Effects of Polymer Modification of an Anti-Cancer Drug with Poly(ethylene oxide)

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Summary: The hydrophobic anti-cancer drug paclitaxel was modified with the hydrophilic poly(ethylene oxide) to a water-soluble predrug. The modification of the polymer chain results in a different solution behaviour of the macromolecules compared with the unmodified polymer. The phase diagram of the (quasi)binary system predrug-water was investigated, and molecular simulations of the predrug were executed in vacuum and in the presence of water. The results are important for further engineering on active drug systems.

Keywords: mPEG-paclitaxel; simulation; solution; structure

Introduction

The application of some pharmaceutically active substances such as the anti-cancer drug paclitaxel – see fig. 1 – suffer from their low solubility in body fluids and serious side effects [1-4]. Paclitaxel (Taxol®) is active against a number of cancer types such as breast-, ovary-, lung cancer and some leukaemias [5-7]. It interferes in the process of mitosis by destabilisation of the microtubuli. The drug can be extracted from the bark of the Asian *Taxis brevifolia.* To overcome the solubility problem different approaches have been used. A presently used clinical method is a Cremophor® EL-ethanol preparation, at the cost, however, of severe side effects such as neurotoxicity and hypersensibility introduced by the emulgator. Other approaches try to avoid negatively interacting additives and couple the Paclitaxel molecule through one (or more) of its reactive groups with the water-soluble poly(oxy ethylene) (PEO) [8, 9]. The active principle of this predrug has first to be released first by an esterase. However, the first predrugs proved to be too stable and most of the drug was excreted before it could become bioavailable. The introduction of the self-immolating linker by B.-W. Jo [10] overcame this disadvantage. In order to improve the drug delivery to the

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sites where it is required and to increase its bioavailability more knowledge of the structure and behaviour of the predrug in aqueous solution is required.

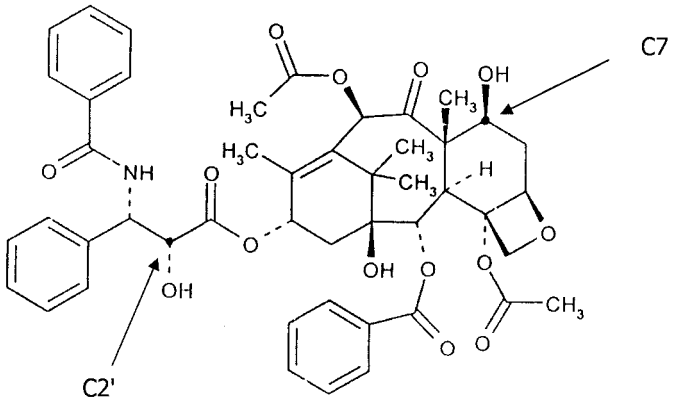


Fig. 1. Structure of paclitaxel. Coupling of the drug through the self-immolating linker (see below) with PEO at carbon 7 leads to the predrug PP7.

In earlier publications we have already reported on the concentration and temperature dependence of the predrug in D_2O [11], measurements of the surface tension and viscosity in water [12, 13]. No superstructures, like micelles, have been found in the predrug investigated by us to date. However, the formation of supramolecular structures may be advantageous because they mean a higher local concentration and the circulation time of the predrug could probably be enhanced. This is particularly desirable since synthesis and purification of the predrug are rather costly.

Recently, core-crosslinked polymeric micelles from crosslinkable block copolymers that act as carriers for paclitaxel were synthesised. In this case, the lipophilic paclitaxel is encapsulated in the hydrophobic core of the (crosslinked) micellar structures [14]. On the contrary, our approach is to synthesize supramolecular structures like micelles from paclitaxel derivatives. In other words, the suprastructure should consist of the predrug and should not just be filled with the lipophilic paclitaxel. Therefore, studies of the behaviour of the predrug in aqueous solution and in isotonic solutions are required to determine structure and interactions of the prepolymer in solution. We report here about recent results of the structure

of one particular predrug – PP7 – in water-containing systems and molecular simulations of the structure in vacuum and in the presence of water.

Materials and Methods

Materials

Paclitaxel was obtained from Brystol-Myers Squibb Co., NY, USA. It was modified with α-methyl terminated ω-OH-functional PEO (Fluka) ($\langle M_w \rangle / \langle M_n \rangle = 1.05$ and molar mass average of 5,000 g mol^{-1}) and linked to carbon 7 (see Fig. 1) with succinic acid through a self-immolating linker. The modified drug is termed PP7, and the α-methyl- and ω-succinyl-terminated PEO is termed PEOS in the following text. The additive "–5000" indicates the molar mass of the PEO, since other degrees of polymerisation can also be used. However, the yield decreases with increasing length of the polymer chain.

Synthesis of the Predrug

The predrug PP7 (7mPEO 5000-succinyloxymethyloxycarbonyl-paclitaxel) was synthesised as follows:

1.057 mmol 7-chloromethoxycarbonyl-paclitaxel was dissolved in anhydrous benzene. 1.057 mmol monomethoxy polyethyleneglycol succinate, 3.171 mmol sodium iodide, 1.902 mmol potassium carbonate and 0.739 mmol 18-crown-ether-6 were mixed in the resulting solution. The mixture was stirred for 36 hours under reflux and then dried under reduced pressure to remove the benzene. The product was re-dissolved in dichloromethane, filtered, and the filtrate was washed twice with water. The organic layer was dried with anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the product was recrystallised from isopropanol. The final yield after purification by preparative chromatography was about 68%. The success of the reaction was confirmed by NMR-spectroscopy. Reaction of the hydroxyl-group on the C2' was sterically hindered.

Methods

Calorimetric measurements were carried out with a Perkin-Elmer DSC-7 differential scanning calorimeter. A total mass of approximately 20 mg was placed in a sealed pressure pan and heated up to 80°C to ensure complete solution. After keeping the solution at 80°C for about 5 min, it was cooled down to –20°C, at a cooling rate (CR) of 20 $K \cdot min^{-1}$. The glass transition temperature of the pure PEO ($\langle M_w \rangle$= 5,000 $g \cdot mol^{-1}$) at – 63°C was not reached. The samples

were heated at a heating rate (HR) of 20 $K \cdot min^{-1}$. A cold crystallisation during heating was not observed.

The molecular dynamics calculations were performed on a silicone graphics (sgi) octane with a MIPS R 12000 processor, OS irix 6.5 at the Seoul National University, Seoul, Korea, using the cerius 2 version 4.0 program. The NVT-molecular dynamics simulation used a Dreiding force field, 1 fs time steps, and a total simulation time of 50 ps or 100 ps. The temperature was set a 300 K.

Results and Discussion

Earlier investigations [11, 12] have shown that there is no significant surface activity of PP7 (5,000 g/mol) and that the shape of the predrug is an ellipsoid rather than a hard sphere [11, 13]. The phase diagram of PP7 (5,000 g/mol) shows a behaviour that is very similar to pure PEO, see fig. 2.

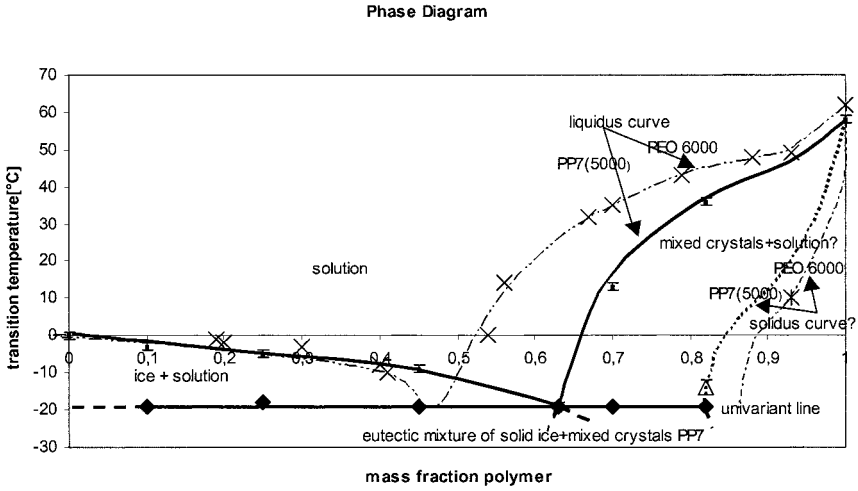


Fig. 2. Phase diagram of the system PP7 (5,000 g/mol)-H_2O.

It is a typical eutectic phase diagram with the probable formation of a mixed crystal PEO-H_2O on the right hand side (predrug-rich end) of the diagram. However, it is difficult to determine the formation of mixed crystals unequivocally with a calorimeter. The assumption of the existence of mixed crystals is mostly based on earlier results obtained by Dobnik [15] from a large number of PEO-samples, covering a broad range of polymers with a narrow distribution of molecular masses.

One important result of the present investigations is that there is no hint of melting, crystallization or glass transition, as is known to occur with the pure paclitaxel which melts around 220°C and can show a glass transition around 160°C. The glass transition of PEO could not be observed with the equipment used in this investigation because the temperature was too low. The predrug PP7 (5,000 g/mol) behaves like pure PEO, but with a molar mass of only about 1,000 g/mol. A detailed discussion of the phase-diagram will be discussed elsewhere.

A summary of the experimental knowledge of the behaviour of PP7 in aqueous solution leads to the conclusion that the hydrophobic paclitaxel molecule is almost completely camouflaged by the PEO-chain. The situation appears to be comparable to the conformation of proteins with a high number of hydrophobic amino acids, such as gly, ala, val, leu and ile. In these cases it is frequently found that the protein coils to a tertiary structure in such a way that the hydrophobic chains are predominantly localized in the inner part of the coil while the hydrophobic amino acid sequences form a hydrophilic shell. The hydrophobic inner part thus can often act as a docking site in enzyme reactions or form a type of water-soluble hydrophobic reactor. The solubility of the latter of which is provided by the hydrophilic shell.

Such a core-shell structure does not allow intermolecular contact between the individual paclitaxel molecule parts of the predrug, hence the formation of crystalline or glassy paclitaxel phases is prevented. Further advantages of such a hydrophilic PEO shell are the targeting effects [16] that have been observed with PEO and the retarding effect of a surface modification by PEO that hinders opsonization.

Nanosize structures are eliminated from the body by uptake by the mononuclear phagocytes system (MPS). This effect causes a half-life time of such carriers (emulsions, liposomes, nanoparticles) from 1 min to 10 min [17]. In order to decrease the ability of the immune system to remove these particular nanostructures from the blood-circulation system the particles have to be camouflaged. Opsonization means interaction of opsonins (=glycoproteins of the blood plasma) with "foreign bodies". Opsonization makes the "foreign bodies" attractive for macrophages and hence prepares the field for phagocytosis by leucocytes. Fibronectine and substances like the serum factor (complement) C3b. Immunoglobulins (antibodies) bind specifically the antigene (opsonization) and prepare the "foreign particle" for the phagocytosis There are different parameters influencing opsonization (and hence uptake by macrophages) and a surface modification of a nanoparticle

or a molecule like paclitaxel can reduce opsonization and hence enhance the circulation time of the drug in the body and therefore its bioavailability.

Another important property can be influenced by surface modification of molecules or nanoparticles, namely the interaction with other molecules of the same type or constituents of the plasma-liquid such as lipid, proteins or polysaccharides.

To further verify the PP7-structure proposed above, studies of the molecular dynamics (MD) were performed (by J.-S. S.). The simulations were conducted without further specific interactions between paclitaxel and PEO (except for the chemical bond on carbon 7). Different degrees of polymerization for PEO were considered and the conformation of the predrug was calculated in vacuum and in the presence of 300 or 500 water molecules. The simulations started from the extended chain. The conformational energy was minimized and the radius of gyration was calculated.

Fig. 3 shows an energy-minimised wire-model of the paclitaxel molecule. For hydrophilisation, the molecule is substituted with PEO on the carbon 7 as described in the Experimental Section in that a self-immolating linker is formed which includes a succinic acid ester group on the one end of the ω-monomethyl-poly (ethylene oxide).

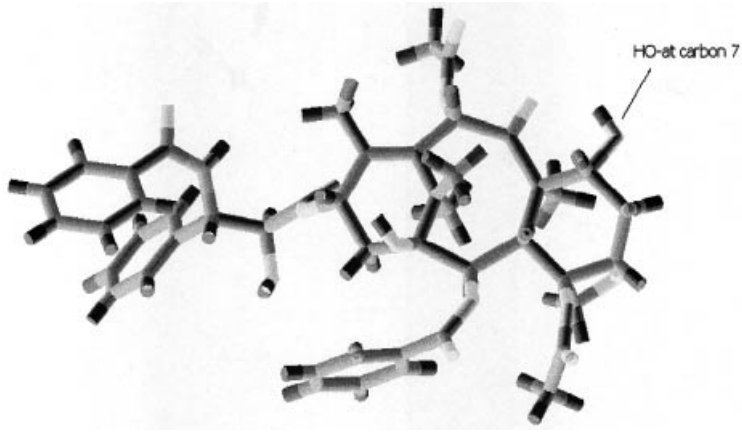


Fig. 3. Energy-minimised "wire" model of Paclitaxel.

The following figures 4-6, show the coiling of the PEO-chain in vacuum with increasing simulation time. No specific interactions were introduced. In general it is found that the

polymer chain and parts of the paclitaxel molecule are very flexible. In particular, the neighbouring phenyl rings perform a kind of "clapping-mode" not unlike hands.

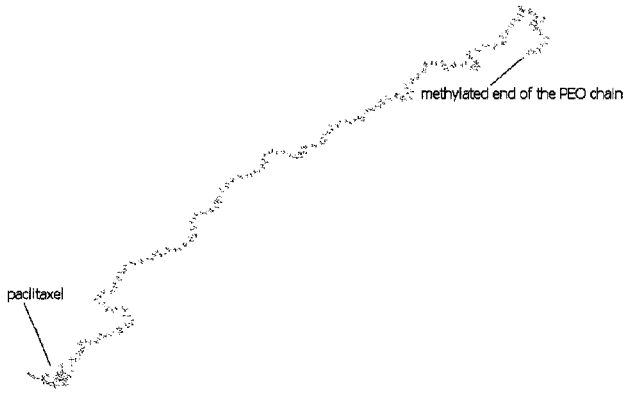


Fig. 4. A MD of PP7(5000) (after 11 ps, started from an extended chain conformation in vacuum).

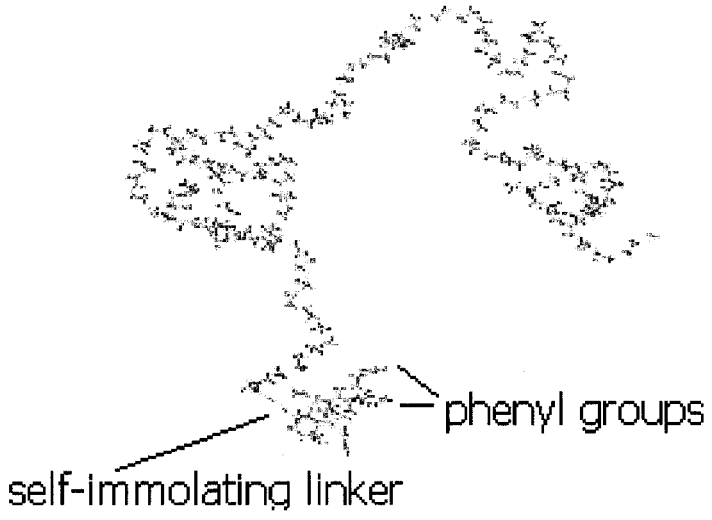


Fig. 5. A MD of PP7(5000) (after 50 ps, started from an extended chain conformation in vacuum). There is much coiling in the middle of the chain and at the distant end.

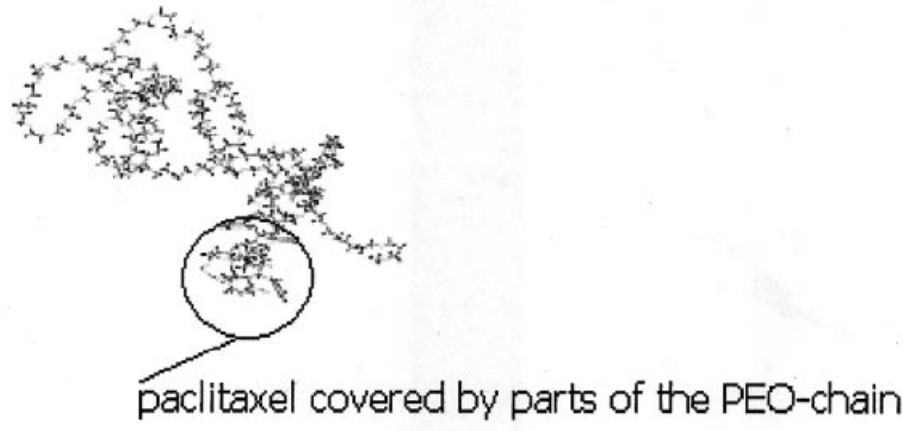


Fig. 6. A MD of PP7(5000) (after 100 ps, started from an extended chain conformation in vacuum). Part of the PEO-chain coils around the paclitaxel molecule. This can be more clearly seen in Fig. 7.

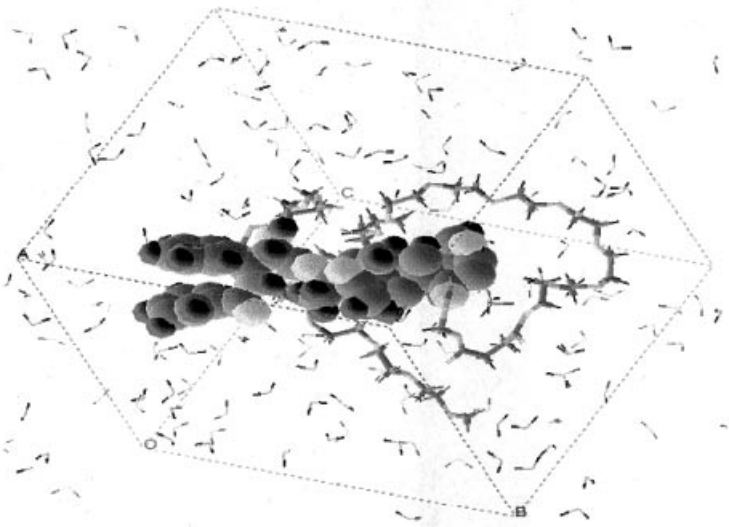


Fig. 7. PP7(1000), a short PEO chain, coils around the lipophilic paclitaxel molecule, 300 water molecules.

The radius of gyration, Rg, that is calculated during the simulation is close to the value that was calculated from measurements of the self-diffusion coefficient of a single PP7(5000) molecule in aqueous solution according to the Stokes-Einstein equation [11]. The course of Rg with simulation time is shown in Fig. 8.

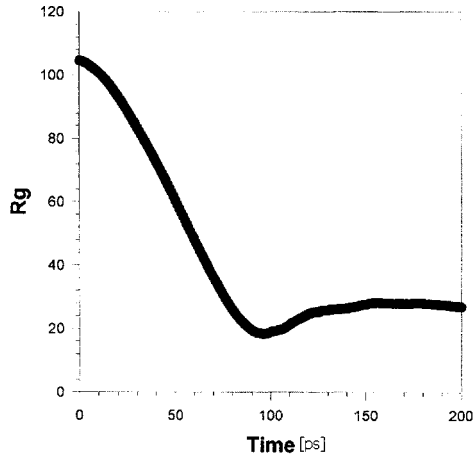
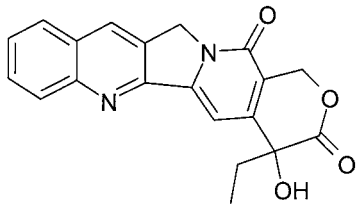


Fig. 8. Development of the radius of gyration, Rg, of PP7(5000) during the molecular dynamics simulation in vacuum. The graphic shows the course of Rg with coiling of the PEO-chain towards the equilibrium conformation.

Another anti-mitotic compound of the taxane family is campthotecin. See Fig. 9. It is also frequently used in anti-cancer therapy. The taxane family contains of about 30 substances found in the taxis plant family.



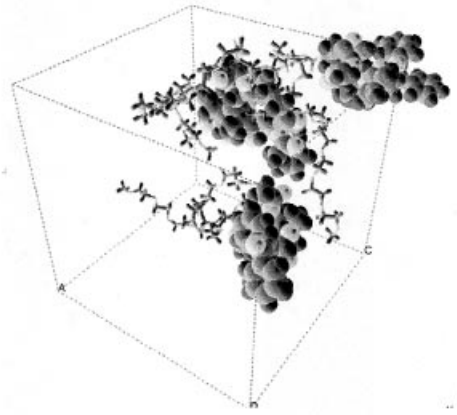
Campthotecin

Fig. 9. Structure of campthotecine, another anti-cancer drug of the taxel family.

Isolation, derivatization and purification of the PEO-derivative of campthotecin (PC) is costly and elaborate, hence we did not have substantial amounts of the material available. Molecular dynamics studies for comparison with PP7 were nonetheless performed. The following Figure 10a, b, shows simulations with 3 drug-molecules and a short PEO chain (Fig. 10a PP7 and Fig. 10b PC). Again, no specific interactions were introduced.

With shorter PEO-chains (because of the limited number of atoms that can be handled by the software) we tried to simulate the behaviour of a small number of pre-drug molecules PP7 and PC, respectively. As shown in Figures 10a and b, the campthotecin derivative tends to aggregate, in contrast to PP7, where such a tendency appears to be absent. This was also found experimentally [11-13]. The reason for this behaviour might be the anisotropy of shape of the rather plane campthotecin molecule which supports mutual alignment.

10a



10b

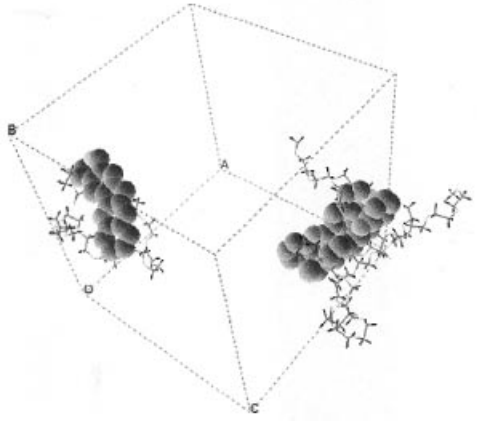


Fig. 10. a) group of three PP7(550) molecules showing no significant aggregation after 320 ps b) group of three CP(550) molecules, two of them form a dimer after 320 ps simulation time.

Conclusion

The properties of drugs in aqueous solution, in particular in the blood plasma, are of great importance for their fate in the body. They influence the circulation time in the blood, interaction with receptors, membrane transport, homo-aggregation and hetero-aggregation, obsonization and immune response. Molecular engineering such as solubilization, micelleization, or the formation of nanoparticles with specific surface properties, or the introduction of self-immolating linkers, are some examples for tailoring drugs besides their pharmaceutical activity. In particular, the formation of supramolecular structures that increase the local concentration of the drug combined with targeting properties of the agent appear to be a useful approach to increase drug efficiency and bioavailability. Engineering of the surface of molecules and supramolecular assemblies can, for example, be accomplished with the tool-box of macromolecular chemistry in utilizing the varieties of properties that can be introduced by copolymerizing hydrophilic and hydrophobic monomers (in particular to block-copolymers). Recent advances in simulation techniques, such as the recently developed method of dissipative particle dynamics (DPD) [18-20], overcomes the problem of the disparate time scales in the dynamics of complex colloidal fluids and can be of great advantage in developing the desired structures without excessive synthetical trial and error experiments. While in the case of paclitaxel some more engineering on the molecular structure of the pre-drug has to be applied, to cause self-aggregation of the molecules, the campthotecin molecule seems to offer simpler concepts for self-aggregation since the shape of the molecule already seems to support the formation of supramolecular structures.

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[1] M. Suffness, *Ann. Report Med. Chem.* **1993**, *18*, 305
[2] R. M. Straubinger, in: M. Suffness, (ed.) *Taxol Science and Applications*, CRC-Press, Boca Raton 1995 p. 237
[3] Mazzo, D., Nguyen Hu. J. J., Pagniez, S., Denis, P., *Am. J. Health Synth. Pharm.* **1997**, *54*, 566
[4] R. Panchagnula, *Int. J. Pharm.* **172**, 1 (1998)
[5] M.C. Wani, H.L. Taylor, M.E. Wall, P. Coggon, A.T. McPhail, *J. Am. Chem. Soc.* **1981**, *93*, 2325
[6] E.K. Rowinsky, L.A. Cazenave , R.C. Donehower, *J. Natl. Cancer Inst.* **1990**, *82,* 1247
[7] W.P. McGuire, E.K.Rowinsky, N.B. Rosenheim, F.C. Grumbine, D.S. Ettinger, D.K. Armstrong, *Ann. Intern. Med.* **1989**, *111*, 273
[8] R.B. Greenwald, **1997** U.S. Pat. 5,614,549; (1999) US Pat 5,880,131; (1999) U.S Pat 5,965,566
[9] Chung Li, **1999** US Pat 5,977,163

[10] B.-W. Jo, **2000** Korean Patent 2000-0019873
[11] B.-W. Jo, M. Hess, M. Zähres, *Mater. Res. Innovat.* **2003**, *7*, 178
[12] M.Hess, J.-S. Sohn, S.-K.Choi, B.-W. Jo, *Macromol. Symp.* **2003**, *201*, 163
[13] J.-S. Sohn, S.-K. Choi, B.-W. Jo, M. Zähres, *Mater. Res. Innovat.* **2004**, *8*(4) in press
[14] X. Shuai, T. Merdan, A. K. Schaper, F. Xi, T. Kissel, *Bioconjugate Chem.* **2004**, *15*, 441
[15] E. Dobnik, thesis, Duisburg, **1991**
[16] R B. Greenwald,Y. H. Choe, J. McGuire, C.D. Conover, *Adv. Drug Delivery Rev.* **2003**, *55*, 217
[17] R.L. Juliano, *Adv. Drug Del. Rev.* **1988**, *2*, 31
[18] P. Espagnol, P., Warren, P. B, *Europhys. Lett.* **1995**, *30*, 191
[19] R.D.Grootland, P.B. Warren, *J. Chem. Phys.* **1997**, *105*, 4423
[20] E. Ryjkina, H. Kuhn, H.F. Rehage, Müller, Peggau, *J. Angew. Chem.* **2002**, ***114***(6), 1025