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Polyvinyl butyral DMN-conjugates for the controlled release of singlet oxygen in medical and antimicrobial applications

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Abstract. Covalent attachment of 1, 4-dimethylnaphthalene (DMN) based endoperoxide forming subunits to a polyvinyl butyral (PVB) backbone has been achieved. The functionalized polymer materials prepared and characterized here can serve as biocompatible carrier systems for studying cellular uptake, intermediate storage and delayed release of singlet oxygen, which opens up new doors for optimizing a variety of medical applications of photogenerated DMN-endoperoxides such as antiviral, antibacterial, antiplasmodial and antitumor activity.

Keywords: dimethyl naphthalene; polyvinyl butyral; endoperoxides; singlet oxygen; drug carriers

1. Introduction

Strategies for the specific targeting and release of biologically active compounds play an important role in the fields of pharmacokinetics and drug design. In this context, the development of polymer-based carrier systems bears an enormous potential for future chemotherapy and nano-medicine (Duncan 2006, Wang *et al.* 2014). Since the pioneering studies on pharmacologically active polymers (Ringsdorf 1975), there has been a continuous improvement of covalently and non-covalently bound carrier-drug conjugates (Khandare and Minko 2006, Larson and Ghandehari 2012). Although in the mean time many of such polymer-drug conjugates have already progressed into clinical development, in some cases their non-biodegradable nature may still be considered as an important drawback. It is therefore important to keep on searching for alternative biocompatible drug delivery systems.

We have recently presented a novel strategy for controlling the delayed release of singlet oxygen ($^1O_2$) in the context of different biomedical applications including cancer chemotherapy or the deactivation of bacteria, viruses and parasites (Posavec *et al.* 2012). The singlet oxygen releasing system applied is based on the intermediate storage and transport of metastable endoperoxides photochemically generated from functionalized dimethylnaphthalene derivatives

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such as 1-(1, 4-dimethyl-naphthalen-2-yl)-ethanol (DMN-OH) and related compounds (Fig. 1).

As an extension of our previous work (Posavec *et al.* 2012), we wish to report here our results on the covalent attachment of such functional 1,4-dimethyl-naphthalen-2-yl subunits to a polyvinylbutyrate (PVB) polymer backbone. PVB has been selected as a carrier medium for drug delivery because of its excellent biocompatibility, non-toxicity and the possibility of preparing spherical PVB nanoparticles that have been shown to be efficiently taken up without further surface modifications by human cancer cells (Posavec *et al.* 2011). This novel approach enables to reach a very high local concentration of endoperoxide-forming groups in the target region. At the same time, the endoperoxide decay process and thus also the $^1O_2$-release period will be drastically prolonged by an incorporation of the DMN-derivatives into the polymer matrix and an additional adaptation modifying the functional structures present in the material system, which is a crucial factor for many biomedical and therapeutical applications (Posavec *et al.* 2012, Bogner *et al.* 2011).

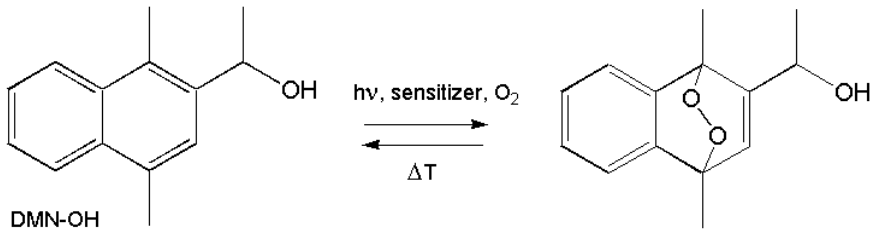

Fig. 1 Photochemical formation and thermally induced decay of DMN-OH endoperoxide (Posavec *et al.* 2012)

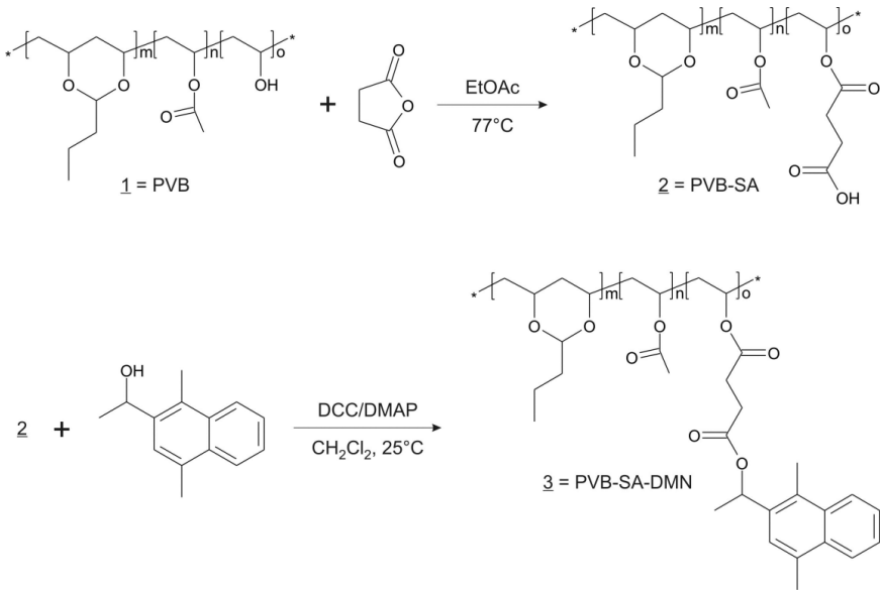

Fig. 2 Binding of DMN-OH to the PVB backbone using succinic acid as a linker molecule

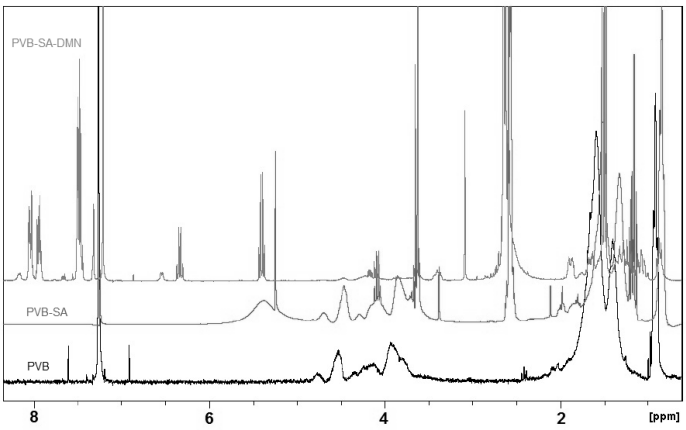

Fig. 3 ^{1}H-NMR spectra in $CDCl_3$ demonstrating the reaction of PVB (bottom) with succinic anhydride forming the PVB-SA intermediate (center), and the esterification with DMN-OH to yield the PVB-SA-DMN polymer product (top)

2. Results and discussion

Covalent binding of DMN-OH to the hydroxyl groups of the PVB polymer was performed by a two-step reaction sequence according to Fig. 2. In the first step, the hydroxyl groups of PVB were modified with succinic anhydride resulting in the formation of PVB succinic acid ester (PVB-SA). In the second step, the carboxylic acid groups of the PVB-SA intermediate were activated by the DCC/DMAP-activation method (Neises and Steglich 1978, Antoni *et al.* 2009) and coupled via esterification to the hydroxyl group of DMN-OH resulting in the polymer PVB-SA-DMN.

In the following figures, the comparative characterization of the product PVB-SA-DMN (3), the PVB-SA intermediate (2), and the PVB polymer educt (1) with ^{1}H-NMR- (Fig. 3), UV-Vis- (Fig. 4), and FTIR-spectroscopy (Fig. 5) is described. The proton NMR-spectrum of the polyvinyl butyral polymer starting material in $CDCl_3$ is shown at the bottom of Fig. 3. When the PVB-SA intermediate is formed, a strong new peak occurs at 2.6 ppm, corresponding to the four CH_2 protons of the attached succinic acid moiety (Fig. 3, center). After the second step of the reaction sequence described above, the PVB-SA-DMN product with characteristic aromatic ^{1}H-NMR peaks in the 7.2-8.1 ppm region appears (Fig. 3, top), corresponding to the naphthalene subunit of DMN.

The UV-Vis absorption spectra of the same three polymers are compared in Fig. 4. Clearly, in the case of the PVB-SA-DMN product, additional absorption bands show up with maxima at around 230 nm and 290 nm, which are expected to occur in the presence of a 1,4-dimethylnaphthalene chromophore (Posavec *et al.* 2012). This result also strongly indiates that a successful attachment of DMN-OH to the backbone of PVB was achieved.

The characteristic carbonyl stretching band at 1778 cm-1 (Schramm and Rinderer 2008) appears only in the PVB-SA and PVB-SA-DMN infrared spectra, and is not present in the PVB starting material (Fig. 5, bottom). Note that the apparently negative peaks in the PVB-SA and PVB-SA-DMN spectra shown in Fig. 5 are artefacts, which most probably result from variations of solvent concentration in the samples (the peak at 3388 cm-1 corresponds to the O-H stretching vibration of the ethanol solvent, the peak at 2975 cm-1 corresponds to CH2- stretching of ethanol, and 1072 cm-1 and 1028 cm-1 peaks correspond to C-O- stretching vibration of the solvent).

3. Conclusion and outlook

In the present work, we have synthesized and characterized the novel polyvinyl butyral derived polymer PVB-SA-DMN, where the alcohol groups in the PVB core structure have been decorated with covalently bound endoperoxide-forming 1,4-dimethylnaphthalene side-groups.

Based on our previously described results on the successful exploitation of functionalized DMN-derivatives for several biomedical applications (Posavec *et al.* 2012, Posavec *et al.* 2011, Bogner *et al.* 2011), endoperoxide loaded films or nanoparticles of the polymer PVB-SA-DMN could potentially be used as novel materials with interesting anticancer and antibiotic effects. In this context it is important to note that the concentration of endoperoxide forming groups in PVB-SA-DMN is expected to be sufficient for inducing a significant cytostatic effect (estimated concentration necessary is >15 μM) (Posavec *et al.* 2012). Further research efforts in this direction are currently underway.

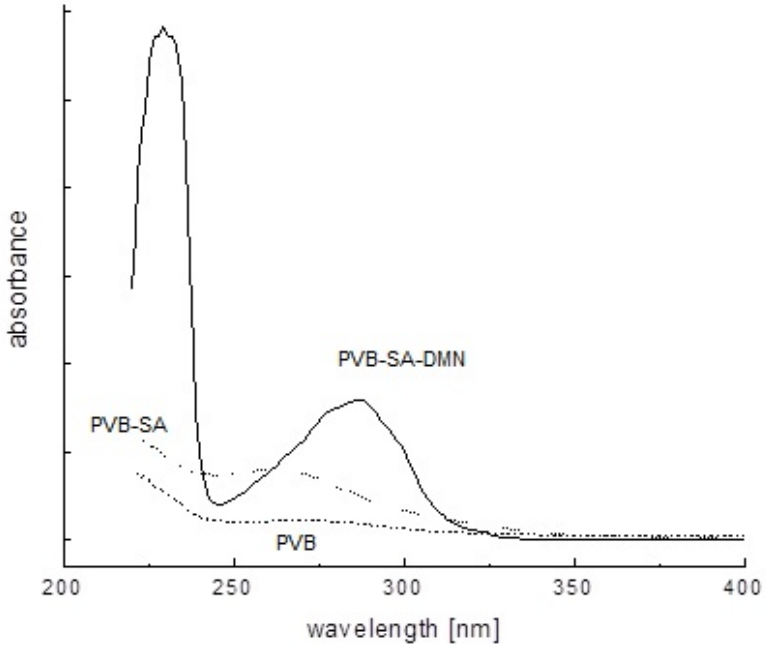

Fig. 4 UV-Vis absorption spectra of PVB (bottom), PVB-SA (center), and PVB-SA-DMN (top)

4. Experimental

4.1 Materials and methods

All commercially available chemicals and solvents used for the present work were applied as received without any further purification. LP B 16H polyvinyl butyral (PVB) powder was from Kuraray Specialities Europe GmbH, Frankfurt, Germany. Solvents ethyl acetate, hexane and dichloromethane were purchased from Sigma-Aldrich, Munich, Germany. Succinic anhydride

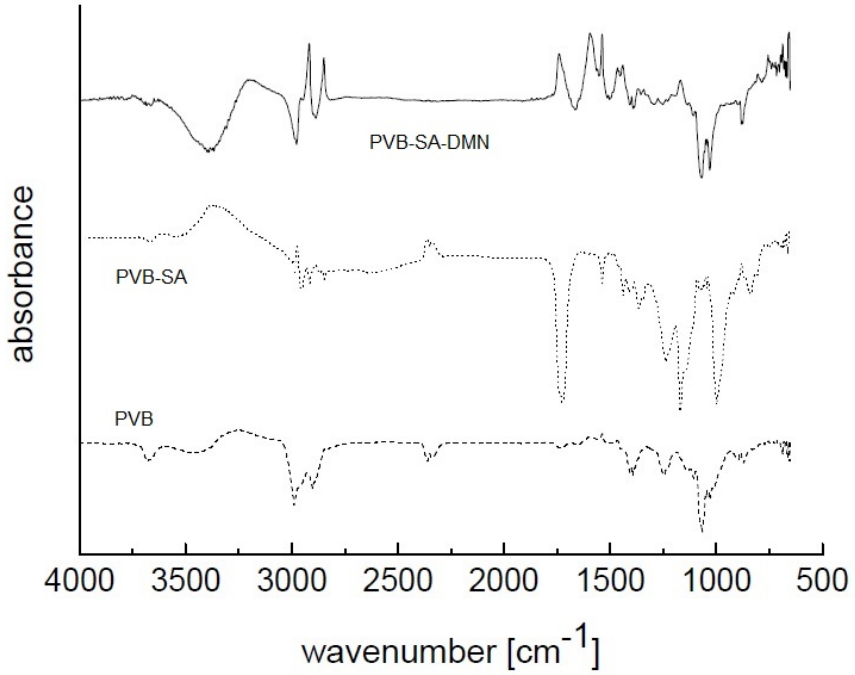

Fig. 5 FTIR-spectra of PVB educt, PVB-SA intermediate and PVB-SA-DMN product in ethanol. The solvent spectrum was used as a reference

(SAH), *N, N´*-dicyclohexylcarbodiimide (DCC), and N,N-dimethylaminopyridine (DMAP) were also from Sigma-Aldrich, Munich, Germany. The functionalized 1, 4-dimethylnaphthalene derivative 1-(1, 4-dimethyl-naphthalen-2-yl)-ethanol (DMN-OH) was prepared and characterized as previously described (Posavec *et al.* 2012). UV-Vis absorption spectra were recorded with a Varian Cary 300 Bio UV/Vis Spectrometer with a 1-cm quartz cell (Hellma GmbH & Co. KG, Müllheim, Germany) and Uvasol® quality solvents from Sigma-Aldrich (Munich, Germany). ^{1}H-NMR measurements were carried out by the Centre for Chemical Analysis of the Faculty of Chemistry and Pharmacy of the University of Regensburg. FTIR spectra were recorded with a Jasco (Tokyo, Japan) FTIR-610 spectrometer using a ZnSe-ATR device of Pike Technologies (Madison, USA).

4.2 Synthesis

Step 1: 1.5 g of LP B 16H PVB powder (6.14 mmol of PVB OH-groups) was dissolved in 120 ml of ethyl acetate at b.p. (with reflux) and stirred for 1 hour. 6.14 mmol (0.614 g) of succinic anhydride was dissolved in 70 ml of ethyl acetate using an ultrasonic bath for 5 minutes, and added to the PVB solution. This mixture was stirred over night at ethyl acetate b.p. of 77℃ (with reflux) and later cooled slowly to room temperature (RT). Hexane was added to the mixture until a white suspension appeared. The suspension was filtrated leaving a white product (PVB-SA). Product characterization was performed with ^{1}H NMR, FTIR and UV-Vis absorption spectroscopy.

Step 2: 187 mg of PVB-SA was dissolved in 5 ml of dichloromethane, to which DCC (158 mg, 0.767 mmol), DMAP (6.25 mg, 0.0511 mmol), and finally, the DMN-OH derivative (104.5 mg, 0.767 mmol) were added. The mixture was vigorously stirred at RT for 72 h. Hexane was added until white DCC-urea precipitated. The solution was filtrated and hexane evaporated until a white oily product (PVB-SA-DMN) remained. Product characterization was performed with ^{1}H NMR, FTIR and UV absorption spectroscopy.

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