Polymer-Modification of an Anti-Cancer Drug: Effects on Hydrodynamic Properties

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Summary: Coupling of a hydrophobic pharmaceutical agents with the hydrophilic poly(ethylene oxide) chain can make the drug water soluble which is necessary for some applications. The modification of the polymer chain results in a different solution behaviour of the macromolecules compared with the unmodified polymer. There are strong indications that shape and rigidity of the modified molecule differ significantly from the simple chain. Some results from measurements of the self-diffusion coefficient and solution viscosity are discussed. The results are important for further engineering on active drug systems.

Keywords: active drug systems, hydrophilic poly(ethylene oxide) chain, macromolecules, polymer chain, self-diffusion coefficient

Introduction

Lipophilic pharmaceuticals have to be made water soluble before they can be applied to the body by injection. One way to accomplish this is to hydrophilise the drug, for example with a hydrophilic polymer like poly (ethylene oxide) (PEO). The prodrug obtained this way will usually have an altered activity, mobility in solution, etc. and even targeting effects of the polymer has been observed in some cases. Long-time stability of a prodrug might be desired, in some cases, however, it keeps the drug in solution too long so that a too high amount of the drug is released from the body on the natural way before it has the chance to become active on its target, e. g. a tumour. Therefore an adjusted life time of a modified drug can be desired, so that it remains water soluble just long enough to provide a good bioavailability.

While water-solubility can be accomplished by e. g. modifying the drug with PEO, the life time of these prodrugs can be controlled by the linkage between the polymer and the active agent, for example by a self-immolating linker. In all these cases reactivity, solution-and transport properties can be affected and play an important role in the optimal application of the drug.

 DOI: 10.1002/masy.200351119

Paclitaxel, an anti-tumour active substance abundant in the bark of the Asian *Taxis brevifolia*, represents an example of a drug which has to be modified as described before. The Paclitaxel molecule, see Fig. 1, interferes in the process of mitosis by destabilisation of the microtubuli. In this way it is active against a number of cancer types such as breast-, ovary-, lung cancer and some leucemias.

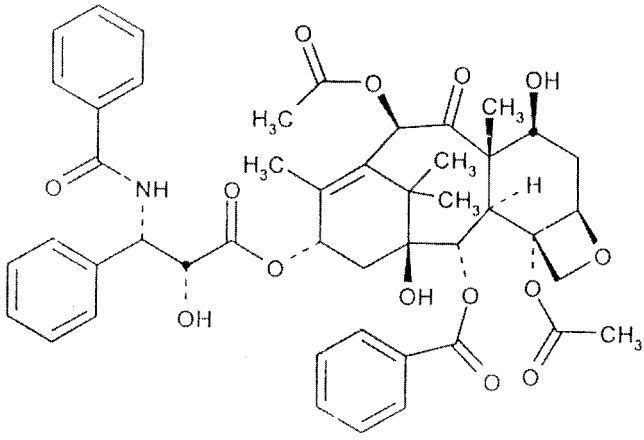

Fig. 1. Paclitaxel, the anti-tumour active principle in the bark of *Taxis brevifolia*. Hydrophilisation can be accomplished, for example, by esterification of the positions 2' or 7 with an appropriate polymer.

Several methods of derivatisation have been described in literature and patented, see for example [1]. An effective self-immolating linker which decomposes just in time and prevents that most of the drug is excreted by the body before it can develop its activity in the tumour, has recently been developed for Paclitaxel by one of us, B.-W. Jo[2]. Now, we have started to study the behaviour of the modified drug (PP7) in solution and compare its behaviour with that of the unmodified PEO, respectively with a PEO (PEOS) which is CH_3-blocked on one end, while the other end is capped by a succinic acid group to provide the coupling with the self-immolating linker, see Fig. 2.

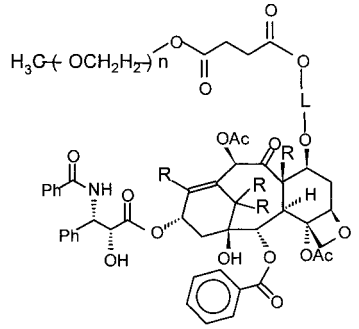

Fig. 2. Paclitaxel coupled at C7 through a self-immolating linker with a functionalised PEO, in the following text termed PP7. The degree of polymerisation in this work is either 113 or 452 according to a molar mass of 5,000 g/mol or 20,000 g/mol, respectively, for the highly uniform PEO which was used here.

Coupling a hydrophilic molecule with a hydrophobic molecule might give rise to an amphiphilic molecule able to form colloidal associates in solution such as micelles. Therefore, we have studied the concentration dependent surface tension of PP7 (5,000 g/mol).

For spherical rigid molecules Einstein[3] has derived an equation which correlates the specific viscosity η_{spec} with the volume fraction of the solute by:

$$\eta_{spec} = \frac{\eta_1 - \eta_{01}}{\eta_{01}} = \nu \cdot \varphi \qquad (1)$$

with the index 1 denoting the solution and 01 the pure solvent. ν is Einsteins "rigidity-factor" and φ is the volume fraction of the solute which is defined through the concentration c and the overlap-concentration c* of the polymer coils. For an ideal rigid sphere ν = 2.5 is valid. Simha [4] expanded the theory for ellipsoids and depending on the aspect ratio for oblate or prolate shapes, respectively.

$$\varphi = \frac{c}{c^*} = \frac{c\frac{4}{3}\pi R_H^3 N_A}{M} \qquad (2)$$

The self-diffusion coefficient D of rigid spheres can be described by the Stokes-Einstein equation and a series expansion of D with respect to the volume fraction is given by:

$$D = \underbrace{\frac{k_b T}{6\pi \eta_{01} R_H^3}}_{D_0}\left(1-\lambda\varphi+...\right) \tag{3}$$

R_H is the apparent hydrodynamic radius of the particle, k_b is the Boltzmann-faktor, T denotes the thermodynamic temperature, the expansion factor λ is sometimes called the rigidity factor and depends on the hydrodynamic and the pair interactions between the particles, therefore on their shape and the rigidity. For an ideal, hard sphere $\lambda = 2$ is valid [5-7]. D_0 is the self-diffusion coefficient of a single particle, that is in an infinitely dilute solution where φ of the solute becomes zero. The denominator in the expression for D_0 is called friction factor f.

For rigid rods of aspect ratio a Dhont e. a. [8] have expanded the theory: λ increases with a. Also, λ becomes larger than the theoretical value when there are attractive interactions. On the (time) average two interacting particles share a longer time in an attractive field than in a neutral or a repulsive one so that the diffusion is slowed down.

Materials and Methods

Paclitaxel was modified with methyl terminated PEO (uniformity of 1.05 and molar mass averages of 5,000 g mol^{-1} and 20,000 g mol^{-1}) and attached to carbon 7 (see Fig. 2) with succinic acid through a self-immolating linker as described elsewhere, Jo [9]. The modified drug is termed PP7, the α-methyl- and ω-succinyl-terminated PEO is termed PEGS in the following text.

The measurements of the surface tension were performed with a Wilhelmy balance and also controlled by the contact angle. The solution viscosity was determined with an Ubbelohde viscosimeter. All experiments were carried out temperature-controlled at 25°C.

The ("long-time", that means > 10^{-4}s) self-diffusion coefficient was calculated from pulsed-gradient-field NMR spectroscopic measurements (Hahn-echo and stimulated echo) on a Bruker DRX 500 with a water cooled Diff30 z-gradient, gradient strength 0.3 T m^{-1} A^{-1} in a standard bore Bruker 11.74 T magnet with a pulsed field gradient magnitude g up to 6 T m^{-1}. In order to avoid undesired convection in the sample, the temperature of the sample was controlled by the water jacket of the z-gradient coil. The samples reached equilibrium after around 30 min.

Results and Discussion

The surface tension of the prodrug PP7 (5,000 g mol^{-1}) behaves over a wide concentration range within experimental error almost like the corresponding PEO chain, as it is shown in Fig. 3.

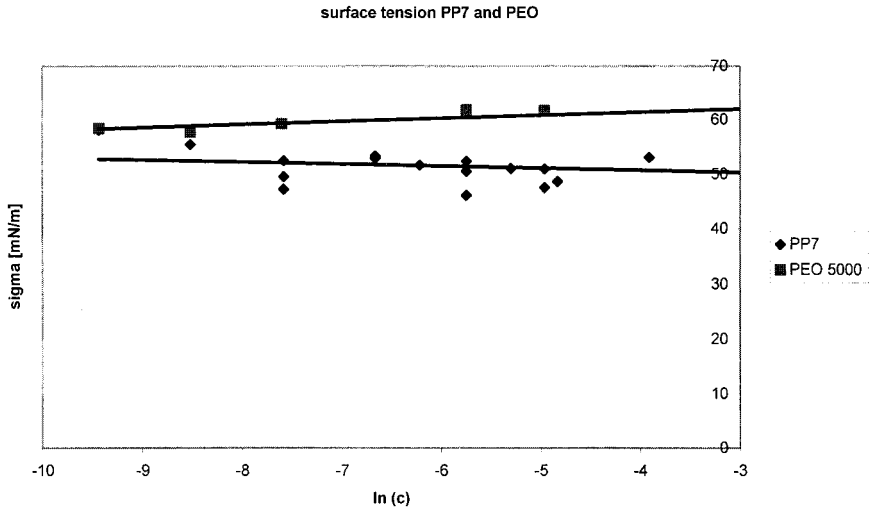

Fig. 3. surface tension vs. The concentration which is normalised by the unit of the concentration to give the argument of the logarithm the unit 1. Both substances show nearly the same behaviour only on a different level.

Assuming that the prodrug, as schematically shown in Fig. 2, consists of a hydrophobic "head" and a hydrophilic "tail", one might expect a detergent-like behaviour with the formation of aggregates of the prodrug like micelles. Apparently, however, there is no such aggregation. Concluding from the concentration dependence of the surface tension the prodrug does not show a detergent-like anisotropy of shape, even at a chain length of PEO as short as 113 repetition units.

The specific viscosity vs. the volume fraction of the solute shows the linear behaviour predicted by eq. 1, see Fig. 4. However, the slope of the function is steeper than the theoretical value of 2.5. According to Simha [4] this can be interpreted as a (prolate or oblate) deviation from a spherical shape of the molecules in solution.

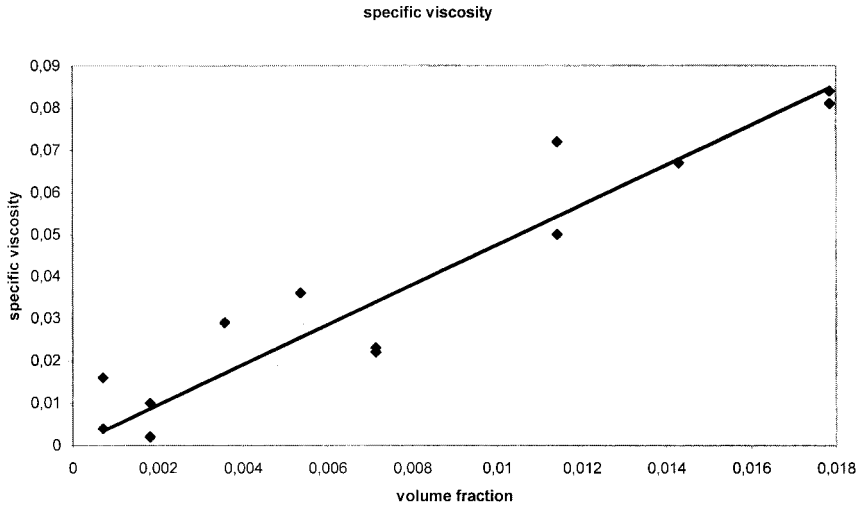

Fig. 4. specific viscosity of PP7 vs. the volume fraction of the solute. Within experimental error the behaviour is linear, although the slope is significantly larger than the theoretical value of 2.5.

In the present case this would mean an axial ratio of about 4.5 (prolate) or 6.2 (oblate), respectively. Which of these alternative molecule shapes is formed cannot yet be decided without further investigations. However, the results of the linear behaviour of the solution-viscosity with the volume fraction supports the interpretation of the surface tension measurements in that there is no discontinuity in the viscosity with the composition of the solution that would indicate a formation of larger aggregations.

Further information can be drawn from the temperature-, time- and concentration dependence of the self-diffusion coefficient of the prodrug in aqueous solution. The interpretation of the NMR pulsed gradient field experiments in terms of the self-diffusion coefficient are shown in Fig. 5. The Self-diffusion coefficient D can be determined from the attenuation of spin-echo experiments in a gradient of the magnetic field along the z-axis of the sample. No time-dependence of the self-diffusion coefficient could be found. The energy of activation does not change between 15°C and 33°C and hence indicating no change of the transport mechanism in this temperature range.

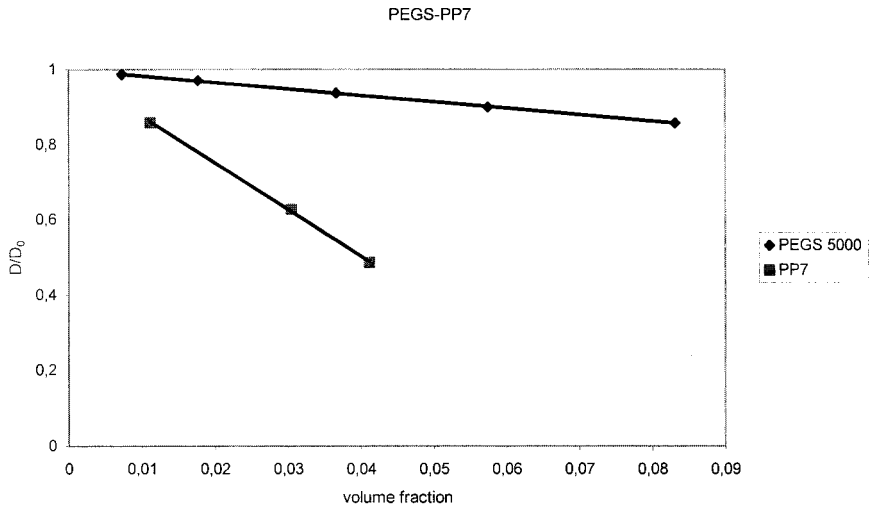

Fig. 5. the ratio of the concentration-dependent self-diffusion coefficient D and the self-diffusion coefficient in an infinitely dilute solution D_0 is plotted vs. the volume fraction of the solute PEGS (5,000) and PP7 (5,000), respectively.

Analysis of the data according to eq. 3 reveals that the effective hydrodynamic radius R_H of PP7 (5,000 g mol^{-1}) and PEGS (5,000) at infinite solution is very similar. The difference in their self-diffusion coefficient is mainly caused by differences in the λ-term. The λ-term is much larger for PP7. As already concluded from the results of the viscosity measurements this indicates a deviation from the behaviour of a rigid sphere as it is shown by PEGS or PEO. There is definitely a significant change of the interaction potential after the PEO chain is coupled with the drug. This becomes clear by comparison with the behaviour of PEO [10]. According to Faraone e. a. [11] pure PEO shows no interaction in solution and behaves like a rigid sphere. PP7, on the other hand, does not behave like a rigid sphere. But there are apparently also no a strong interaction between the PP7 molecules leading to larger aggregations in solution, see the behaviour of the surface tension. Also, the self-diffusion coefficient of an isolated PP7 molecule is not too much different from that of PEO. That leads to the conclusion that the surface of the molecule prodrug still is rather PEO-like. However, the shape of PP7 seems to be different from PEO, at least a deformed sphere. This deformation appears not to be so strong that the anisotropy of the shape causes lyotropic behaviour. The present explanation of the experimental results is that the PEO-chain coils-up around the lipophilic core of the paclitaxel in a way comparable to the tertiary structure of

proteins where the hydrophilic parts of the polymer chain form a conformation which makes the surface hydrophilic while the inner part of the shell is lipophilic and covers the hydrophobic core. The hydrophobic interactions between core and shell could also explain the small decrease in the apparent hydrodynamic radius which is observed comparing PP7 with PEOS. Further investigations, in particular the influence PEO-chains on the hydrodynamic properties and a more detailed analysis of the hydrodynamic radius are planned to determine structure and interaction of the polymer modified drug in solution. In particular this will be of importance concerning possible interactions with components of the body fluid like proteins and polysaccharides and with respect of a possible targeting effect.

Conclusion

Molecular engineering on the paclitaxel molecule not only changes its solubility, a number of other properties such as activity, targeting and the transport properties in solution are affected by the modification. Examination of the details of these structure property relations can improve the applicability and the efficiency of the drug. Conformation of the polymeric solubiliser and the stability of the polymer-drug linkage play a crucial role in these interactions.

Acknowledgement

Financial support of the Deutsche Forschungsgemeinschaft (DFG), Bonn, and the Korean Science Foundation (KOSEF), Seoul, is greatly acknowledged.

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