

Paclitaxel-loaded microparticles and implants for the treatment of brain cancer: Preparation and physicochemical characterization

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Abstract

The aim of this study was to prepare different types of paclitaxel-loaded, PLGA-based microparticles and lipidic implants, which can directly be injected into the brain tissue. Releasing the drug in a time-controlled manner over several weeks, these systems are intended to optimize the treatment of brain tumors. The latter is particularly difficult because of the blood–brain barrier (BBB), hindering most drugs to reach the target tissue upon systemic administration. Especially paclitaxel (being effective for the treatment of ovarian, breast, lung and other cancers) is not able to cross the BBB to a notable extent since it is a substrate of the efflux transporter P-glycoprotein. Both, biodegradable microparticles as well as small, cylindrical, glycerol tripalmitate-based implants (which can be injected using standard needles) were prepared with different paclitaxel loadings. The effects of several formulation and processing parameters on the resulting drug release kinetics were investigated in phosphate buffer pH 7.4 as well as in a diethylnicotinamide (DENA)/phosphate buffer mixture. Using DSC, SEM, SEC and optical microscopy deeper insight into the underlying drug release mechanisms could be gained. The presence of DENA in the release medium significantly increased the solubility of paclitaxel, accelerated PLGA degradation, increased the mobility of the polymer and drug molecules and fundamentally altered the geometry of the systems, resulting in increased paclitaxel release rates.

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1. Introduction

Malignant gliomas represent 13–22% of the brain cancers. Regardless of the treatment method the median survival time is less than 1 year (Benoit et al., 2000). Despite surgery, external beam radiation therapy and systemic chemotherapy, these tumors tend to recur within centimeters of their original location. The low efficiency of systemic chemotherapy is due to the presence of the blood–brain barrier (BBB) (Hammarlund-Udenaes et al., 1997). Only low molecular weight, lipid-soluble molecules and a few peptides and nutrients can cross the BBB to a significant extent, either by passive diffusion or using specific transport mechanisms. Thus, for most drugs it is difficult to

achieve therapeutic levels at the target site. Paclitaxel in particular is not able to cross the BBB to a notable extent (Heimans et al., 1994; Sparreboom et al., 1996; Gallo et al., 2003), because it is a substrate of the efflux transporter P-glycoprotein (P-gp) (Kemper et al., 2003; Rice et al., 2003). Furthermore, highly potent drugs which are to be delivered to the Central Nervous System, often cause serious toxic side effects when administered systemically.

Various approaches have been proposed to overcome these restrictions (Tamargo and Brem, 1992; Pardridge, 1997), including the: (i) administration of the drug directly into the brain tissue (intracranially), (ii) disruption of the BBB using intracarotid administration of vasoactive molecules such as bradykinin or hypertonic solutions, (iii) creation of prodrugs in which water-soluble drugs are attached by cleavable bonds to lipid-soluble carriers such as dihydropyridine, (iv) attachment of the drug to a vector that crosses the BBB, such as 83–14 murine monoclonal

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antibody to the human insulin receptor, (v) intravenous injection of surfactant-coated nanoparticles (Kreuter et al., 1997), (vi) infusion pumps delivering drugs into the cerebrospinal fluid, (vii) implantation of tissue or cells into the brain, and (viii) gene therapy. The first approach (intracranial drug administration) has shown very promising results. However, its major disadvantage is the elevated risk of infections. Generally, the half-life of the drug within the brain tissue is short, and frequent injections are required to assure sufficiently high drug levels over prolonged periods of time. An interesting possibility to overcome this restriction is the intracranial administration of controlled drug delivery systems, such as microparticles and implants. The latter are stereotaxically injected, directly into the targeted brain regions. As the release of the incorporated drug from these systems can be controlled over periods of weeks to months, one or two administrations are generally sufficient. Thus, the risk of infections can significantly be reduced (Benoit et al., 2000).

Different types of drugs, including 5-fluorouracil (5-FU) and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) have been incorporated into such intracranial controlled drug delivery systems (Brem and Gabikian, 2001). GliadelTM was the first product of this type available on the market (Brem et al., 1995a,b; Valtonen et al., 1997; Westphal et al., 2000). It consists of flat poly[bis(*p*-carboxyphenoxy) propane-*co*-sebacic acid]-based discs loaded with BCNU. These discs are placed onto the wall of the resection cavity of the tumor. 5-FU-loaded, poly(lactic-*co*-glycolic acid) (PLGA)-based microparticles have been proposed by the groups of Benoit and Menei (Menei et al., 1999, 2004, 2005). They can either be injected into the wall of the resection cavity (if the tumor is operable), or into the tumor itself (if the latter is inoperable). Clinical trials with both types of treatment methods showed promising results (Menei et al., 2004, 2005). Nevertheless, since not all tumors respond to these drugs (e.g., due to DNA-repair enzyme-mediated resistances), there is still a significant need to identify further drugs which can be used for this type of local brain tumor treatment.

Paclitaxel is a potent anti-cancer drug and promising candidate. It promotes the assembly of microtubules (Schiff et al., 1979). Importantly, the microtubules which are formed in the presence of paclitaxel are abnormally stable, resulting in a loss of the natural microtubules' dynamics. As the latter are essential for cell division and other vital processes, the cells die. Paclitaxel has shown to be effective for the treatment of different types of tumors, including ovarian, breast and lung cancer (Harper et al., 1999; Johnston, 2004; Watatani et al., 2004). The group of Horikoshi proposed PLGA-based, paclitaxel-loaded microparticles (which are intravenously injected) for the treatment of lung cancer (Sato et al., 1996; Wang et al., 1996, 1997). Isopropyl myristate was added to the microparticles to adequately increase the resulting drug release rate. Helen Burt and co-workers (Dordunoo et al., 1995; Liggins et al., 2000; Liggins and Burt, 2001) encapsulated paclitaxel into poly(ϵ -caprolactone)- and poly(L-lactic acid) (PLA)-based microparticles and studied the ability of the systems to prevent tumor growth in the peritoneal cavity of rats.

One of the major challenges when developing paclitaxel formulations is the very low water-solubility of the drug: 0.30 $\mu\text{g/ml}$ at 37 °C (Lee et al., 2003). Cremophor[®] EL (polyoxyethylated castor oil) and ethanol are used to dissolve it in the commercially available product Taxol[®]. However, severe side effects (in particular hypersensitivity reactions) can be caused by Cremophor[®] EL (Paradis and Page, 1998). To avoid this risk, the group of Kinam Park synthesized several potential hydrotropes for paclitaxel (Lee et al., 2003). *N,N*-diethylnicotinamide (DNA) was found to be particularly efficient to increase the solubility of paclitaxel. In a recent study, they proposed an interesting accelerated *in vitro* drug release test based on the presence of DNA in the release medium. Significantly higher release rates from thin, paclitaxel-loaded, PLGA-based films were observed in DNA/phosphate buffer mixtures compared to phosphate buffer alone.

The major aim of the present study was to prepare and physicochemically characterize different types of paclitaxel-loaded, PLGA-based microparticles and lipidic implants, which can be directly injected into the brain tissue. The effects of several formulation and processing parameters on the resulting drug release kinetics were to be investigated in pure phosphate buffer pH 7.4 as well as in a DNA/phosphate buffer mixture. Using optical and scanning electron microscopy, size exclusion chromatography and differential scanning calorimetry also deeper insight into the underlying drug release mechanisms was to be gained.

2. Materials and methods

2.1. Materials

Paclitaxel (Zyo Pharma Trade, Hamburg, Germany), poly(D,L-lactic-*co*-glycolic acid) (PLGA; Resomer RG 503; PLGA 50:50; containing 25% D-lactic units, 25% L-lactic units and 50% glycolic units; Boehringer Ingelheim Pharma KG, Ingelheim, Germany), glycerol tripalmitate (Dynasan[®] 116; Sasol GmbH, Witten, Germany), *N,N*-diethylnicotinamide (DNA; Sigma–Aldrich, Taufkirchen, Germany), polyethylene sorbitan monooleate (Tween 80; Sigma–Aldrich Chemie GmbH, Steinheim, Germany), polyvinyl alcohol (PVA, Mowiol 40–88; Aventis SA, Frankfurt/Main, Germany).

2.2. Microparticle preparation

Drug-free and paclitaxel-loaded, PLGA-based microparticles were prepared with an oil-in-water (O/W) solvent extraction/evaporation method. Four different types of paclitaxel-loaded systems were prepared, differing in the theoretical drug loading (20 or 40% w/w) and PVA concentration in the outer aqueous phase during microparticle preparation (1 and 5% w/v). The exact amounts of polymer and drug used for the preparation of each type of system are indicated in Table 1.

Either 160 or 120 mg PLGA was dissolved in 5 ml methylene chloride (magnetic stirrer: 400 rpm). Then, 40 or 80 mg paclitaxel was dissolved in this organic phase. The latter was emulsified into an outer aqueous PVA solution (100 ml; 1 or 5% w/v as indicated in Table 1; magnet stirrer: 600 rpm, 3 min).

Table 1
Formulation parameters and characteristics of the investigated paclitaxel-loaded, PLGA-based microparticles

PVA (%)	Theoretical loading (%)	PLGA (mg)	Paclitaxel (mg)	Practical loading, % (\pm s)	Encapsulation efficiency, % (\pm s)	Particle size, μ m (\pm s)
1	20	160	40	18.0 (\pm 0.1)	90.0 (\pm 0.3)	32.2 (\pm 12.9)
	40	120	80	35.8 (\pm 0.1)	89.4 (\pm 0.2)	33.8 (\pm 15.1)
5	20	160	40	17.2 (\pm 0.1)	86.0 (\pm 0.3)	18.8 (\pm 10.0)
	40	120	80	33.0 (\pm 1.5)	82.5 (\pm 3.7)	17.3 (\pm 8.50)

Upon contact with the outer aqueous phase, the organic solvent diffused into the water. Due to convection (and diffusion) the methylene chloride was distributed throughout the aqueous phase and evaporated at its surface. Thus, the organic solvent's concentration in the inner phase of the emulsion decreased with time, and the polymer concentration increased. At a certain time point, the PLGA precipitated and encapsulated the drug: the microparticles were formed. The latter were hardened by adding 500 ml de-ionized water and further stirring (600 rpm, 2 min). The microparticles were separated by filtration under nitrogen pressure, washed with de-ionized water, re-suspended in 500 μ l de-ionized water, frozen in liquid nitrogen and freeze-dried (Freeze-drier RP2V; Serail, Argenteuil, France). Drug-free microparticles were prepared accordingly without adding paclitaxel.

2.3. Implant preparation

Two types of glycerol tripalmitate-based, paclitaxel-loaded implants were prepared, differing in the initial drug loading (29 and 60% w/w, respectively). The implants (weight = 6.0 ± 0.5 mg) consisted only of paclitaxel and glycerol tripalmitate. The drug and lipid (in total 100 mg) were dissolved in 25 ml chloroform. This solution was filtered (sterile PTFE filter, 0.2 μ m pore size; Corning, New York, USA), frozen in liquid nitrogen and freeze-dried under aseptic conditions. The obtained dry powder was compressed at 250 N (for 10 s) using a self-made, manual compression tool [with cylindrical matrices (diameter = 2 mm) and a hydraulic press (Perkin-Elmer, Rodgau-Jügesheim, Germany)]. The sterility of the implants was evaluated according to the European Pharmacopoeia (all tested samples were found to be sterile).

2.4. Determination of the practical drug loading

In the case of PLGA-based microparticles, 2–3 mg samples were dissolved in 1 ml methylene chloride (1 min vortex mixing and 3 min ultrasonication). A 10 ml of a 50:50 (v/v) mixture of acetonitrile and water was added to this solution, and the latter vortexed for 1 min. A nitrogen stream was introduced to evaporate the methylene chloride at room temperature, resulting in the precipitation of PLGA. Further 35 ml of a 50:50 (v/v) acetonitrile:water mixture was added and the sample placed in an ultrasound bath for 5 min and then centrifuged at 4000 rpm for 10 min. The content of paclitaxel and of its major degradation product 7-epi-taxol (Baek et al., 2004) in the supernatant was determined by HPLC analysis: 10 μ l were injected

into an Zorbax SB–CN column (5 μ m, 250 mm \times 4.6 mm). A 45:55 (v/v) mixture of methanol and water was used as mobile phase (flow rate: 1 ml/min). The column effluent was detected UV spectrophotometrically ($\lambda = 227$ nm). Analogous to Baek et al. (2004), the peaks of paclitaxel and 7-epi-taxol were considered when calculating the percentage of drug release. The percentage of 7-epi-taxol did never exceed 20% referred to the total “paclitaxel + 7-epi-taxol” content. For reasons of simplicity, only paclitaxel is mentioned in the following. Experiments with spiked samples showed that all drug could be recovered using this method.

In the case of glycerol tripalmitate-based implants, 5–9 mg samples were dissolved in 1 ml methylene chloride (1 min vortex mixing and 3 min ultrasonication). A 10 ml of a 50:50 (v/v) mixture of acetonitrile and water was added to this solution, and the latter again vortexed for 1 min. A nitrogen stream was introduced to evaporate the methylene chloride at room temperature, resulting in the precipitation of the glycerol tripalmitate. Further 35 ml of a 50:50 (v/v) acetonitrile:water mixture was added and the sample placed in an ultrasound bath for 5 min and then centrifuged at 4000 rpm for 10 min. The drug content in the supernatant was measured by HPLC analysis as described above. Experiments with spiked samples showed that all drug could be recovered using this method.

2.5. In vitro drug release studies

Drug release was measured in two types of media: (i) pure phosphate buffer pH 7.4, and (ii) a 2 M solution of DENA in phosphate buffer pH 7.4. To provide sink conditions [paclitaxel solubilities at 37 $^{\circ}$ C: 0.45 μ g/ml in phosphate buffer pH 7.4; 980 μ g/ml in 2 M DENA in phosphate buffer pH 7.4 (Baek et al., 2004)], approximately 2 mg microparticles/one implant were/was suspended in 45 ml/500 ml phosphate buffer pH 7.4, and approximately 10 mg microspheres/one implant were/was exposed to 40 ml DENA/phosphate buffer in screw-capped tubes/bottles. The latter were placed in a horizontal shaker (80 rpm, 37 $^{\circ}$ C; GFL 3033; Gesellschaft für Labortechnik GmbH & Co., KG, Burgwedel, Germany).

In the case of pure phosphate buffer pH 7.4, the microparticle-containing tubes were centrifuged (4000 rpm, 10 min) after 1, 3, 7, 14, 21 and 28 d and the supernatant was completely replaced with fresh medium. Analogously (but without centrifugation), the release medium of bottles containing implants was completely exchanged with fresh medium after 1, 3, 7, 14 and 21 d. As the drug concentrations in the buffer were very low, the amount of paclitaxel released was indirectly determined by

measuring the amount of drug remaining within the microparticles/implants. At pre-determined time points, tubes/bottles were withdrawn ($n = 3$) and the supernatant removed (in the case of microparticles after centrifugation at 4000 rpm for 10 min). The remaining microparticles/implants were dried and their drug content determined as described in Section 2.4.

In the case of DENA/phosphate buffer, the drug concentrations in the release medium were sufficiently high to allow accurate direct measurements: at pre-determined time points, 0.5 ml samples were withdrawn (replaced with fresh medium) and their drug content measured by HPLC analysis as described in Section 2.4.

Furthermore, drug-free microparticles were exposed to both types of release media (as described above). At pre-determined time points, tubes were withdrawn and centrifuged (4000 rpm, 10 min). The supernatant was removed, the remaining microparticles dried and stored for further analysis (DSC, SEC and optical microscopy).

2.6. Determination of the average polymer molecular weight

The average polymer molecular weight of PLGA was determined by size exclusion chromatography (SEC). Samples were dissolved in dimethylsulfoxide (0.5% w/v). One volume part of this solution was mixed with three volume parts of the mobile phase (tetrahydrofurane:methanol:acetic acid, 85:15:0.8 v/v/v). Approximately 200 μ l of this mixture were injected into a size exclusion (gel permeation) chromatography apparatus equipped with a precolumn (Shodex KGF, Waters, Saint Quentin en Yvelines, France), two main columns (Styragel HR 1, Waters; PL-gel 5 μ m 10^E 4A, Polymer Laboratories, Marseille, France) and a refractometric detector (RID-10A, Shimadzu, Touzart et Matignon, Courtaboeuf, France). All measurements were performed at a flow rate of 1 ml/min at room temperature. The system was calibrated with polystyrene standards (PS-2, Polymer Laboratories, Marseille, France). The indicated molecular weights are weight-average molecular weights (M_w).

2.7. Determination of the glass transition temperature

The glass transition temperature of the polymer (T_g) was determined by differential scanning calorimetry (DSC, DSC821e; Mettler Toledo, Viroflay, France). Approximately 5 mg samples were heated in sealed aluminum pans (investigated temperature range: -30 to $+330$ °C, heating rate: 10 °C/min, two heating cycles).

2.8. Optical microscopy studies

The morphology of PLGA-based microparticles was studied by optical microscopy (Olympus BH2; OSI, Tokyo, Japan). Samples were dispersed in a water droplet on a glass slide and immediately observed (video camera COHU; computerized image analyzer: Microvision Instruments, Evry, France).

2.9. Particle size analysis

Mean particle sizes were determined using a Coulter Counter (Multisizer, Coultronics, Margency, France). Microspheres (approximately 10 mg) were suspended by ultrasonication for 10 min in an aqueous solution of Tween 80 (0.02% w/v) and assayed after dilution in Isoton[®] II (Coultronics, Margency, France).

3. Results and discussion

3.1. Paclitaxel release from lipidic implants

As it can be seen in Fig. 1, paclitaxel release from glycerol tripalmitate-based implants (initial loading = 29% w/w) was very slow in phosphate buffer pH 7.4: except from an initial burst of approximately 12%, almost no drug was released within the first 3 weeks. The burst effect can be attributed to the release of drug present in cavities which are directly connected to the surface of the implants. The release of further paclitaxel was effectively slowed down by the lipidic matrix. In contrast, drug release was much faster in a mixture of DENA and phosphate buffer pH 7.4 (Fig. 2). Within only 1 week, approximately 73 and 87% paclitaxel were released (depending on the initial drug loading). This can at least partially be attributed to the tremendous increase in drug solubility [by a factor >2000: from 0.45 μ g/ml in pure phosphate buffer pH 7.4 to 980 μ g/ml in 2 M DENA/phosphate buffer (Baek et al., 2004)]. Diffusional mass transport can be expected to play a major role in the control of drug release from this type of delivery system (Guse et al., 2006). Thus, the dramatic increase in paclitaxel solubility results in a significant increase in the concentration of dissolved drug within the implants. Consequently, the paclitaxel gradients (being the driving forces for diffusion) steeply increase, resulting in increased drug release rates.

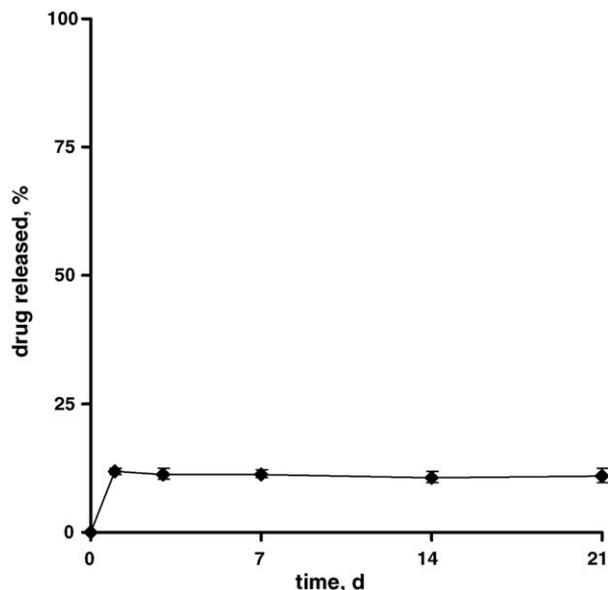


Fig. 1. In vitro release of paclitaxel from glycerol tripalmitate-based, cylindrical implants in phosphate buffer pH 7.4 (initial loading = 29% w/w).

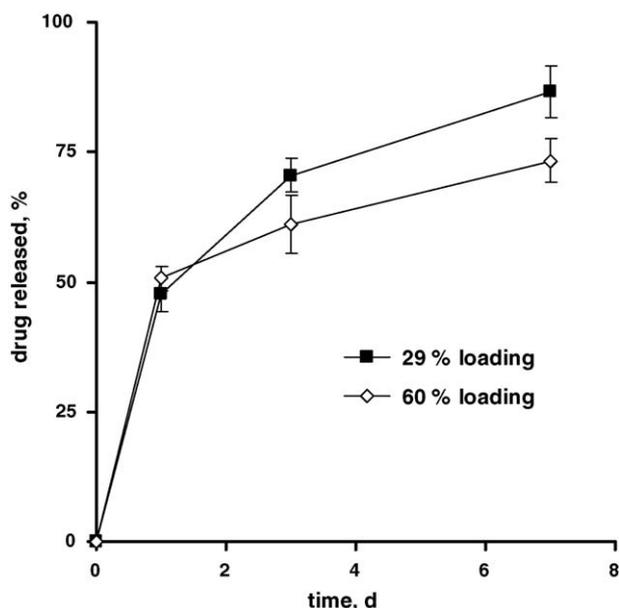


Fig. 2. In vitro release of paclitaxel from glycerol tripalmitate-based implants in DENA/phosphate buffer (the initial drug loading is indicated in the figure).

Fig. 2 also shows that the relative paclitaxel release rate slightly decreased with increasing initial drug loading. This can probably be attributed to the (still) limited solubility of paclitaxel (980 $\mu\text{g}/\text{ml}$ in 2 M DENA/phosphate buffer): as the initial drug loading is rather high (29 and 60% w/w, respectively), not all of the drug is immediately dissolved upon imbibition of the release medium. Thus, dissolved and un-dissolved paclitaxel co-exist in the matrix. Importantly, only dissolved drug is available for diffusion. Thus, increasing the initial drug loading from 29 to 60% w/w does not increase the concentration of drug that can diffuse out of the implant. Consequently, the absolute paclitaxel release rate remains about constant, while the 100% reference value for complete drug release increases. Hence, the relative paclitaxel release rate decreases. It has to be pointed out that also the porosity of the lipidic matrix upon drug exhaust increases and that this phenomenon is more pronounced at high initial drug contents. Increased matrix porosities lead to increased drug mobilities and, thus, increased absolute and relative release rates with increasing drug loadings. This effect competes with the above described “limited drug solubility effect”. Obviously, the latter predominates in the present system.

3.2. Encapsulation efficiency and size of PLGA-based microparticles

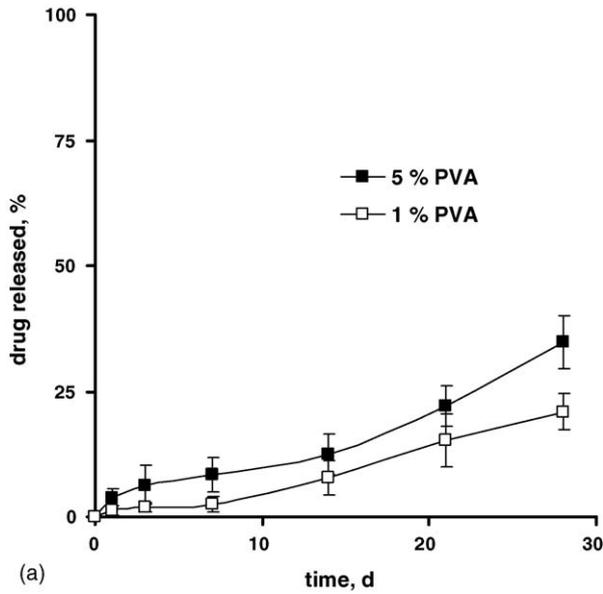
The encapsulation efficiency of paclitaxel in the PLGA-based microspheres was rather high (82.5–90.0%), irrespective of the theoretical drug loading and PVA concentration in the outer aqueous phase during microparticle preparation (Table 1). This can be explained by the high lipophilicity of the drug, minimizing paclitaxel loss into the external water phase. Clearly, the PVA concentration in the latter had a significant effect on the resulting microparticle size: At 1% (w/v) PVA, much larger microparticles

were obtained (mean size = 32–34 μm) than at 5% (w/v) PVA (mean size = 17–19 μm). This can be attributed to the more pronounced decrease in surface tension between the organic and aqueous phase during microparticle preparation (PVA acting as a surfactant) and to the increase in viscosity of the external water phase (PVA acting as a viscosity inducing agent) with increasing PVA concentration. Both effects result in a decrease in droplet size of the organic phase and, thus, in decreased amounts of polymer and drug per microparticle. The decrease in microparticle size with increasing PVA concentration in the outer aqueous phase has an important consequence for the resulting encapsulation efficiencies of paclitaxel: the decrease in particle size leads to a decrease in the length of the diffusion pathways and, hence, to an increase in drug loss into the external water phase during microparticle preparation. Irrespective of the theoretical drug loading, the encapsulation efficiency of smaller microparticles (prepared with 5% PVA in the outer aqueous phase) was lower than that of larger microparticles (prepared with 1% PVA) (Table 1).

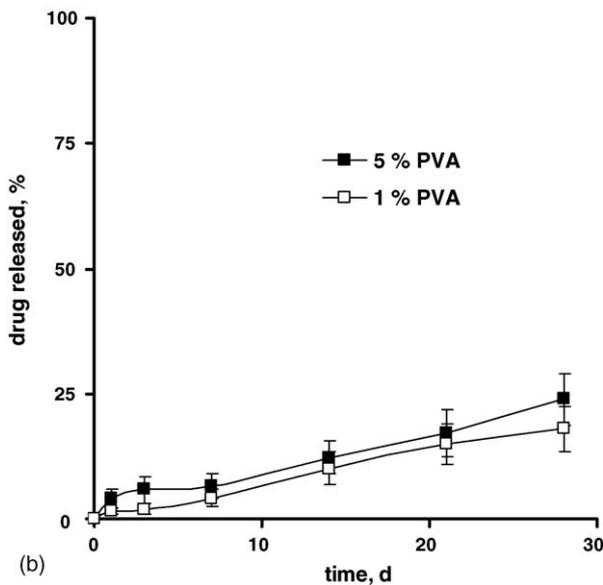
3.3. Paclitaxel release from PLGA-based microparticles

Fig. 3a and b shows the observed paclitaxel release kinetics in phosphate buffer pH 7.4 from PLGA-based microparticles with 20 and 40% theoretical drug loading, respectively. Similar to the glycerol tripalmitate-based implants, drug release was very slow: less than 35% paclitaxel was released within the first 4 weeks, irrespective of the type of system. Furthermore, the effects of the PVA concentration in the outer aqueous phase during microparticle preparation on drug release are shown in Fig. 3. Irrespective of the initial drug content, the relative release rate slightly increased with increasing PVA concentration. This can at least partially be explained by the decrease in microparticle size, resulting in decreased diffusion pathway lengths. However, the shape of the observed curves indicates that paclitaxel release from the investigated PLGA-based microparticles is not purely diffusion-controlled (the release rate from purely diffusion-controlled drug delivery systems monotonically decreases with time). Interestingly, very similar drug release patterns were observed for the investigated two theoretical drug loadings (20 and 40%; corresponding to 17–18% and 33–36% practical drug loading) (Fig. 3a versus b). Thus, also the limited solubility of paclitaxel cannot be the solely drug release rate controlling mechanism (if this was the case, a significant decrease in the relative drug release rate should have been observed when doubling the initial drug content, for the same reasons as discussed above with lipidic implants).

Importantly, the addition of DENA to the release medium tremendously increased the resulting paclitaxel release rate (Fig. 4 versus Fig. 3) (please note the different scaling of the x -axes). Irrespective of the PVA concentration in the outer aqueous phase during microparticle preparation and the theoretical drug loading, paclitaxel release was complete within only 10 d. Similar to drug release in pure phosphate buffer pH 7.4, there was no major effect of the theoretical (and practical) drug loading on the resulting paclitaxel release rate (Fig. 4a versus b). Furthermore, the increase in PVA concentration in the



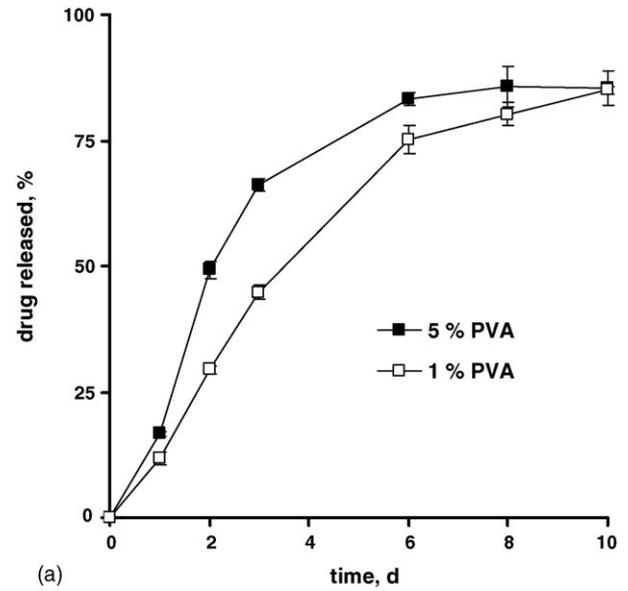
(a)



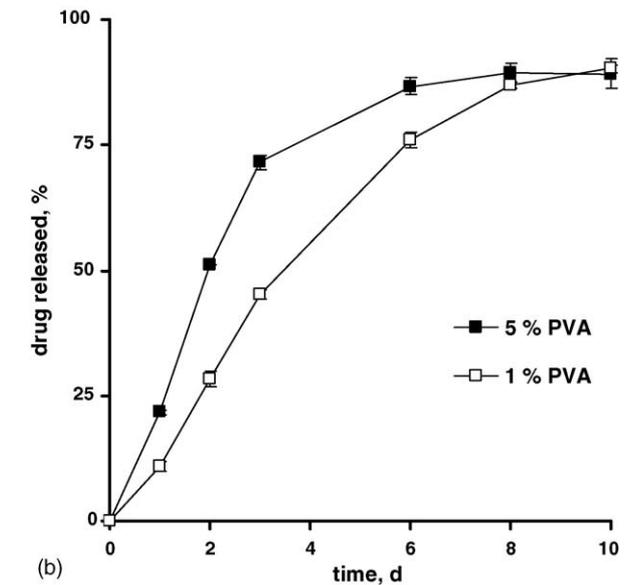
(b)

Fig. 3. Paclitaxel release from PLGA-based microparticles in phosphate buffer pH 7.4: (a) 20% theoretical drug loading and (b) 40% theoretical drug loading (the PVA concentration in the outer aqueous phase during microparticle preparation is indicated in the figures).

outer aqueous phase during microparticle preparation resulted in a moderate increase in the relative drug release rate. Analogous to paclitaxel release in pure phosphate buffer, this can (at least partially) be attributed to the decrease in microparticle size, resulting in decreased diffusion pathway lengths. As in the case of the glycerol tripalmitate-based implants, the tremendous increase in drug release upon addition of DENA can (at least partially) be attributed to the more than 2000-fold increase in drug solubility. However, the presence of DENA can be expected to have also other effects, e.g., on the degradation behavior of the matrix former PLGA (Baek et al., 2004). To better understand the involved phenomena, size exclusion chromatography, differential scanning calorimetry and optical microscopy were used to monitor changes in the physicochem-



(a)



(b)

Fig. 4. Paclitaxel release from PLGA-based microparticles in DENA/phosphate buffer: (a) 20% theoretical drug loading and (b) 40% theoretical drug loading (the PVA concentration in the outer aqueous phase during microparticle preparation is indicated in the figures).

ical properties of the investigated microparticles upon exposure to pure phosphate buffer and to DENA/phosphate buffer, respectively.

3.4. Effects of the presence of DENA on PLGA degradation and microparticle morphology

As it can be seen in Fig. 5, the decrease in the average polymer molecular weight of PLGA (M_w) was much more rapid in DENA/phosphate buffer than in pure phosphate buffer. In the latter case, the average polymer molecular weight decreased from 40 to 34 kDa within the first week. This is in good agreement with results reported on other types of PLGA-based drug delivery systems (Charlier et al., 2000). In contrast, a drastic

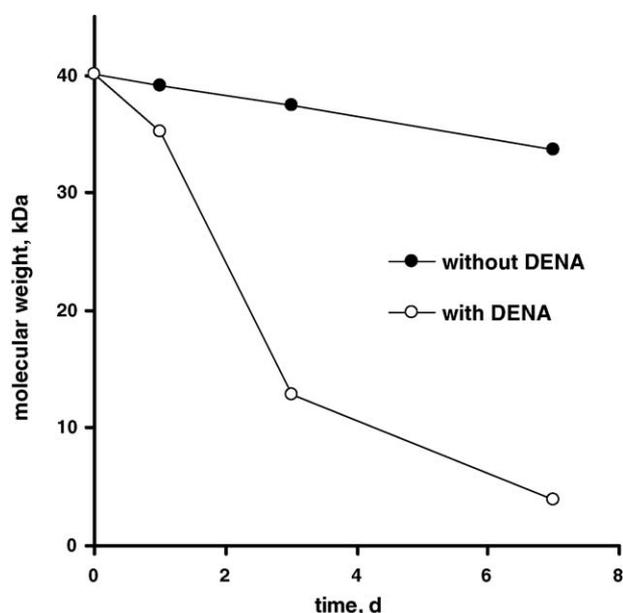


Fig. 5. Effects of the presence of DENA in the release medium (phosphate buffer) on the decrease in the average polymer molecular weight (M_w) of PLGA-based microparticles (measured by size exclusion chromatography).

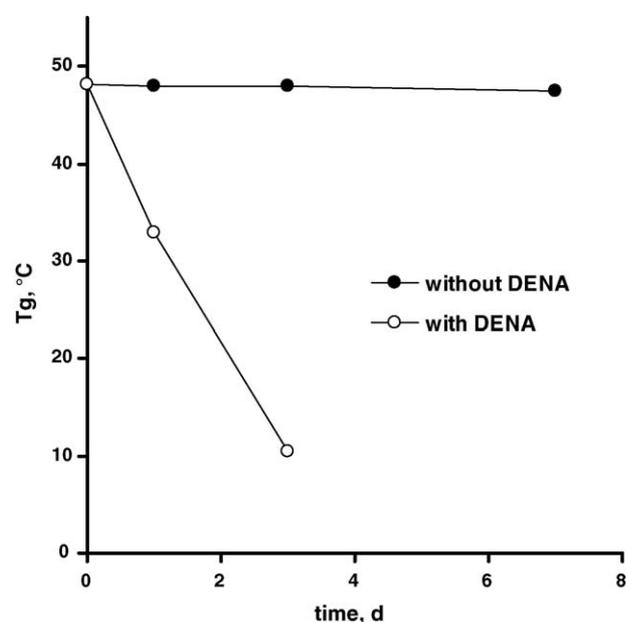


Fig. 6. Effects of the presence of DENA in the release medium (phosphate buffer) on the decrease in the glass transition temperature (T_g) of PLGA-based microparticles (measured by differential scanning calorimetry).

decrease (from 40 to 4 kDa after 7 d exposure) was observed in DENA/phosphate buffer. This can be explained by the basic nature of DENA and the fact that PLGA chain cleavage is induced by bases (ester hydrolysis). Baek et al. (2004) observed similar effects with thin, PLGA-based films. The significant difference in polymer degradation kinetics in the presence/absence of DENA has important consequences for the resulting drug release patterns: with decreasing average molecular weight, the degree of polymer chain entanglement decreases and, thus, the mobility of the macromolecules increases. According to the free volume theory of diffusion, this results in increased drug diffusivities and, thus, increased absolute and relative release rates.

The glass transition temperature (T_g) of a polymer can be used as a measure for the mobility of the macromolecules. A decrease in T_g corresponds to an increase in the mobility of the polymer chains. Fig. 6 shows the experimentally determined decrease in the glass transition temperature of PLGA in the investigated microparticles upon exposure to pure phosphate buffer pH 7.4 and DENA/phosphate buffer, respectively. Clearly, the T_g decreased very rapidly in the latter case (due to accelerated polymer degradation, Fig. 5), whereas it remained about constant within the observation period in pure phosphate buffer pH 7.4. This has important consequences for the mobility of the paclitaxel molecules within the polymeric matrices: the diffusion coefficient of the drug can be expected to be much higher if DENA is present in the release medium (and penetrates into the microparticles). Thus, the observed significant differences in paclitaxel release in pure phosphate buffer versus DENA/phosphate buffer (Fig. 3 versus Fig. 4) cannot only be attributed to the difference in drug solubility, but at least partially also to a marked increase in polymer chain and drug mobility in the microspheres.

It has to be pointed out that dried microparticles were used for the DSC measurements to determine the glass transition temperatures shown in Fig. 6. In wet systems, the T_g can be expected to be lower due to the plasticizing effect of water. It has recently been shown that the glass transition temperature in PLGA-based microparticles rapidly decreases below 37 °C upon water imbibition (Faisant et al., 2002). Thus, the polymer is in the rubbery state during drug release (resulting in much higher diffusion rates compared to the glassy state), irrespective of the type of release medium.

The fundamental increase in polymer chain mobility upon exposure to DENA/phosphate buffer [indicated by the polymer degradation kinetics (Fig. 5) and changes in glass transition temperature (Fig. 6)] not only affects the mobility of the drug molecules, but also the morphology of the delivery systems. Fig. 7 shows optical microscopy pictures of the investigated microparticles before and after 1, 3 and 7 d exposure to pure phosphate buffer pH 7.4 and DENA/phosphate buffer, respectively. Clearly, the spherical microparticles remained intact and separated upon exposure to pure phosphate buffer. In contrast, the systems started to fuse together after only 1 d exposure to DENA/phosphate buffer. After 3 d, no more individual microparticles could be distinguished, and after 7 d a continuous polymeric film had been formed. Thus, the presence of DENA in the release medium not only increases the solubility and mobility of the drug, but also markedly changes the morphology and geometry of the delivery system. Hence, the conditions for paclitaxel release are fundamentally altered.

To hinder microparticle aggregation upon exposure to DENA/phosphate buffer, 0.5% Tween 80 was added to this release medium and paclitaxel release in the presence and absence of the surfactant was compared (Fig. 8). As it can be seen, the release rate was only slightly increased in the presence

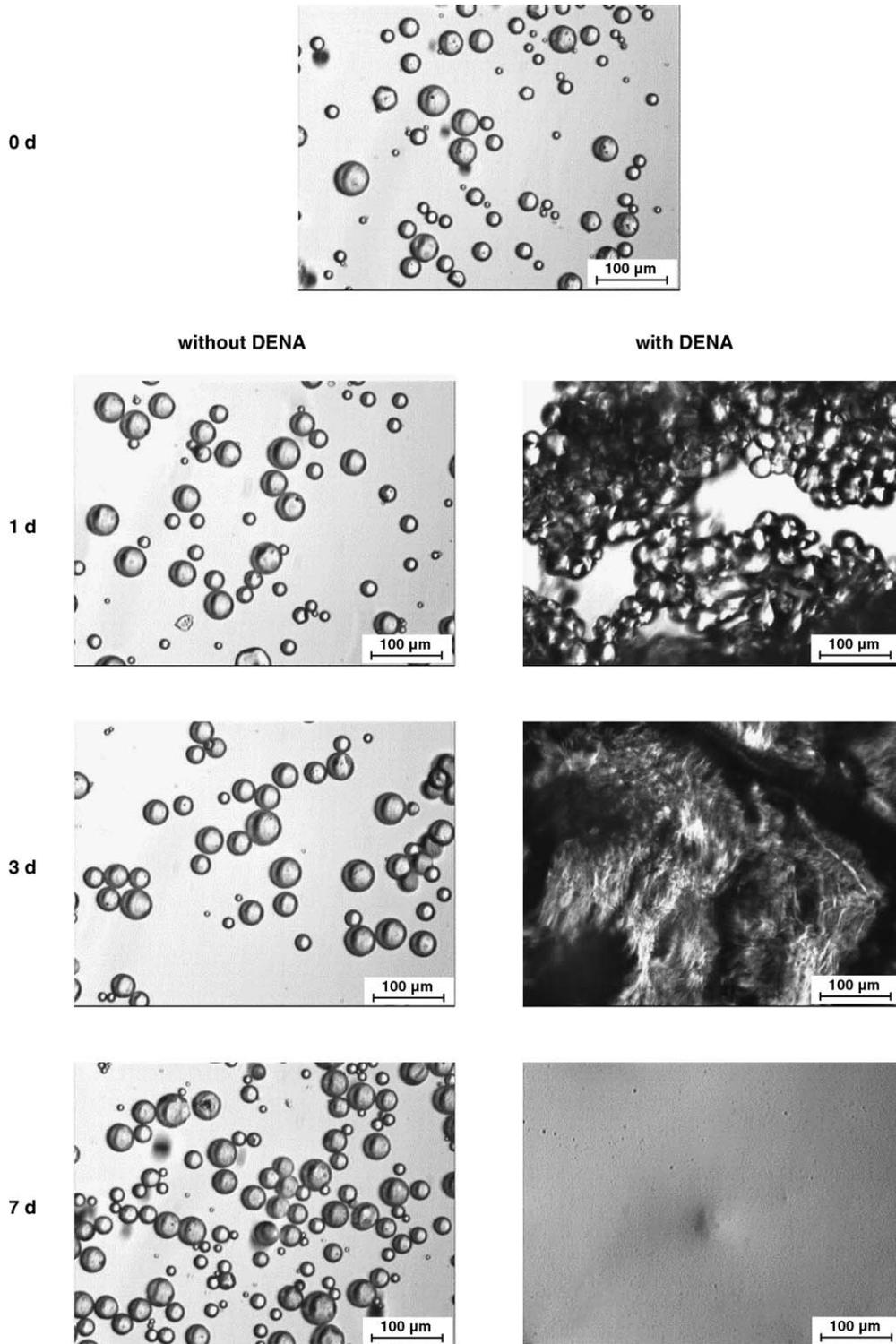


Fig. 7. Optical microscopy pictures of PLGA-based microparticles before and after 1, 3 and 7 d exposure to pure phosphate buffer pH 7.4 and DENA/phosphate buffer, respectively.

of Tween 80. This might be attributable to an increase in the wettability of the microparticles, an increase in drug solubility (resulting in increased concentration gradients) and/or to reduced microparticle aggregation. Importantly, the effect on drug release was not very pronounced.

3.5. Potential short term test for *in vitro* drug release

Obviously, the biological fluids in the brain tissue to which the microparticles and implants are exposed upon intracranial administration are more similar to pure phosphate buffer pH 7.4

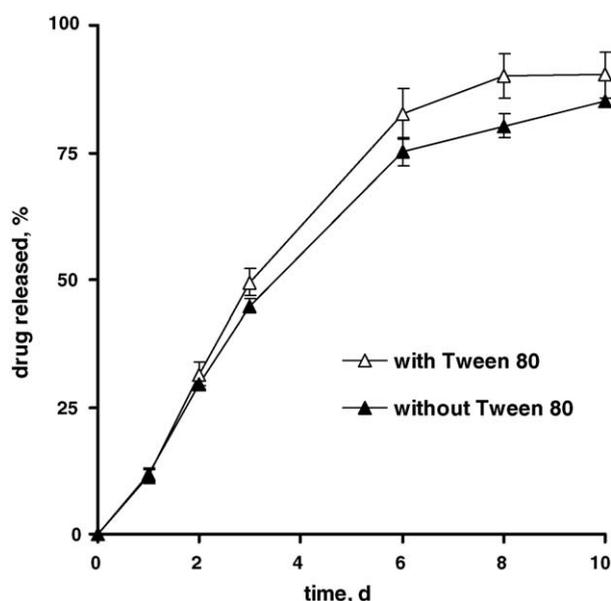


Fig. 8. Effects of the presence of 0.5% Tween 80 in the release medium (DENA/phosphate buffer) on paclitaxel release from PLGA-based microparticles (20% theoretical drug loading, 1% PVA in the outer aqueous phase during microparticle preparation).

than to the investigated DENA/phosphate buffer mixture. Thus, the observed tremendous effects of DENA on polymer degradation and drug release are non-physiologic. Upon administration of the drug delivery systems *in vivo*, paclitaxel release kinetics more similar to those shown in Figs. 1 and 3 can be expected (the drug being slowly released during several weeks). Thus, care has to be taken when drawing conclusions from drug release profiles determined upon exposure to DENA/phosphate buffer mixtures on the resulting *in vivo* tissue concentrations. However, the addition of DENA can be very helpful to provide a suitable short term test *in vitro* for this type of controlled drug delivery systems (Baek et al., 2004), similar to *in vitro* drug release studies performed at elevated temperature (Shameem et al., 1999). For example, the effects of different formulation and processing parameters on the resulting drug release kinetics can be much more rapidly investigated than upon exposure to pure phosphate buffer pH 7.4 (within a few days versus several weeks/months). This can be of great help to facilitate and speed up the development of new products and/or the optimization of existing ones. In addition, this type of short-term test could be of major importance for industrial production: much more rapid feedback on the characteristics of a specific implant/microparticle batch can be provided.

4. Conclusion

Different types of paclitaxel-loaded, lipidic implants and PLGA-based microparticles with controlled release kinetics during several weeks have been prepared and characterized *in vitro*. Importantly, these devices can directly be injected into the brain tissue (intracranial administration), overcoming the restriction that paclitaxel cannot cross the blood–brain barrier to a significant extent upon systemic administration. Thus, this type of

controlled drug delivery system might be helpful to improve the local treatment of operable and inoperable brain tumors.

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References

- Baek, N., Lee, J., Park, K., 2004. Aqueous *N,N*-diethylnicotinamide (DENA) solution as a medium for accelerated release study of paclitaxel. *J. Biomater. Sci. Polym. Edn.* 15, 527–542.
- Benoit, J.P., Faisant, N., Venier-Julienne, M.C., Menei, P., 2000. Development of microspheres for neurological disorders: from basics to clinical applications. *J. Control. Release* 65, 285–296.
- Brem, H., Ewend, M.G., Piantadosi, S., Greenhoot, J., Burger, P.C., Sisti, M., 1995b. The safety of interstitial chemotherapy with BCNU-loaded polymer followed by radiation therapy in the treatment of newly diagnosed malignant gliomas: phase I trial. *J. Neurooncol.* 26, 111–123.
- Brem, H., Gabikian, P., 2001. Biodegradable polymer implants to treat brain tumors. *J. Control. Release* 74, 63–67.
- Brem, H., Piantadosi, S., Burger, P.C., Walker, M., Selker, R., Vick, N.A., Black, K., Sisti, M., Brem, S., Mohr, G., Muller, P., Morawetz, K., Schold, S.C., 1995a. Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas. *Lancet* 345, 1008–1012.
- Charlier, A., Leclerc, B., Couarraze, G., 2000. Release of mifepristone from biodegradable matrices: experimental and theoretical evaluations. *Int. J. Pharm.* 200, 115–120.
- Dordunoo, S.K., Jackson, J.K., Arsenault, L.A., Oktaba, A.M.C., Hunter, W.L., Burt, H.M., 1995. Taxol encapsulation in poly(ϵ -caprolactone) microspheres. *Cancer Chemother. Pharmacol.* 36, 279–282.
- Faisant, N., Siepmann, J., Benoit, J.P., 2002. PLGA-based microparticles: elucidation of mechanisms and a new, simple mathematical model quantifying drug release. *Eur. J. Pharm. Sci.* 15, 355–366.
- Gallo, J.M., Li, S., Guo, P., Reed, K., Ma, J., 2003. The effect of P-glycoprotein on paclitaxel brain and brain tumor distribution in mice. *Cancer Res.* 63, 5114–5117.
- Guse, C., Koennings, S., Kreye, F., Siepmann, F., Goepferich, A., Siepmann, J., 2006. Drug release from lipid-based implants: elucidation of the underlying mass transport mechanisms. *Int. J. Pharm.* 314, 137–144.
- Hammarlund-Udenaes, M., Paalzow, L.K., de Lange, E.C.M., 1997. Drug equilibration across the blood–brain barrier—pharmacokinetic considerations based on the microdialysis method. *Pharm. Res.* 14, 128–134.
- Harper, E., Dang, W., Lapidus, R.G., Garver, R.I., 1999. Enhanced efficacy of a novel controlled release paclitaxel formulation (paclimer delivery system) for local-regional therapy of lung cancer tumor nodules in mice. *Clin Cancer Res.* 5, 4242–4248.
- Heimans, J.J., Vermorken, J.B., Wolbers, J.G., Eelink, C.M., Meijer, O.W., Taphoorn, M.J., Beijnen, J.H., 1994. Paclitaxel (taxol) concentrations in brain tumor tissue. *Ann. Oncol.* 5, 951–953.
- Johnston, S.R., 2004. Ovarian cancer: review of the National Institute for Clinical Excellence (NICE) guidance recommendations. *Cancer Invest.* 22, 730–742.

- Kemper, E.M., van Zandbergen, A.E., Cleypool, C., Mos, H.A., Boogerd, W., Beijnen, J.H., van Tellingen, O., 2003. Increased penetration of paclitaxel into the brain by inhibition of P-glycoprotein. *Clin. Cancer Res.* 9, 2849–2855.
- Kreuter, J., Petrov, V.E., Kharkevich, D.A., Alyautdin, R.N., 1997. Influence of the type of surfactant on the analgesic effects induced by the peptide dalargin after its delivery across the blood–brain barrier using surfactant-coated nanoparticles. *J. Control. Release* 49, 81–87.
- Lee, J., Lee, S.C., Acharya, G., Chang, C.J., Park, K., 2003. Hydrotropic solubilization of paclitaxel: analysis of chemical structures for hydrotropic property. *Pharm. Res.* 20, 1022–1030.
- 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.
- 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.
- Menei, P., Capelle, L., Guyotat, J., Fuentes, S., Assaker, R., Bataille, B., Francois, P., Dorwling-Carter, D., Paquis, P., Bauchet, L., Parker, F., Sabatier, J., Faisant, N., Benoit, J.P., 2005. Local and sustained delivery of 5-fluorouracil from biodegradable microspheres for the radiosensitization of malignant glioma: a randomized phase II trial. *Neurosurgery* 56, 242–248.
- Menei, P., Jadaud, E., Faisant, N., Boisdrion-Celle, M., Michalak, S., Fournier, D., Delhay, M., Benoit, J.P., 2004. Stereotaxic implantation of 5-fluorouracil-releasing microspheres in malignant glioma. *Cancer* 100, 405–410.
- Menei, P., Venier, M.C., Gamelin, E., Saint-Andre, J.P., Hayek, G., Jadaud, E., Fournier, D., Mercier, P., Guy, G., Benoit, J.P., 1999. Local and sustained delivery of 5-fluorouracil from biodegradable microspheres for the radiosensitization of glioblastoma: a pilot study. *Cancer* 86, 325–330.
- Paradis, R., Page, M., 1998. New active paclitaxel amino acids derivatives with improved water solubility. *Anticancer Res.* 18, 2711–2716.
- Pardridge, W.M., 1997. Drug delivery to the brain. *J. Cereb. Blood Flow Metab.* 17, 713–731.
- Rice, A., Michaelis, M.L., Georg, G., Liu, Y., Turunen, B., Audus, K.L., 2003. Overcoming the blood–brain barrier to taxene delivery for neurodegenerative diseases and brain tumors. *J. Mol. Neurosci.* 20, 339–343.
- Sato, H., Wang, Y.M., Adachi, I., Horikoshi, I., 1996. Pharmacokinetic study of taxol-loaded poly(lactic-co-glycolic acid) microspheres containing isopropyl myristate after targeted delivery to the lung in mice. *Biol. Pharm. Bull.* 19, 1596–1601.
- Schiff, P.B., Fant, J., Horwitz, S.B., 1979. Promotion of microtubule assembly in vitro by taxol. *Nature* 277, 665–667.
- 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.pharmsci.org/>).
- Sparreboom, A., Tellingen, O.V., Nooijen, W.J., Beijnen, J.H., 1996. Tissue distribution, metabolism, and excretion of paclitaxel in mice. *Anticancer Drugs* 7, 78–86.
- Tamargo, R.J., Brem, H., 1992. Drug delivery to the central nervous system: a review. *Neurosurg. Quart.* 2, 259–279.
- Valtonen, S., Timonen, U., Toivanen, P., Kalimo, H., Kivipelto, L., Heiskanen, O., Unsgaard, G., Kurne, T., 1997. Interstitial chemotherapy with carmustine-loaded polymers for high-grade gliomas: a randomized double-blind study. *Neurosurgery* 41, 44–49.
- 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 and in vivo evaluation of taxol release from poly(lactic-co-glycolic acid) microspheres containing isopropyl myristate and degradation of the microspheres. *J. Control. Release* 49, 157–166.
- Watatani, M., Ueda, K., Daito, K., Azumi, T., Hirai, T., Yamato, M., Hojo, T., Hirai, K., Inui, H., Shiozaki, H., 2004. Clinical experience of weekly paclitaxel-based treatment as preoperative chemotherapy for patients with primary breast cancer. *Breast Cancer* 11, 187–193.
- Westphal, M., Delavault, P., Hilt, D., Olivares, R., Belin, V., Dumas-Duport, C., 2000. Placebo controlled multicenter double-blind randomized prospective phase III trial of local chemotherapy with biodegradable carmustine implants (Gliadel™) in 240 patients with malignant gliomas: final results. *Abstr. Neurooncol.* 2, 301.