm for 60 s in the o-phase using a Tempest I.Q.^{2} rotor-stator homogenizer (Virtis, Gardiner, NY, USA). Concentration units during microparticle formulation were practically expressed based on percentage of polymer weight added to the solvent volume approximately assuming equivalent density, i.e., 20% PLGA denotes a polymer solution prepared with 500 mg PLGA and 2.5 ml methylene chloride. The o-phase or s/o-phase, respectively, was then emulsified in 5 ml of 5% (w/v) PVA solution by vortexing for 20 s and poured into 75 ml of a magnetically stirred 0.5% (w/v) PVA solution for solvent evaporation at room temperature. After 3 h, particle fractions were collected on test sieves (Newark Wire Cloth Company, Clifton, NJ, USA), washed with water, and lyophilized. The weight of the dried particles in each fraction was determined on an analytical balance.

In some cases, fenretinide was ground/micronized in a pre-cooled agate mortar on dry ice for usage in the s/o/w technique. As fenretinide is not hygroscopic, the procedure was performed in a normal lab atmosphere and the ground material was subsequently dried at 37 °C to constant weight.

2.5 *In situ* implants

Fenretinide was dissolved in N-Methylpyrrolidone (NMP) to obtain a 100 mg/ml stock solution. Different amounts of PLGA polymer were placed in 5 ml glass vials and the required volumes of drug stock solution and additional pure NMP were added to obtain solutions with different polymer concentrations (see section 2.4 for explanation of concentration units) and a final loading of 7 mg fenretinide/200 µl in situ implant forming solution. For high polymer concentrations, a fast dissolution of the polymer in NMP required incubation at 37 °C. The

resulting drug + polymer/NMP solutions were slowly injected with 1-ml syringes (BD 309602, Becton Dickinson, Franklin Lakes, NJ, USA) and 23G needles (BD 305193) into the release medium to form implants. In order to determine the precise amount of injected drug solution, the weight of the syringe was determined before and after injection.

2.6 Scanning electron microscopy and digital photography

For analysis by scanning electron microscopy (SEM), the microparticles or implant samples were placed on a sample holder with conductive double-adhesive tape and sputtered with gold (Desk II, Denton Vacuum, Moorestown, NJ, USA). In order to study the inner structure of implants, cross sectioning was performed with a razor blade after lyophilisation of the samples. Micrographs were taken with a Hitachi S3200 (Pleasanton, CA, USA) or a Philips XL 30 ESEM (Bothell, WA, USA).

Digital photography (EOS 350D, Canon, Krefeld, Germany) was used to document the macroscopic appearance of the implants after 32 days incubation in release medium.

2.7 Encapsulation efficiency, recovery assay, and HPLC analysis

In order to determine the encapsulation efficiency or the remaining drug in microparticle pellets from the release study, the polymer had to be removed from the samples prior to HPLC analysis. Lyophilized samples were dissolved in 0.5 ml of tetrahydrofuran and PLGA was precipitated by the addition of 9.5 ml of ethanol. After centrifugation (5 min, 16,100·g, Centrifuge 5415D, Eppendorf, Hamburg, Germany) and dilution if required, the samples were analyzed on a Waters HPLC system (Waters 1525 pumps, Waters 717plus Autosampler, Waters 2487 Dual λ absorbance detector) with a Nova-Pak® C18 column. The observed limit of quantification

corresponded to a sample concentration of 0.1 µg/ml. Additionally, the absence of degradation products was found in control studies using the same assay on a Waters 2695 Alliance system with a Waters 2996 photodiode array detector.

2.8 Release studies

Different experimental configurations were evaluated for release studies of microparticle samples. The highest reproducibility and practical use was realized with a procedure, in which 5 mg of microparticles were placed in 50-ml plastic tubes and suspended in 50 ml of a sterilized solution of 1% (v/v) Polysorbate 80 in phosphate buffered saline (PBS) at pH 7.4. The tubes were incubated at 37 °C on a rocking platform shaker at 25 rpm, VWR, West Chester, PA, USA). Samples in triplicate were removed at predefined time points, filtered through a 0.45-µm membrane filter, and both the pellet after lyophilisation and the filtrate were analyzed for drug content.

Drug release from *in situ*-forming implants was determined after injecting the implants inside filter bags with a 1-µm pore size (NMO1SBF cut to size, Midwest Filter Corporation, Lake Forest, IL, USA), which were placed in 50-ml plastic tubes containing 45 ml of a sterilized solution of 1% (v/v) Polysorbate 80 in PBS, pH 7.4. During sampling, 35 ml were withdrawn for HPLC analysis and replaced by fresh medium to maintain sink conditions.

3. Results

3.1 Fenretinide physicochemical properties

In order to follow a rational microencapsulation approach, the solubility of fenretinide was first determined in aqueous and organic solvents, and organic solvent mixtures. As can be seen from Table I, the drug showed limited solubility in useful nonpolar, water-immiscible carrier solvents for encapsulation like methylene chloride (2.5 mg/ml) or ethyl acetate (21 mg/ml). However, the solubility in some fully water-miscible organic solvents was considerably higher (Table I). Methanol and tetrahydrofuran (THF) were selected as cosolvent candidates for drug dissolution in the carrier solvent, and the dissolving power of their mixtures with methylene chloride is given in Table II. Elevated drug solubility (> 140 mg/ml) in just 8:92 v/v methanol/methylene chloride was observed, indicating methanol to be a very efficient cosolvent for fenretinide. By contrast, THF was much less efficient, requiring 20% cosolvent to attain a solubility of > 100 mg/ml.

Providing estimates on the physicochemical properties of new compounds is of major interest for a rational formulation development for economical reasons. Based on the determined solubilities, good solvents for fenretinide were defined practically as those solvents capable of dissolving ~100 mg/ml or more. When comparing the Hildebrand solubility parameter δ_t of good with those of poor solvents, no correlation between δ_t values and the dissolving power could be established (Table I). For instance, the poor solvents acetonitrile and 1-propanol (both $\delta_t = 24.6$ $MPa^{1/2}$) have an almost identical Hildebrand solubility parameter as dimethylformamide ($\delta_t = 24.8$ $MPa^{1/2}$), but much less dissolving power for fenretinide. This illustrates, that the Hildebrand theory that was developed primarily for non-polar, non-hydrogen-bonding interactions between solvents and compounds is not so useful for fenretinide. By including such additional interactions as provided by the Hansen theory of solubility parameters, solubility parameters of fenretinide (δ_d; δ_p; δ_h ($MPa^{1/2}$): 17.8; 6.7; 8.8) were estimated by a group contribution method by means of the HSPiP software. The graphical plot as depicted in Fig. 1 more clearly illustrates some clustering of good

solvents in the Hansen space compared to that which may be deduced from the values of Hansen solubility parameters as provided in Table I. When calculating the Hansen solubility parameter of fenretinide by a fit of the experimental data for pure good solvents (δ_d; δ_p; δ_h ($MPa^{1/2}$): 17.1; 10.8; 12.2), a clear shift particularly in the contributions from polar and hydrogen-binding forces towards higher values were observed. With the only exception of methylene chloride that was wrongly suggested as good solvent, the fit allowed differentiation between good and poor solvents.

For the drug solubility in aqueous media, two cases of media types are of particular importance, i.e., detergent solutions used as a hardening bath during the microparticle preparation and media used in release studies. Two extreme temperatures for solvent evaporation techniques, 4 °C and 30 °C, were chosen to detect the range of drug solubility in aqueous PVA solutions that may be used as the external phase. As seen in Fig. 2a, the solubility of fenretinide in PVA solutions is very low at both temperatures. Thus, the loss of drug to the water-phase (w-phase) driven by its solubility in aqueous PVA solutions was not expected to be to a concern when maximizing encapsulation efficiency. By contrast, another detergent, Polysorbate 80, strongly increased the fenretinide solubility (Fig. 2b) and was considered to serve for drug solubilization in release studies.

3.2 Impact of the microencapsulation method on the PLGA microparticle properties

Based on the solubility characteristics of fenretinide, two microencapsulation methods were selected for further evaluation, namely, the o/w cosolvent and s/o/w techniques. However, although the cosolvent was kept at a minimum level required to completely dissolve the drug, low encapsulation efficiencies of only 30-40 % were observed for both cosolvents, THF and methanol. Increasing the speed of polymer precipitation at the surface of nascent microparticles

by higher polymer concentration in the o-phase, e.g., for Resomer® RG 503 an increase from 16 to 20%, did not have a large impact on the encapsulation efficiency (32.6% vs. 35% for methanol as cosolvent). Due to the poor encapsulation efficiency by cosolvent techniques and the required high loading, the s/o/w-technique was used in all further experiments.

Increasing the polymer concentration is known to often result in a denser particle matrix with a lower burst and a more prolonged release profile. When the PLGA concentration (Resomer® RG 503) in the o-phase was increased from 15 to 25%, a shift in the particle size distribution toward the larger size fraction was observed (Fig. 3). Therefore, in some cases and particularly for formulations with a further increase in polymer concentration to, e.g., 35%, it was necessary to collect a particle size fraction of 20-63 µm or 20-90 µm to ensure a significant yield of particles in the fraction for further analysis. Both, the encapsulation efficiency and the overall yield of microparticles were relatively independent of the polymer concentration in the o-phase and covered a range from 70-80% for the encapsulation efficiency and 80-85% for the overall yield. Some of the missing material was observed to stick to the stirring bar, probably due to aggregation of nascent particles in the early stage of their hardening.

The analysis of the microparticle shape and surface structure by scanning electron microscopy (SEM) revealed a significant amount of broken, collapsed, or poorly formed particles at low polymer concentrations (15% PLGA). Higher polymer concentrations (25% PLGA) resulted in smoother particle surfaces, although a certain number of microparticles exhibited an ellipsoid shape with an occasional surface perforation by spiky drug needles (Figs. 4a-c). As expected from the differences in particle morphology, a faster release from microparticles with lower polymer concentration and imperfect polymer coatings of drug crystals was observed compared to those with a higher polymer concentration and fully embedded drug (Fig. 4d).

In the next series of experiments, a further increase in the polymer concentration from 25 to 35 % did not result in a more spherical shape of the particles. As can be seen in Figs. 5a-c, non-

encapsulated ends of drug needles were still present in few cases, even though the drug used in these experiments had a crystal size below 20 µm and the size range of the collected main fraction was extended to 20-90 µm. Encapsulated drug near the microparticle surface resulted in bulging of the particle surfaces. The release rates for the 20-90 µm fraction of the 25% PLGA formulation (Fig. 5d) were in good agreement with the release rates for the 20-45 µm fraction (Fig. 4d), at least during the first week of the study. In the following weeks, the 20-90 µm samples showed an ongoing fenretinide release, whereas the 20-45 µm particles exhibited a lag phase. Higher polymer concentrations (30%) resulted in a slower release and release rate was not strongly affected for microparticles prepared above this concentration at 35%. In conclusion, the 25% PLGA formulation was expected to provide the required drug release over 2-4 weeks if < 20 µm drug crystals were used.

However, the apparent size of drug crystals is not an invariable property of a specific substance but is largely influenced by various process parameters during crystallization, aggregation of drug particles, and possibly due to impurities present in drug substance. For a more robust microparticle formulation that is not affected by the variability in drug particle size from batch to batch, ground fenretinide was encapsulated into microparticles with 25% PLGA in the o-phase in the next set of experiments. This procedure resulted in particles with a perfect spherical shape (Fig. 6). However, a small portion of non-encapsulated drug that may contribute to a burst release could not be removed during the washing procedure and remained at the particle surface (Figs. 6c and d). As expected, the embedding of smaller, likely well separated drug crystals into the polymer matrix resulted in a slower drug release from 25% RG 503 microparticles (Fig. 6e). In order to increase release rate, porosity was induced by adding different concentrations of phosphate buffered saline (PBS) in the inner water phase in a $(w_1+s)/o/w_2$ procedure. From systematic screening experiments with blank particles (exemplary images in Figs. 6a and b), a formulation was selected that produced highly porous particles (w_1: 5X PBS). Fenretinide

experienced a dramatically increased release from this formulation, which was practically completed after 7 d in the employed medium with elevated detergent concentration. Additionally, another PLGA material with lower molecular weight and uncapped end groups, Resomer® RG 502H, was evaluated for the encapsulation of ground drug. However, fenretinide release rates could not be altered substantially by increasing the RG 502H concentration within its solubility range (Fig. 6e).

3.3 *In situ* forming implants

Besides microparticles, *in situ* forming implants are an interesting alternative drug carrier concept. Since fenretinide was highly soluble in N-methylpyrrolidone (NMP) (Table I), the solvent used in FDA-approved products (e.g., Eligard®), several formulations of drug and polymer in NMP were evaluated in this study. However, unloaded implants were first prepared with varying concentrations of PLGA (Resomer® RG 503) in NMP. The implants were lyophilized after 3 days and their microstructure was studied by SEM, as shown in Fig. 7. The implant prepared using 10% PLGA had collapsed and basically consisted of a shell with a hollow core rather than a matrix structure (data not sown). The usage of higher polymer concentrations ranging from 15-35% PLGA provided implants of different internal porosities (Fig. 7). In the 15% PLGA formulation, it appeared that a significant amount of water was able to enter the implant. This influx resulted in a large pocket filled with spontaneously formed microparticles (Fig. 7a). In contrast, the implant with 35% PLGA in NMP consisted of a denser matrix with a thin shell (see insert of Fig. 7c). NMP diffusion out of the implant was limited and thus some residual NMP was noticeable in the core still after 3 days of incubation. For both, the 25% and the 35% RG 503 formulations, no pores were observed on the outside of the implant.

In addition to Resomer® RG 503, Resomer® RG 502H was used as the matrix of fenretinide-loaded *in situ* implants. Because of the lower molecular weight of RG 502H, higher polymer concentrations of up to 70% were used for this PLGA grade. As can be seen from Fig. 8, a clear relationship between the polymer concentration, i.e., the density of the precipitated matrix and the release profile was detected for Resomer® RG 503 with the slowest release observed for the 35% RG 503 formulation. Surprisingly, no such systematic effect of increasing Resomer® RG 502H concentrations on fenretinide release rates was observed. However, Fig. 8 indicates a lower burst and desirable slow and continous release profile of fenretinide from RG 502H compared to RG 503 in the drug-solubilizing release media.

Considerable differences in the shape of the implants were apparent when samples were harvested at the end of the release study (day 32). As documented by digital photography (Fig. 9), all RG 502H samples exhibited a significantly swollen, occasionally translucent matrix. Some residual yellow drug aggregates were still encapsulated in the implant core, which appeared to be comparatively less swollen. By contrast, the RG 503 samples showed some swelling but maintained the irregular shape that was initially formed during the injection and *in situ* precipitation/encapsulation process.

4. Discussion

4.1 Requirements for fenretinide carriers

Fenretinide is a BCS class IV drug with very low oral bioavailability. Therefore, high daily doses of 200 mg/day in chemoprevention (corresponding to 2 capsules of drug formulation) (25) (26) and up to 4000 mg/m^2/day in a Phase 1 study on neuroblastoma in children (~40 capsules/day) were evaluated. Since only marginal amounts of the orally administered drug are absorbed, it remains unclear which parenteral daily dose would be useful for long-term injectable controlled-

release dosage forms with much higher bioavailability. Recent publications with intravenously injected drug-polymer conjugates or micelle systems followed the general expectation that much lower parenteral doses will be required and therefore, injections of 0.2 (19) to 1 mg (21) of fenretinide equivalents in mice were used. However, when considering the low volumes that are possible for intramuscular or subcutaneous injection and the desired timeframe of sustained release over 1 to 4 weeks, a high loading of the drug carrier was set as a desired formulation criteria in the present study.

4.2 Solubility issues impact microencapsulation strategies

Hydrophobic drugs such as fenretinide are most commonly encapsulated by o/w emulsion techniques into PLGA microparticles (7). Due to the low aqueous solubility, the drug escape to aqueous PVA solutions used as external water phase can be considered as marginal (Fig. 2a), e.g., a total of 6 µg for standard batches with 100 mg drug. In order to achieve the desired high loading in o/w techniques, drug solubility in the o-phase was required to be at least 100 mg/ml. However, non-water miscible solvents provided only low solubility for fenretinide (Table I). Based on the preformulation analysis, a more precise determination of the Hansen solubility parameter of fenretinide could be provided by fitting experimental solubility data compared to a rough estimation from the chemical structure by the group contribution method (Fig. 1). However, wrongly inclusion of methylene chloride in the group of good solvents by the software fitting illustrates that additional, specific interactions which possibly may be relevant for high drug solubility are not included in the Hansen parameters.

Due to the absence of suitable drug solubility in pure solvents commonly used as o-phases (Table I), two alternative techniques have been suggested, i.e., o/w cosolvent methods and the s/o/w-technique. Methanol and tetrahydrofuran (THF) were selected as cosolvent candidates

because they showed a high dissolving power for fenretidine, a high vapor pressure that will ease their removal from the hardening bath by solvent evaporation, and, for methanol, some evidence of successful usage as a cosolvent for the encapsulation of hydrophobic drugs (27). When analyzing drug solubility in mixtures of the cosolvent candidates and methylene chloride, surprisingly, advantageous effects of methanol as fenretinide cosolvent were observed although pure methanol has only 15% of the dissolving power of THF (Table II). As a straight line connecting the two points that correspond to the solvents' Hansen solubility parameters of methylene chloride and methanol closely passes the fitted value of fenretinide, some increase in solubility for methanol/methylene chloride mixtures may be expected from Figure 1.

As totally water-miscible cosolvents will partition into the water-phase during the emulsification procedure (28), drug dissolved in this eluting cosolvent may be lost to the external phase. Although keeping the cosolvent concentration at a minimum, low encapsulation efficiencies were observed with both cosolvent systems as may have been expected by the slower phase separation in the microparticles. A faster solidification of particles as induced by employing higher polymer concentrations (29) showed some minor improvements on encapsulation efficiency, but was not expected to be a feasible approach that could compete with loading levels expected for the s/o/w method.

4.3 Microencapsulation by s/o/w technique and interpretation of release data

By the s/o/w preparation method, microparticle fractions of a particle size suitable for injection were obtained with good reproducibility in all cases (Fig. 3). However, only for higher polymer concentrations such as 25% Resomer® RG 503, leading to an increased viscosity of the polymer solution, a suitable embedding of unmicronized drug needles was achievable (Fig. 4). At the same time and in good agreement with data from the literature (30), a shift towards larger particle

size fraction resulted from such higher polymer concentrations (and higher polymer solution viscosity) (Fig. 3). Additionally, the burst and subsequent release rates were reduced (Fig. 4d) due to a more complete coverage of drug needles with polymer, longer diffusion lengths, and an expected lower inner porosity of the particles (31). However, the maximum effect of alterations of the RG 503 concentration in the o-phase on drug release was observed for the 30% polymer formulation. A further increase from 30 to 35% neither influenced the shape of the particles nor the fenretinide release (Fig. 5).

Although drug crystals used in this study were typically below 20 μm, some microparticles with surfaces penetrated by drug needles were found even in 25 to 35% batches. This was most likely due to flocculated drug particles that could not be separated during the s/o suspension procedure. The perforation of the polymer shell by unmicronized drug needles provided a fast access of medium to the whole payload of the particle during release studies. When employing micronized drug in the 25% RG 503 formulation (Fig. 6), lower release rates were observed as expected, indicating that the drug has been well separated in the polymer and was released gradually by diffusion and/or bulk erosion of the matrix. It is well known, that bulk erosion of the polymer typically contributes to the drug release from PLGA matrices after a critical molecular weight is reached during polymer degradation. Therefore, this well-known induction time to polymer mass loss obviously is shorter for PLGA with initially shorter polymer chains (32) (33). Moreover, water uptake as a precondition for degradation is higher in PLGA with free carboxyl end groups, such as Resomer® RG 502H. Because RG 502H has about half of the inherent viscosity of RG 503 (0.20 vs. 0.42 dl/g for the employed polymer batches), the concentration of RG 502H in the o-phase was increased to 50 or 60%. Although a much faster release was observed for both RG 502H formulations, as desired in principle (Fig. 6), RG 502H allowed less control of the release rate of fenretinide by changes in the polymer concentration according to the in vitro release test.

Higher water uptake into particles can also be achieved by introducing macropores in the matrix. Porosity can be achieved during particle hardening by addition of osmotically active additives such as salt in the inner water phase in $w_1/o/w_2$ techniques (7). From a larger set of experiments with blank particles of different levels of porosity, a highly porous formulation was selected to be used for fenretinide as proof-of-concept. The release from this formulation was linear and much faster than from non-porous 25% RG 503 particles, but still slower than from a physical mixture of fenretinide and PLGA.

Due to the drug's extremely low solubility of ~20 ng/ml in PBS, the design of a suitable release assay under sink conditions was challenging. For example, for 5 mg particles encapsulating a total of 1 mg fenretinide, 500 L of PBS would be required for sink conditions in a closed vessel set-up. This large volume, besides having quantification issues, can be considered to be irrelevant in terms of handling and reproducibility. As drugs are often released faster *in vivo* than *in vitro* (34), there are several approaches reported in the literature to increase drug solubility and/or release rates *in vitro* by, e.g., alcoholic release media, higher temperatures, altered pH values, or substances that increase the polymer hydrolysis (7). Polysorbate 80, commonly used as detergent in release media at low concentrations to improve wetting of polymers and mimic the surface active molecules present at the injection site, showed a dramatic increase in fenretinide solubility in this study. A linear correlation between Polysorbate 80 concentration and drug solubility was observed (Fig. 2b, inset), which indicates that the drug associates with, and was solubilised by, the Polysorbate 80 micelles. The common micellar solubilization was strengthened by a multitude of diffusion studies (*data not shown*), where fenretinide (MW: 391 Da) dissolved in Polysorbate 80-containing release vehicles (with micelle size up to 20 nm (35)) did neither adsorb nor permeate through membranes with, e.g., 50 kDa (~6 nm) pores, but easily went through 1 μm pores.

Therefore, a configuration for release studies from microparticles was selected where microparticles were dispersed in 50 ml tubes with medium of elevated detergent concentration (1% Polysorbate 80), which allowed sink conditions over the entire study. This procedure helped to avoid possible particle loss during medium replacement or alterations of particles during repeated centrifugation and resuspension. Although high media volumes have occasionally been used to study the release of hydrophobic drugs as recently summarized (7), it is obvious that such volumes do not display the conditions at a subcutaneous injection site. In conclusion, it can be discussed that the release from the microparticles might be slower *in vivo* than observed in the release assay with the elevated detergent medium.

4.4 Fenretinide-loaded *in situ* forming implants

Besides preformed microparticles which typically are prepared in costly industrial processes, *in situ* forming implants have recently attracted substantial interest as polymeric controlled delivery matrices due to easy handling, reduced needle size and injection volume, and low manufacturing costs (36) (7). In contrast to conventional implantable rods from dense matrices with long diffusion distances and long release periods particularly for hydrophobic drugs, *in situ* implants are often characterized by a highly macroporous structure (Fig. 7), which is formed during polymer precipitation by solvent exchange, i.e., efflux of NMP as polymer carrier solvent and influx of water as non-solvent to the polymer (37). The efflux of NMP may also contribute to the release profile by increasing the drug's local solubility as the solvent leaves the implant. Additionally, the formation of a large internal interface between the drug loaded PLGA bulk and the water-filled interconnective pore structure may increase diffusion-governed controlled release. When discussing release rates from *in situ* implants, it has to be considered that in contrast to microparticles no aqueous carrier is required during injection. Therefore, for a fixed injection volume, a higher mass of drug per injection may be administered and thus a longer-

lasting release may be achievable when confronted with the inability to incorporate sufficient drug in the depot.

The reverse relationship between the fenretinide release and the polymer concentration for RG 503 implants (Fig. 8) correlates well with differences in matrix porosity (Fig. 7). The observed variability in release can be justified by the irregular implant shapes formed *in vitro* with different diffusion lengths, porosities, and surface area-to-volume ratios. If more compact implant shapes may be formed *in vivo* after injection in a tissue, the contribution of shape differences to the variability in release rates might possibly be reduced. For high RG 503 concentrations, as expected, a long lag phase occurred after the burst release phase. In contrast, more hydrophilic RG 502H matrices allowed a slow and continuous drug release out of the polymer matrix, consistent with the absence of a lag phase from this low molecular weight, free-acid end-capped polymer (32) (33). The observed order in release rates from RG 502H formulations, i.e., 30% > 50% < 70% suggested an interplay of multiple contributions to the release mechanism. Besides the level of macroporosity and water/drug diffusion rates, which would be expected to reduced release rate as initial PLGA/NMP concentration was increased, other affected mechanisms, e.g., reduced diffusion of water-soluble acid-degradation products (38) leading to increased autocatalytic polymer degradation, might be involved in release control. RG 502H implants as isolated at the end of the release study showed differences in their swollen shape (Fig. 9a-c). The 70% RG 502H implants (Fig. 9c) were smaller and appeared to have a less swollen core covered by a strongly swollen shell. Drug encapsulated in this shell can be assumed to be more susceptible to drug release. These observations were consistent with the hypothesized slower efflux of acidic degradation products in the denser 70% RG 502H implants, resulting in faster local polymer degradation and drug release. Finally, it should be noted that photo- and thermolabile fenretinide was stable in all PLGA formulations and no degradation products as

previously characterized by stress tests (*data not shown*) were detected in both the loading assay and the release study.

5. Conclusions

This study provides an in depth evaluation of fenretinide delivery based from PLGA matrices in vitro. Both, preformed microparticles and *in situ* forming implants allowed adjustment of fenretinide release rates depending on the morphology of the carrier. Since low bioavailability and, therefore, high oral doses have so far disadvantageously impacted feasibility of fenretinide oral treatments, such parenteral depot formulations could be a reasonable alternative path to clinical development of this drug. Depending on the indication and the involved mechanism of drug action—e.g., a) prevention or treatment of different forms of cancer by direct intracellular induction of apoptosis or b) ophthalmic diseases with a reduction of vitamin A transport to the eye by intravascular blocking of transport proteins—both, the most suitable site of administration and rate of drug release will have to be addressed in future preclinical and clinical studies.

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References

1. J.M. Brady, D.E. Cutright, R.A. Miller, Battisto.Gc, and E.E. Hunsuck. Resorption Rate, Route of Elimination, and Ultrastructure of Implant Site of Polylactic Acid in Abdominal-Wall of Rat. J Biomed Mater Res. 7:155-166 (1973).
2. M.S. Shive, and J.M. Anderson. Biodegradation and biocompatibility of PLA and PLGA microspheres. Adv Drug Deliv Rev. 28:5-24 (1997).
3. L.R. Beck, D.R. Cowsar, D.H. Lewis, J.W. Gibson, and C.E. Flowers. New Long-Acting Injectable Microcapsule Contraceptive System. Am J Obstet Gynecol. 135:419-426 (1979).
4. T. Tice, and D. Lewis. Microencapsulation process. United States Patent US 4,389,330 (1983).
5. S.P. Schwendeman. Recent advances in the stabilization of proteins encapsulated in injectable PLGA delivery systems. Crit Rev Ther Drug. 19:73-98 (2002).
6. W.L. Jiang, R.K. Gupta, M.C. Deshpande, and S.P. Schwendeman. Biodegradable poly(lactic-co-glycolic acid) microparticles for injectable delivery of vaccine antigens. Adv Drug Deliver Rev. 57:391-410 (2005).
7. C. Wischke, and S.P. Schwendeman. Principles of encapsulating hydrophobic drugs in PLA/PLGA microparticles. Int J Pharm. 364:298-327 (2008).
8. G.L. Amidon, H. Lennernas, V.P. Shah, and J.R. Crison. A Theoretical Basis for a Biopharmaceutic Drug Classification - the Correlation of in-Vitro Drug Product Dissolution and in-Vivo Bioavailability. Pharm Res. 12:413-420 (1995).
9. H. Seong, T.K. An, G. Khang, S.U. Choi, C.O. Lee, and H.B. Lee. BCNU-loadedpoly(D, L-lactide-co-glycolide) wafer and antitumor activity against XF-498 human CNS tumor cells in vitro. Int J Pharm. 251:1-12 (2003).
10. M. Xie, L. Zhou, T. Hu, and M. Yao. Intratumoral delivery of paclitaxel-loaded poly(lactic-co-glycolic acid) microspheres for Hep-2 laryngeal squamous cell carcinoma xenografts. Anticancer Drugs. 18:459-466 (2007).
11. R.C. Moon, H.J. Thompson, P.J. Becci, C.J. Grubbs, R.J. Gander, D.L. Newton, J.M. Smith, S.L. Phillips, W.R. Henderson, L.T. Mullen, C.C. Brown, and M.B. Sporn. N-(4-Hydroxyphenyl)Retinamide - New Retinoid for Prevention of Breast-Cancer in the Rat. Cancer Research. 39:1339-1346 (1979).
12. N. Hail, H.J. Kim, and R. Lotan. Mechanisms of fenretinide-induced apoptosis. Apoptosis. 11:1677-1694 (2006).
13. T.A. Hultin, C.M. May, and R.C. Moon. N-(4-Hydroxyphenyl)-All-Trans-Retinamide Pharmacokinetics in Female Rats and Mice. Drug Metabolism and Disposition. 14:714-717 (1986).
14. U.S. National Institutes of Health. www.clinicaltrials.gov.
15. G. Wolf. Lipofuscin and macular degeneration. Nutr Rev. 61:342-346 (2003).
16. R.A. Radu, Y. Han, T.V. Bui, S. Nusinowitz, D. Bok, J. Lichter, K. Widder, G.H. Travis, and N.L. Mata. Reductions in serum vitamin A arrest accumulation of toxic retinal fluorophores: a potential therapy for treatment of lipofuscin-based retinal diseases. Invest Ophthalmol Vis Sci. 46:4393-4401 (2005).
17. A. Garaventa, R. Luksch, M.S. Lo Piccolo, E. Cavadini, P.G. Montaldo, M.R. Pizzitola, L. Boni, M. Ponzoni, A. Decensi, B. De Bernardi, F.F. Bellani, and F. Formelli. Phase I trial and pharmacokinetics of fenretinide in children with neuroblastoma. Clinical Cancer Research. 9:2032-2039 (2003).
18. B.J. Maurer, O. Kalous, D.W. Yesair, X.Q. Wu, J. Janeba, V. Maldonado, V. Khankaldyyan, T. Frgala, B.C. Sun, R.T. Mckee, S.W. Burgess, W.A. Shaw, and C.P.

Reynolds. Improved oral delivery of N-(4-hydroxyphenyl)retinamide with a novel LYM-X-SORB organized lipid complex. Clinical Cancer Research. 13:3079-3086 (2007).
19. I. Orienti, G. Zuccari, V. Bergamante, R. Carosio, R. Gotti, M. Cilli, and P.G. Montaldo. Fenretinide-polyvinylalcohol conjugates: New systems allowing fenretinide intravenous administration. Biomacromolecules. 8:3258-3262 (2007).
20. C. Zou, M. Brewer, X. Cao, R. Zang, J. Lin, Y. Deng, and C. Li. Antitumor activity of 4-(N-hydroxyphenyl)retinamide conjugated with poly(L-glutamic acid) against ovarian cancer xenografts. Gynecol Oncol. 107:441-449 (2007).
21. T. Okuda, S. Kawakami, Y. Higuchi, T. Satoh, Y. Oka, M. Yokoyama, F. Yamashita, and M. Hashida. Enhanced in vivo antitumor efficacy of fenretinide encapsulated in polymeric micelles. Int J Pharm. 373:100-106 (2009).
22. R.L. Dunn, J.P. English, D.R. Cowsar, and D.P. Vanderbilt. Biodegradable in situ forming implants and methods of producing the same. United States Patent US 4.938,763 (1990).
23. M.A. Royals, S.M. Fujita, G.L. Yewey, J. Rodriguez, P.C. Schultheiss, and R.L. Dunn. Biocompatibility of a biodegradable in situ forming implant system in rhesus monkeys. J Biomed Mater Res. 45:231-239 (1999).
24. HSPiP. Hansen Solubility Parameters in Practice. http://www.hansen-solubility.com
25. F. Formelli, R. Carsana, A. Costa, F. Buranelli, T. Campa, G. Dossena, A. Magni, and M. Pizzichetta. Plasma Retinol Level Reduction by the Synthetic Retinoid Fenretinide - a One-Year Follow-up-Study of Breast-Cancer Patients. Cancer Research. 49:6149-6152 (1989).
26. T. Camerini, L. Mariani, G. De Palo, E. Marubini, M.G. Di Mauro, A. Decensi, A. Costa, and U. Veronesi. Safety of the synthetic retinoid fenretinide: Long-term results from a controlled clinical trial for the prevention of contralateral breast cancer. Journal of Clinical Oncology. 19:1664-1670 (2001).
27. D.T. Birnbaum, J.D. Kosmala, D.B. Henthorn, and L. Brannon-Peppas. Controlled release of beta-estradiol from PLAGA microparticles: The effect of organic phase solvent on encapsulation and release. J Control Release. 65:375-387 (2000).
28. W.-I. Li, K. Anderson, R. Mehta, and P. DeLuca. Prediction of solvent removal profile and effect on properties for peptide-loaded PLGA microspheres prepared by solvent extraction/evaporation method. J Control Release. 37:199 (1995).
29. R. Bodmeier, and J.W. Mcginity. Solvent Selection in the Preparation of Poly(Dl-Lactide) Microspheres Prepared by the Solvent Evaporation Method. Int J Pharm. 43:179-186 (1988).
30. G. Spenlehauer, M. Veillard, and J.P. Benoit. Formation and characterization of cisplatin loaded poly(d,l-lactide) microspheres for chemoembolization. J Pharm Sci. 75:750-755 (1986).
31. S.R. Mao, Y. Shi, L. Li, J. Xu, A. Schaper, and T. Kissel. Effects of process and formulation parameters on characteristics and internal morphology of poly(D,L-lactide-co-glycolide) microspheres formed by the solvent evaporation method. Eur J Pharm Biopharm. 68:214-223 (2008).
32. F.G. Hutchinson. Continuous release pharmaceutical compositions. European Patent EP 58 481 (1986).
33. Y. Ogawa, M. Yamamoto, S. Takada, H. Okada, and T. Shimamoto. 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 (1988).
34. B.S. Zolnik, and D.J. Burgess. Evaluation of in vivo-in vitro release of dexamethasone from PLGA microspheres. J Control Release. 127:137-145 (2008).

35. M. Turk, and R. Lietzow. Stabilized nanoparticles of phytosterol by rapid expansion from supercritical solution into aqueous solution. AAPS PharmSciTech. 5:e56 (2004).
36. C.B. Packhaeuser, J. Schnieders, C.G. Oster, and T. Kissel. In situ forming parenteral drug delivery systems: an overview. Eur J Pharm Biopharm. 58:445-455 (2004).
37. P.D. Graham, K.J. Brodbeck, and A.J. McHugh. Phase inversion dynamics of PLGA solutions related to drug delivery. J Controlled Release. 58:233-245 (1999).
38. A.G. Ding, and S.P. Schwendeman. Determination of water-soluble acid distribution in poly(lactide-co-glycolide). J Pharm Sci. 93:322-331 (2004).
39. A. Doolittle. The technology of solvents and plasticizers. Chapt. 12. Properties of individual solvents, Wiley, New York, 1954.
40. E.A. Grulke. Solubility parameter values. In J. Brandrup, E.H. Immergut, and E.A. Grulke (eds.), Polymer Handbook, John Wiley & Sons, Inc., Hoboken, 1999.

Tables

Table I: Solubility of fenretinide in selected organic solvents (S), solvent miscibility with water, and Hildebrand as well as Hansen solubility parameters.

Solvent (S)	Solubilities			Hildebrand[c]	Hansen[d]		
	drug/S (mg/ml)[a]	S/Water (%)[b]	Water/S (%)[b]	δ_t ($MPa^{1/2}$)	δ_d ($MPa^{1/2}$)	δ_p ($MPa^{1/2}$)	δ_h ($MPa^{1/2}$)
Methylene chloride (DCM)	2.5	1.32	0.20	20.3	17.0	7.3	7.1
Ethyl acetate (EA)	21	8.70	3.30	18.2	15.8	5.3	7.2
Chloroform	7	0.80	0.20	19.0	17.8	3.1	5.7
Methanol (MeOH)	36	miscible		29.7	14.7	12.3	22.3
Ethanol (EtOH)	47	miscible		26.6	15.8	8.8	19.4
1-Propanol (1-Prop)	36	miscible		24.6	16.0	6.8	17.4
2-Propanol (2-Prop)	37	miscible		23.5	15.8	6.1	16.4
Acetone	**100**	miscible		20.1	15.5	10.4	7.0
Acetonitrile (ACN)	3.8	miscible		24.6	15.3	18.0	6.1
Tetrahydrofurane (THF)	**> 200**	miscible		19.4	16.8	5.7	8.0
Dimethylformamide (DMF)	**> 99**	miscible		24.8	17.4	13.7	11.3
Dimethylsulfoxide (DMSO)	**> 95**	miscible		26.6	18.4	16.4	10.2
N-Methylpyrrolidone (NMP)	**150**	miscible		22.9	18.0	12.3	7.2

[a] Solubility as determined at room temperature (see section 2.2); [b] Solubility in % (wt./wt.) at 20 °C (methylene chloride: 25°C) according to (39); [c] Values according to (40); [d] Values according to the HSPiP software, 3rd edition.

Table II: Solubility[a] of fenretinide in methylene chloride : cosolvent mixtures

Methylene chloride : cosolvent ratio	drug solubility (mg/ml)	
	cosolvent MeOH	cosolvent THF
10 : 0	2.5	2.5
9.6 : 0.4	53	—
9.5 : 0.5	62	22
9.4 : 0.6	87	—
9.2 : 0.8	> 140	—
9 : 1	> 150	44
8.75 : 1.25	—	57
8.5 : 1.5	—	69
8.25 : 1.75	—	86
8 : 2	—	103
0:10	36	> 200

[a] Solubility as determined at room temperature (see section 2.2)

Legend to figures

Fig. 1: Plot of Hansen solubility parameters including contributions of dispersive (δ_d), polar (δ_p), and hydrogen-bonding forces (δ_h). Good solvents are typed in red. For fenretinide, the Hansen solubility parameters were either estimated from its structure by a group contribution method or fitted based on the values of good solvents (HSPiP software). According to the fit, methylene chloride (MC) was wrongly expected to be a good solvent. For explanation of solvent abbreviations see Table I.

Fig. 2: Solubility of fenretinide in aqueous media. a) Effect of temperature and polyvinyl alcohol (PVA) concentration (w/v) on drug solubility. b) Drug solubility as a function of Polysorbate 80 percentile concentration (v/v) in phosphate buffered saline (PBS) at 37 °C. Inset: Linear correlation between Polysorbate 80 concentration and drug solubility. (n=3, median, range).

Fig. 3: Particle size distribution as determined by the mass yield of fenretinide loaded s/o/w microparticles in different sieved fractions depending on the concentration of PLGA (Resomer® RG 503) in the o-phase (n=3, median, range).

Fig. 4: Effect of the PLGA (Resomer® RG 503) concentration in the o-phase on the particle morphology and fenretinide release behavior. SEM images are displayed for a) 15% PLGA, b) 20% PLGA, and c) 25% PLGA; d) Drug release of different microparticle formulations was compared to a physical mixture of fenretinide and PLGA in 1% (v/v) Polysorbate 80/PBS pH 7.4 at 37 °C (n=3, median, range). All particles were prepared with the s/o/w method and a size fraction of 20-45 µm was used.

Fig. 5: Effect of the PLGA (Resomer® RG 503) concentration in the o-phase on the particle morphology and fenretinide release behavior. SEM images are displayed for a) 25% PLGA, b) 30% PLGA, and c) 35% PLGA; d) Drug release of different microparticle

formulations was compared to a physical mixture of fenretinide and PLGA in 1% (v/v) Polysorbate 80/PBS (n=3, median, range). All particles were prepared with the s/o/w method and a size fraction of 20-90 µm was used.

Fig. 6: Microencapsulation of ground fenretinide. SEM of a) non-porous blank particles, b) porous blank particles (w_1: 5X PBS), c) non-porous drug loaded particles, and d) porous drug-loaded particles. Samples a-d were prepared with 25% Resomer® RG 503 in the o-phase. Panel e) shows the release of drug from different formulations (using ground drug) with altered polymer types, concentrations, or matrix porosities in 1% (v/v) Polysorbate 80/ PBS pH 7.4 at 37 °C. All data for 20-63 µm particles (n=3, median, range).

Fig. 7: Overview and details of the microstructure of 500 µl *in situ* implants after 3 days of incubation in PBS pH 7.4 at 37 °C. Implants were prepared from a) 15%, b) 25%, and c) 35% Resomer® RG 503 in N-methylpyrrolidone (NMP).

Fig. 8: Effect of PLGA type and concentration on the release of fenretinide from *in situ* forming implants in 1% v/v Polysorbate 80/ PBS pH 7.4 at 37 °C (n=3-5, median, range).

Fig. 9: Representative shapes of fenretinide loaded implants at day 32 of the release study in 1% (v/v) Polysorbate 80/ PBS pH 7.4 at 37 °C. Implants were prepared from a) 30%, b) 50%, and c) 70% Resomer® RG 502 in NMP or from d) 15%, e) 25%, and f) 35% Resomer® RG 503 in NMP. The relative intensity of the yellow color roughly reflects the relative drug remaining in the implant.

Figures

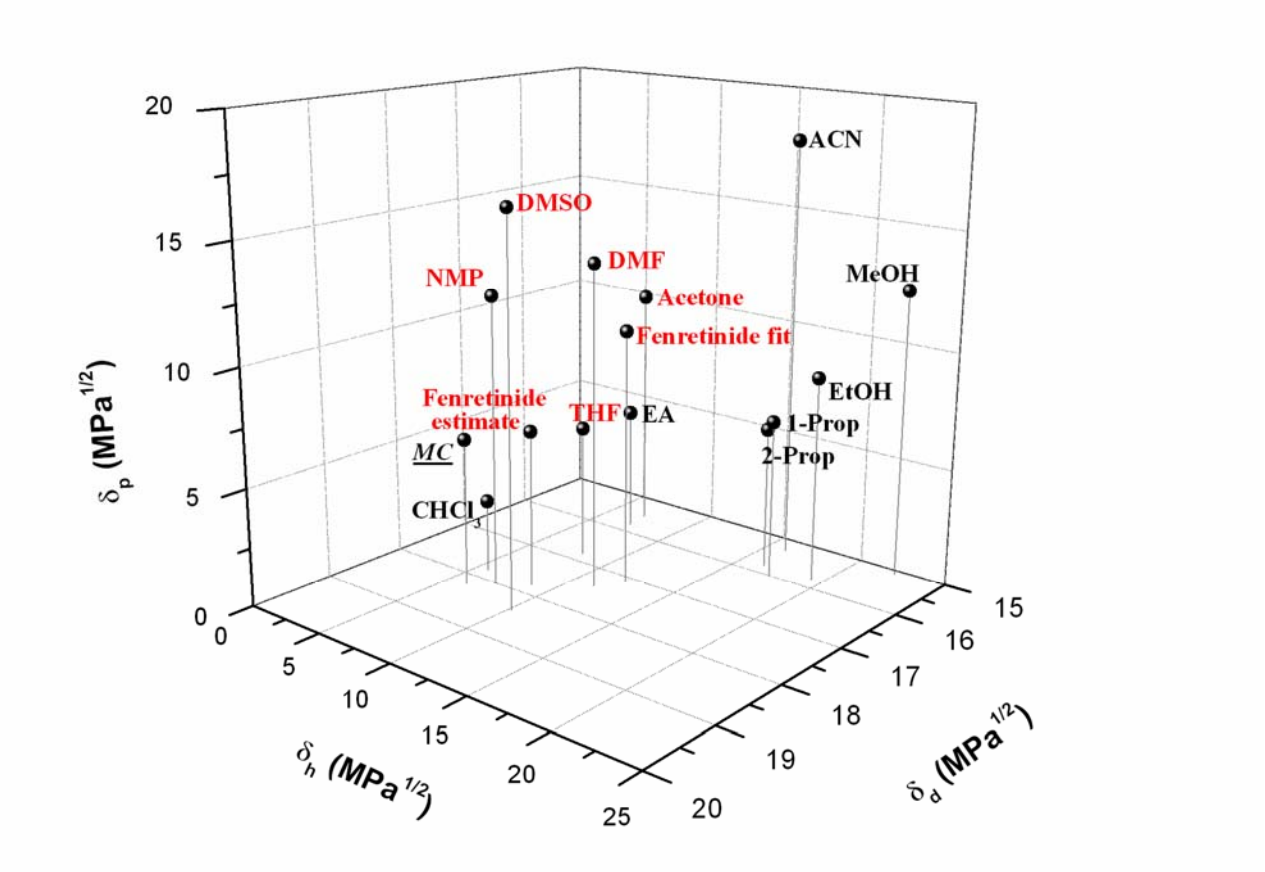

Figure 1

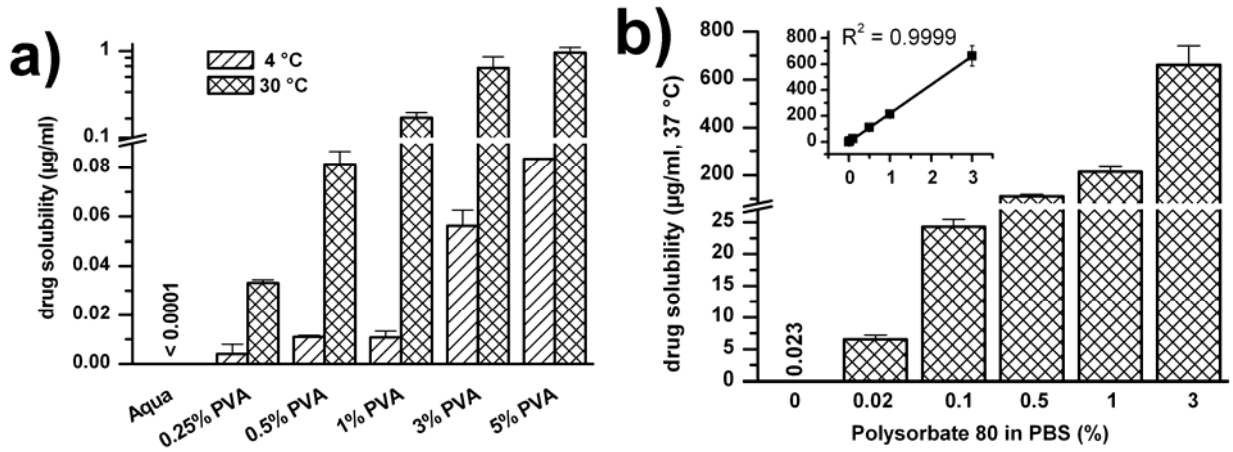

Figure 2

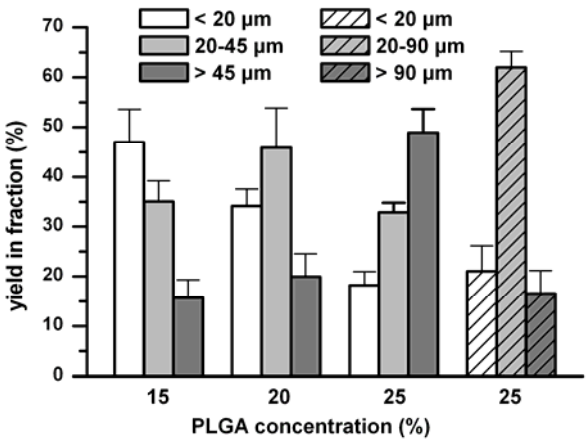

Figure 3

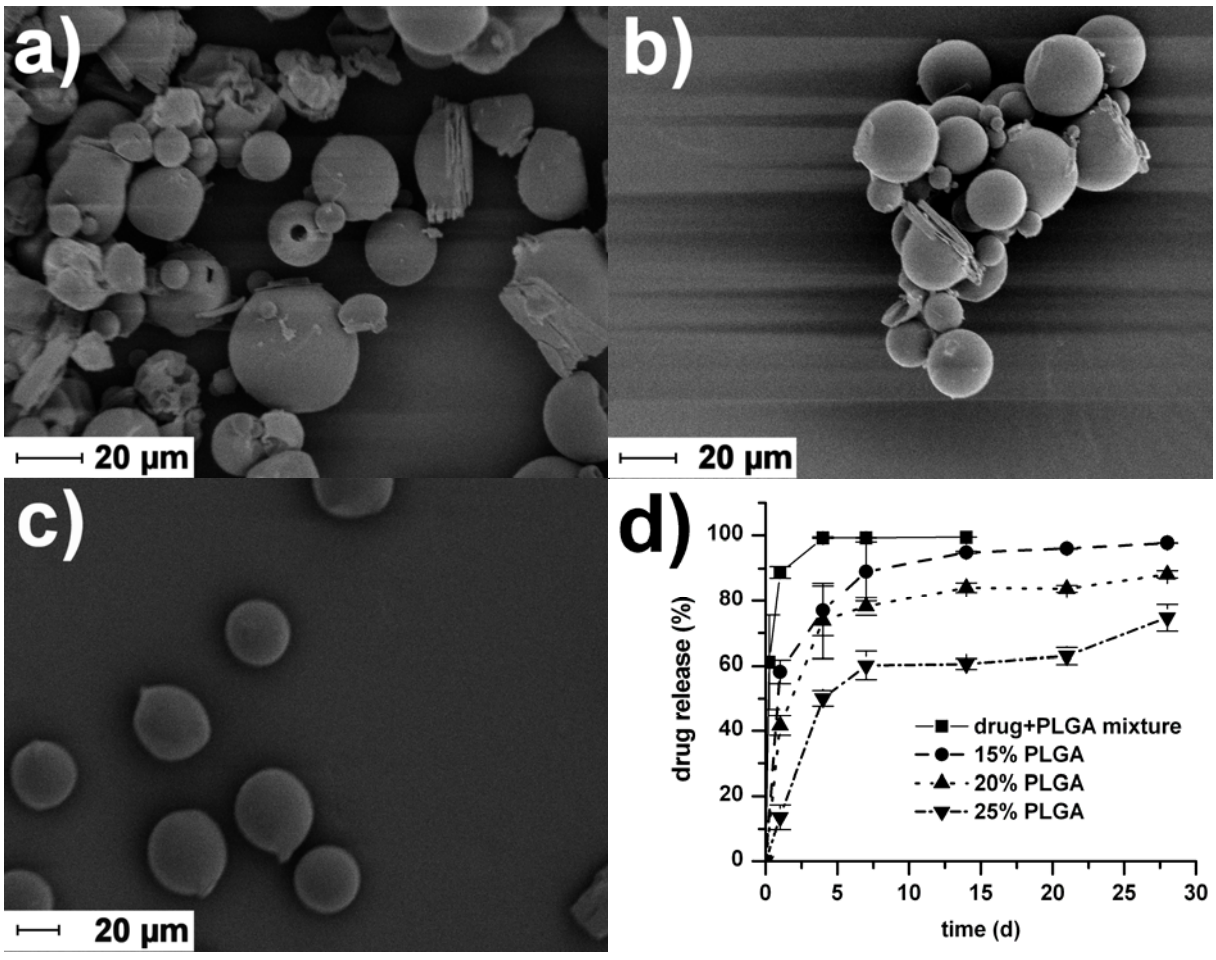

Figure 4

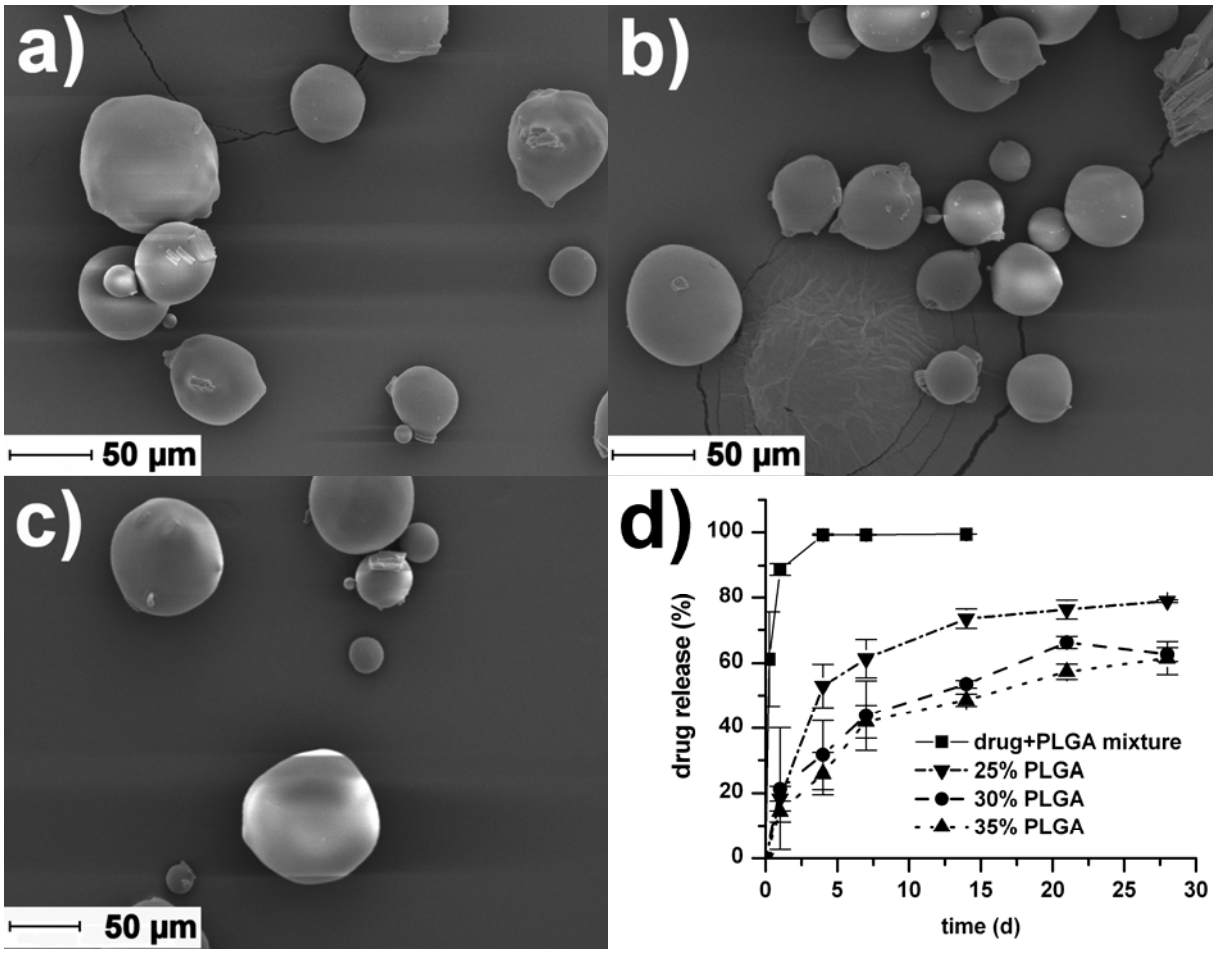
a)
50 µm
b)
50 µm
c)
50 µm
d)
drug release (%)
time (d)
drug+PLGA mixture
25% PLGA
30% PLGA
35% PLGA

Figure 5

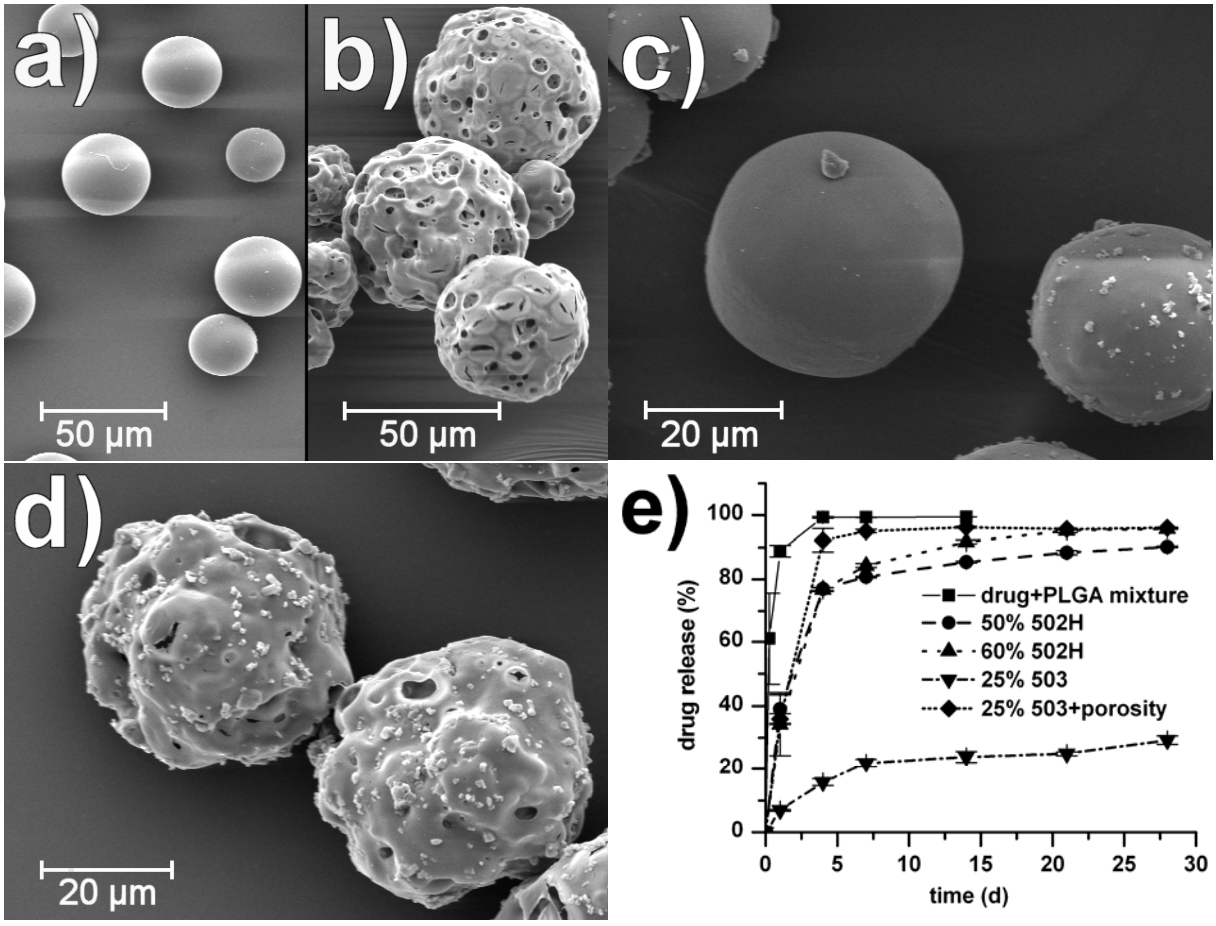

Figure 6

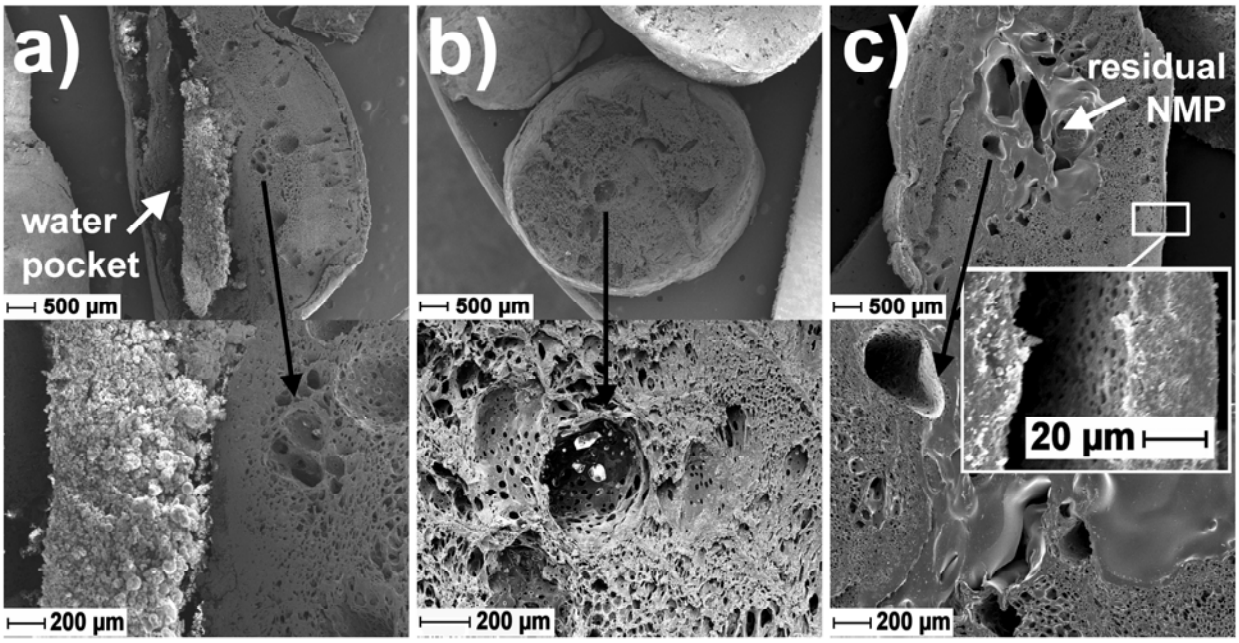

Figure 7

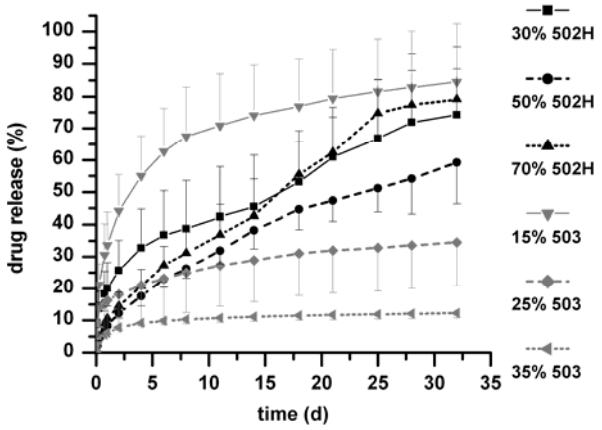

Figure 8

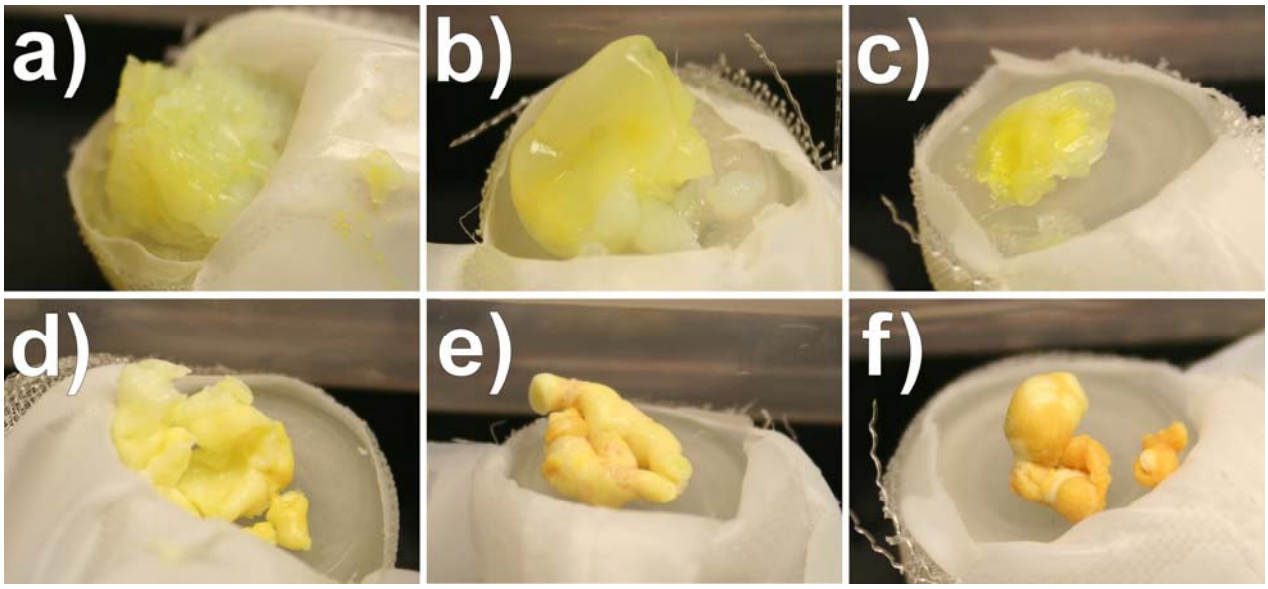

Figure 9