# Self-Diffusion of PEO-Modified Paclitaxel in Aqueous Solution: Hydrodynamic Properties

Dedicated to Prof. *Jung-Il Jin*, Korea University, Seoul, Korea, on the occasion of his 60th birthday

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**Summary**: The anti-cancer drug Paclitaxel has been hydrophilized by coupling with poly(ethylene oxide) through a self-immolating linker to the polymer. The mobility of the functionalized polymer and of the amphiphilic drug-modified polymer in $D_2O$ was studied by the temperature and concentration dependence of the long-time self-diffusion coefficients of the components. The technique applied was pulse-gradient field NMR spectroscopy. The diffusivity of the molecules can be described in terms of the Einstein-Sutherland and the Stokes-Einstein equations. First results indicate that combining the drug with the polymer rather influences the rigidity parameter and the pair-interaction in solution than the shape and the hydrodynamic radius of the polymer coil. Unexpectedly, micelle-formation, although highly probable, was not observed within the concentration range investigated here.

**Keywords:** amphiphilic polymer; mobility; pulse-gradient field NMR

## Introduction

Paclitaxel is a lipophilic anti-cancer active drug that can be isolated from the bark of the pacific yew tree (Taxus *brevifolia*). Its anti-cancer activity concentrates on interfering with the mitosis through destabilization of the microtubuli. Paclitaxel is proven to interact in such a manner with a number of different cancer types, such as lung, breast, ovary, and some leukaemias.

As a lipophilic molecule Paclitaxel cannot be applied directly to the body by injection or orally; it has to be hydrophilized first. There are different potential reactive sites for this on the molecule, see Fig. 1. One successful way to hydrophilize a lipophilic molecule is to attach

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it to a hydrophilic polymer chain like poly(ethylene oxide) (PEO). PEO itself is known for its tumour targeting potential in that PEO accumulates preferably in some malign tissues. The preferable sites for the modification, where PEO can be coupled with Paclitaxel, are positions 2' and 7 are positions 2' and 7, see Fig. 1. This has been successfully done, as described in various patents, e.g.[1]. The disadvantage of these modifications was that the stability of the modified drug was too high. The modified drug has to be released from the polymer by an esterase and, with the types of linkage between the polymer and the drug existing to date, this reaction takes several hours. As a consequence, much of the drug was excreted by the body before it could develop its activity.

Jo et al.[2] recently developed a patented self-immolating link between PEO and the drug which provides a hydrolytic stability of the drug-polymer link in the body liquid of only a few minutes, just sufficient to transport the major amount of the drug to the site where its activity is required.

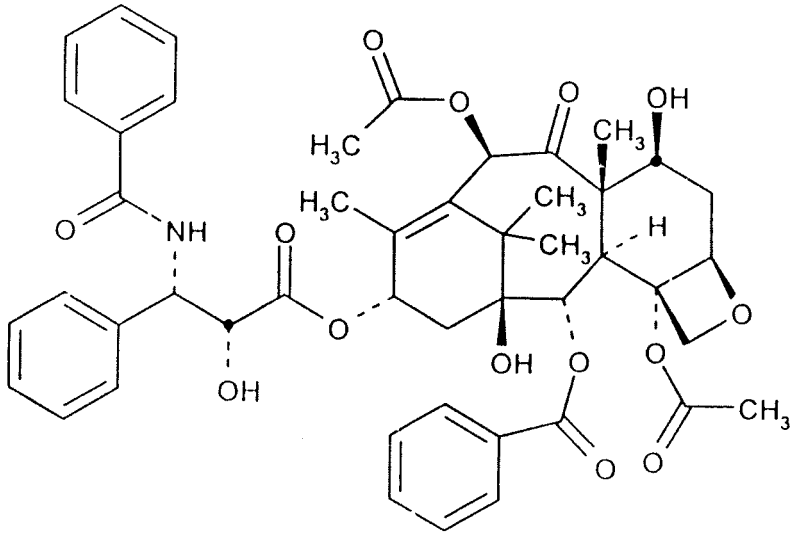


Fig. 1: The anti-cancer active drug Paclitaxel with its potential docking sites on C-2' and on C-7 for a hydrophilic modification with PEO. (The derivative investigated in this study is the pre-drug with substitution at C-7, termed PP7.)

Further studies of the transport behaviour of the drug in the body liquid require detailed knowledge about the mobility of the molecule, its interaction and phase behaviour under different conditions. Investigations into the surface activity, the search for mesophases to be expected because of the likely amphiphilic character of the molecule, and modifications of the substitution pattern of the drug are presently underway. In later stages of the investigations it will be of particular interest to study possible interactions with proteins present in the body fluid and to consider diffusion in confined environments (as they are provided by cells), therefore knowledge of the "simple" pure systems is important.

In a previous publication[3] we reported first results on diffusion of these systems in $D_2O$. We now report further results on the investigation of the hydrodynamic properties of the 7'-substituted Paclitaxel (PP7) and succinic acid-modified PEOS (5000 g/mol and 20000 g/mol) as determined from pulse-gradient field NMR/experiments. The monosuccinyl PEO (PEOS) was chosen to compare the behaviour of the polymer coil with the corresponding behaviour of the modified drug. The pulse gradient field technique has been described elsewhere eg. by Callaghan et al.[4], Steijskal and Tanner[5], Otting[6], Stilbs[7] and Kimmich[8]. The Einstein-Sutherland equation (eq. 1) describes the self-diffusion coefficient of Brownian particles:

$$D = \frac{k_b \cdot T}{f} \tag{1}$$

$D \equiv$ self-diffusion coefficient, $f \equiv$ friction factor, $k_b \equiv$ Boltzmann-factor, $T \equiv$ thermodynamic temperature.

In the case of spheres at infinite dilution (that is, for a single particle) eq. 1 becomes the well-known Stokes-Einstein equation:

$$D_0 = \frac{k_b \cdot T}{6 \cdot \pi \cdot \eta_s \cdot R_H^3} \tag{2}$$

$\eta_s \equiv$ the viscosity of the pure solvent, here $D_2O$, taking into account its temperature dependence; $R_H \equiv$ the hydrodynamic radius of the equivalent sphere.

The volume fraction dependence of the self-diffusion coefficient for a dispersion of colloidal hard spheres can be described by a series expansion in two equivalent ways, see for example Dhont[9] or Russel et al.[10]:

$$D = D_0 (1 - \lambda \cdot \varphi + ...) \tag{3}$$

or through the concentration dependence of the friction factor $f$:

$$f = f_0(1 + \nu \cdot \varphi + ...) \tag{4}$$

where $f_0$ equals $6\pi\eta_s R_H^3$. In the case of polymer chains the volume fraction can best be described by taking the coil volume fraction, which is related to the coil-overlap concentration $c^*$:

$$\varphi = c/c^* \tag{5}$$

with $c \equiv$ the mass concentration and $c^*$ defined by:

$$c^* = \frac{M}{\frac{4}{3}\pi R_H^3 N_A} \tag{6}$$

$M \equiv$ the molar mass; $N_A \equiv$ Avogadro's constant.

At infinite dilution $f$ in eq. 1 is equal to $f_0$. The coefficient $\nu$ is sometimes called the rigidity parameter and depends on the hydrodynamic pair interaction between the particles and thus depends on the shape and rigidity of the particles. For rigid spheres $\nu = 2.00$ in the absence of hydrodynamic interactions, Pyun and Fixman[11], Hanna et al.[12]. Dhont et al.[13] have extended the theory for rod-like particles. In the case of repulsive interactions $\nu$ becomes smaller than 2, whereas it becomes larger in the case of attractions. This can be understood by realising, that in the case of attractions, two particles of the same component will - on the average - stay longer in proximity with each other than with other molecules, thereby slowing down their diffusion.

## Experimental

Paclitaxel, see Fig. 1, was coupled to α-hydro-ω-methyl-polyl(ethylene oxide) (PEO) with a self-immolating succinic acid spacer (abbreviation of the PEO with the linker: PEOS) to obtain the water soluble product PP7, as described elsewhere[14]. A highly uniform PEO of molar mass Mw = 5000 g/mol (PEOS 5000) was used to create the desired water solubility.

The non-uniformity of the molar mass was 1.05 for all polymers, the succinate derivative, PEOS and the hydrophilized drug PP7. There was also a PEOS of molar mass Mw = 20000 g/mol, with the same narrow distribution of the molar mass, available for comparative studies. The modified drug PP7, is however, presently only available attached to the 5000 g/mol polymer.

The experiments were carried out on a Bruker Avance DRX 500 equipped with a water cooled Diff30 probe with z-gradient and a 40 A gradient amplifier. The gradient strength was 0.3 T $m^{-1}$ $A^{-1}$. The measurements were done in a Bruker 11.74 T standard bore magnet. The pulsed magnetic field gradient magnitude g was varied up to 6 T/m. The gradient was calibrated by comparison with literature data of $D_2O$ and was accurate within 1.5%. The sample temperature was determined after calibration with the temperature dependence of the peak shift in methanol. Because of the development of undesired temperature gradients in the probe head which caused convection in the sample, the sample temperature was controlled by the water jacket of the z-gradient coil. The samples were allowed to equilibrate for 30 min prior to the experiments being carried out. The variance in the determined values of the diffusivity was always of the order of magnitude of $2x10^{-13}$ $m^2$ $s^{-1}$. The experiments were carried out in $D_2O$ of purity > 99.95 D%.

## Results and Discussion

The diffusion time $\Delta$ during which the NMR experiments were carried out delivered the so-called "long-term" diffusion coefficient which means that the time scale of the motion is large compared with the relaxation times of the particles[11], that means $t > 10^{-6}$ s. During a diffusion time $\Delta$ = 350 ms the diffusion coefficients were determined with an accuracy of about $2 \cdot 10^{-13}$ $m^2s^{-1}$. Fig. 2 shows the concentration and temperature dependence of the diffusion coefficient of PEOS 5000 and PP7. Fig. 3 shows the influence of the higher molar mass on the self-diffusion coefficient of PEOS. Clearly, the molecules interact as indicated by the decreasing self-diffusion coefficient with the polymer concentration. At low concentrations there is an almost perfect linear dependence, whereas at higher concentrations higher coefficients of the expansion have to be considered. The concentration dependence of the self-diffusion coefficient is stronger in case of the drug-modified PEO, indicating attractive interactions between the molecules which lead to an increase of the $\lambda$-parameter and thus to an increase in the self-diffusion coefficient. However, in the concentration range investigated here, there is

no evidence of critical micell formation. No lyotropic phases were observed in this concentration range.

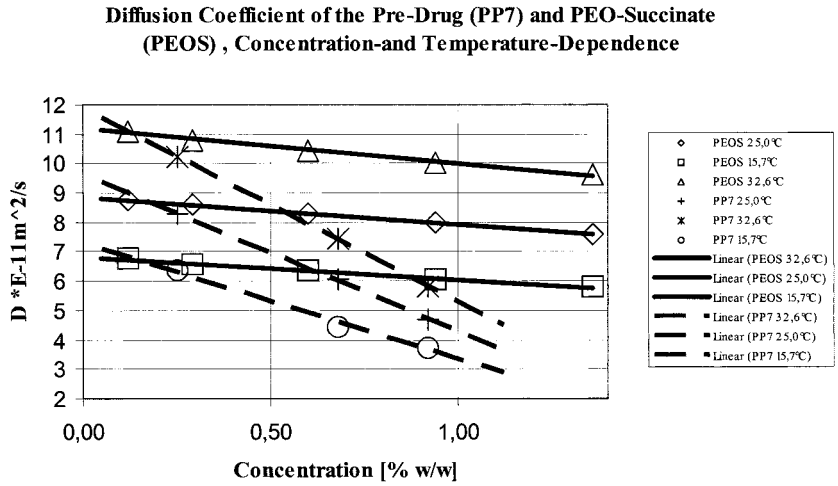


Fig. 2: Diffusion coefficient D vs. composition with the temperature as parameter. The molar mass of the PEO chain is 5000 g/mol

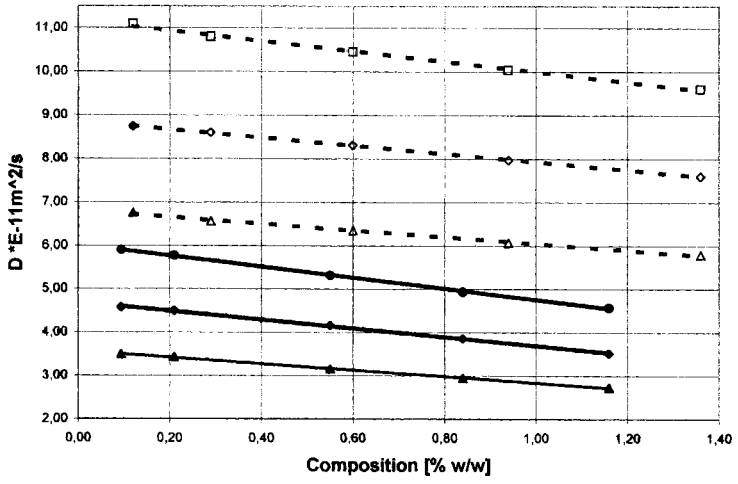


Fig. 3: Diffusivity and molar mass of PEOS. Composition dependence of the diffusion coefficient with the temperature as parameter. dotted lines: PEOS 5000 g/mol; full lines: PEOS 20000 g/mol, open squares: T = 32,6°C, open lozenges: T = 25°C, open triangles: T = 15°C, filled circles: T = 32,6°C, filled lozenges: T = 25°C, filled triangles: T = 15°C

The temperature dependence of the self-diffusion coefficient is of an Arrhenius-type, with an apparent energy of activation constant within experimental accuracy for all samples in the investigated concentration/temperature range. This indicates no significant changes in the transport mechanism. There is also no significant change in the size and shape of the molecules. Hence, the formation of larger aggregations or micelles could not be proven yet. The hydrodynamic radius $R_H$ at 25°C is calculated with eq. 2 and the results are shown in Table 1.

Table 1: Hydrodynamic radius and series expansion coefficient λ in eq. 3

| | $R_H$/nm | $\lambda$ |
|---|---|---|
| PEOS 5000 | 2.23 | 1.2 |
| PEOS 20000 | 4.13 | 6.2 |
| PP7 | 2.06 | 12 |

The fact that the single-particle self-diffusion coefficient for dilute solutions, $D_0$, does not change drastically form PEOS to PP7 indicates that the shape of the molecules does not change significantly and that both types of molecules nearly behave like rigid spheres, as is expected and known for pure PEO[15]. Analysis of the concentration dependence of the self-diffusion coefficient in terms of eq. 3 shows an about 10 times larger expansion coefficient $\lambda$ compared with the drug-free PEOS 5000, see Fig. 4. Since the shape of the molecules is not significantly altered by the coupling of the drug with the polymer chain as pointed out above, an explanation might be found in a change in the interaction potential[16]. The magnitude of the effect could be explained by effective attractions between the modified drug molecules. The behaviour of PEOS 20000 can be understood as that of a soft sphere.

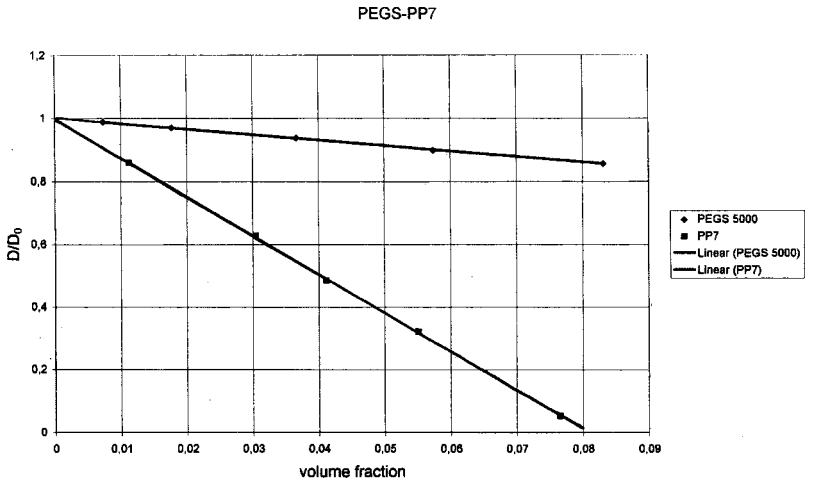


Fig. 4: Concentration dependence of the diffusion coefficient of PEOS 5000 and PP7 at 25°C in terms of eq. 3

## Conclusion

Coupling the hydrophobic Paclitaxel with PEO via a self-immolating linker suggests an amphiphilic macromolecular product consisting of the **hydrophobic** drug as the **"head"** and the **hydrophilic** macromolecular **"tail".** However, the rather similar $D_0$-values of PEOS and PP7 (see Fig. 2) indicate that the pre-drug does not behave differently to that of a rigid spheroid like PEO (or PEOS). The apparent hydrodynamic radius of PP7 is only slightly smaller compared with PEOS 5000. The major difference in the diffusion behaviour appears to be caused by the friction factor. This means there are stronger interactions in pre-drug solutions compared with PEOS solutions. Since there were no lyotropic phases or micelles observed in the concentration range investigated here, a probable interpretation is that the polymer chain attached to the pre-drug coils around the drug, resulting in a core-shell-like structure comparable to the folding of many proteins (i. e: the tertiary structure), having a hydrophilic surface that keeps them water soluble but a hydrophobic interior. The attractive interactions between the Paclitaxel molecule and the surrounding PEO chain could even explain the reduced hydrodynamic radius observed in PP7 (5000 g/mol) compared with PEOS (5000 g/mol). This interpretation has recently been supported by investigations of the surface tension and the viscosity of aqueous solutions of PP7[17,18]. This would have consequences for the targeting effect of the polymer mentioned above and probably also on the reaction rate of

the esterase. Therefore, a broader concentration range in pure aqueous solution has to be investigated and different methods of binding Paclitaxel to the polymer have to be examined in the future in order to understand shape, transport and reaction mechanism of Paclitaxel in the complex environment where it is finally supposed to be bioavailable and active.

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