JOURNAL OF OPTOELECTRONICS AND ADVANCED MATERIALS Vol. 9, No. 6, June 2007, p. 1816 - 1820

Porphyrin encapsulation in nanostructured hydrogels

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Nanoporous hydrogels have been prepared by freezing-thawing method. Three types of porphyrins have been encapsulated into PVA hydrogels. It was demonstrated that the PVA hydrogels represent an efficient encapsulation vehicle for porphyrins. Hydrogels prepared from high molecular mass PVA have a better sorption profile for porphyrins, and are better suited for the preparation of controlled release vehicles.

(Received November 14, 2006; accepted April 26, 2007)

Keywords: Hydrogel, Porphyrin, Encapsulation

1. Introduction

Hydrogels constitute an important advance in present-day biomedical and environmental technology. They have important applications in water decontamination, biosensors and implantable devices. Among them, hydrogels from poly (vinyl alcohol) are especially important due to their advantages of being water soluble, non-toxic, non-carcinogenic and biodegredable. The PVA hydrogel has excellent biomedical properties regarding mechanical, water absorption and swelling characteristics.

Hydrogels have also important applications in the field of drug release devices [1, 2, 3, 4]. Their nanostructured characteristics have been confirmed by various methods, including SEM microscopy [5,6]. Different release vehicles can be built by using either cylindrically shaped, membranar or multilaminate devices [7,8,9,10]. Also, multilaminate devices can be built in which the degree of crosslinking differs from one membrane to another. This kind of devices enable directional release, either normal (release in water) or accelerated (release in ionic media).

Porphyrins constitute a class of chemical compounds with many applications including photonics, pigments, nanomaterials and oncology. Alternative cancer therapies have been developed recently, and among them the photodynamic therapy (PDT) is especially important. Photodynamic therapy (PDT) is a treatment that uses a photosensitiser, that when irradiated at a specific wavelength, transfers the energy stored in its molecular structure, to another molecule (specifically, the oxygen in tissue), generating an active species (singlet oxygen, with free-radical character) that oxidizes the tumour and accelerates tumour apoptosis by mitochondrial damage. The porphyrin is a compound with a long-lived triplet excited state, soluble in aqueous media and in biological fluids.

Today there is a growing need for the development of controlled release formulations in the pharmaceutical industry, especially in complex therapeutic areas such as oncology. In this direction, a stabilization of the porphyrin in the hydrogel matrix represents a step forward in PDT.

5,10,15,20-tetra-sulfonato-phenyl porphyrin [TSPP] is a photosensitizer used mainly in the photodynamic therapy of various types of cancer (skin cancer, eye cancer, non small cell lung cancer not treatable by chemotherapy or cobaltotherapy), but also in cancer diagnosis (functionalized TSPP accumulates specifically in tumors and the fluorescence resulting from irradiation can be used to detect tumoral tissue) and in the treatment of viral infections such as herpes simplex infection.

The aim of this study is to find the way of porphyrins' encapsulation in an ecological and biocompatible PVA hydrogel matrix, in order to stabilize them to the action of light. We tried to encapsulate two types of porphyrins: water soluble and water insoluble ones. Two types of PVA have been used in order to check the influence of the PVA molar mass on the hydrogel capacity for porphyrins' encapsulation.

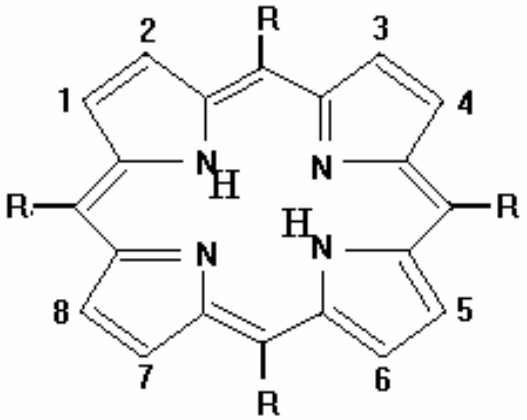
2. Experimental

2.1. Materials and methods

2.1.1. Porphyrins

For the experiments, three types of porphyrins have been used: two soluble in organic solvents such as dimethylsulfoxide (5,10,15,20-tetra-pyridil porphyrin, TPyP and 5,10,15,20-tetra-phenyl porphyrin, TPP) and one water-soluble (TSPP). TSPP serves as a therapeutic agent in photodynamic therapy. The porphyrins were

synthesized at ICECHIM institute in Bucharest. They were protected from light during storage as well as during all steps of sample or solution preparation.



TPyP: R=-C_5NH_4 ; TPP: R=-C_6H_5 ;TSPP: R= -C_6H_4-$SO_3^-Na^+$

Fig. 1. Structure of the porphyrins TPyP, TPP and TSPP.

2.1.2. Preparation of the porphyrin solutions

For the preparation of the porphyrin solutions a Kern precision balance with a precision of 0.1 mg was used. For liquid handling (pipetting) operations, Eppendorf high precision pipettes (1 mL with 1 μL precision, 1 mL with 100 μL precision and 1-5 mL) were used.

Solutions with a concentration of 10^{-3} M have been prepared for all the studied porphyrines. As solvents, DMSO for TPP and TPyP, and water for TSPP have been used.

2.13. Poly(vinyl alcohol) [PVA].

Two types of PVA samples (origin: Rasnov, Romania) have been used: PVA 90-98 (degree of polymerization 900, degree of hydrolysis 98%) and PVA 30-98 (degree of polymerization 300, degree of hydrolysis 98%). They are industrial grades and have been used without further purification.

2.1.4. Preparation of the PVA solution

The PVA solution was prepared by dissolving PVA 90-98 or PVA 30-98 sample in distilled water under stirring and constant heating (90^0C), for 3 hours. The hot solution was filtered and its content in solids has been determined.

2.1.5. Preparation of the hydrogels

PVA hydrogels have been synthesized starting from two PVA grades: PVA 30-98 and PVA 90-98 and they have been prepared by the freeze-thawing method. Three twelve-hours cycles of freeze-thawing have been employed. Freezing was carried out by using a criostat (cycles of -25^0C to 25^0 C). The resulting hydrogels have been conditioned in distilled water.

2.1.6. Sorption experiments protocol

Two ways for porphyrins sorption on the PVA hydrogels have been proposed:

a/. sorption of the water soluble porphyrin (TSPP) has been achieved by directly immersing of the pre-weighed PVA hydrogel tablet (1.5 g) into 5 mL of the porphyrin solution.

b/. sorption of water insoluble porphyrins (TPP and TPyP) has been made in two consecutive steps: (1) the immersion of the pre-weighed PVA hydrogel tablet (1.5 g) into 5 mL of the

porphyrin solution in DMSO and (2) the elimination of the DMSO from the hydrogel by extraction with distilled water.

2.1.7. Measurement of the porphyrins degree of absorption

Samples were kept away from light during handling and inter-vessel transfer.

The degree of porphyrins sorption on PVA hydrogels was monitored for 30 minutes.

The degree of porphyrins sorption has been monitored in the porphyrin solution by using UV-VIS spectrophotometry with a Perkin Elmer Lambda 25 Spectrophotometer (Perkin Elmer, CT, USA).

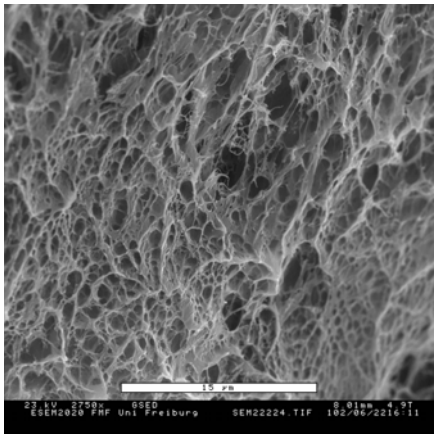
(λ_{TPP}= 414 nm; λ_{TPyP}= 417 nm; λ_{TSPP}= 434 nm**)**

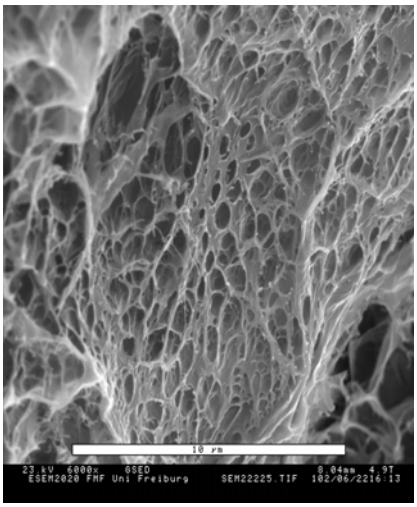
3. Results

Through freeze-thawing technique, heterogeneous, white, opaque and mechanical resistant PVA hydrogels have been obtained. SEM analysis showed a porous structure of the hydrogel, evidencing interconnected pores with a size distribution in the range of 80-950 nm (Fig. 1).

The porphyrins nano-cycles could be well entrapped by the hydrogels pores.

To encapsulate water insoluble porphyrins (TpyP, TPP) into the hydrogels, we had to immerse PVA hydrogel into the porpyrins' DMSO solution. In the presence of DMSO, the hydrogel had evidenced a supplementary swelling. It became transparent and colored in the porphyrin specific color. The sorption curves for TpyP, TPP are presented in Fig. 2.





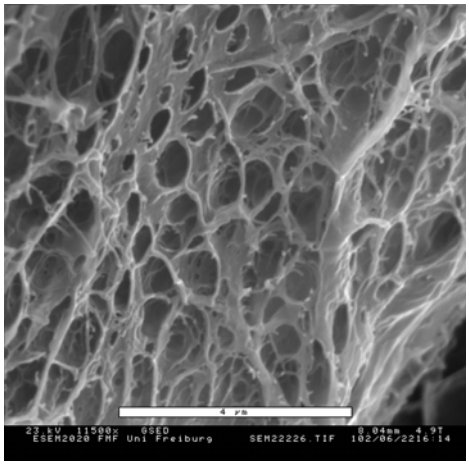
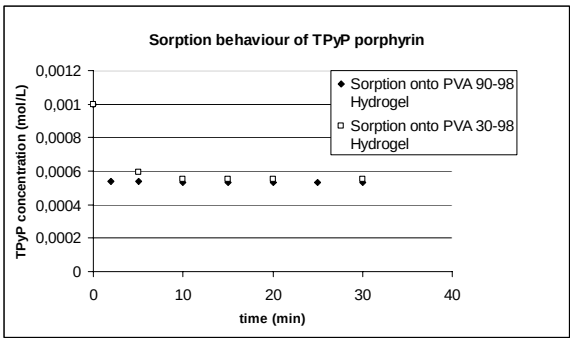


Fig. 1. SEM images of PVA 90-98 hydrogel.

For TPyP and TPP, a slightly stronger sorption on the PVA 90-98 hydrogel has been observed, by comparing with the sorption on PVA 30-98 hydrogel.



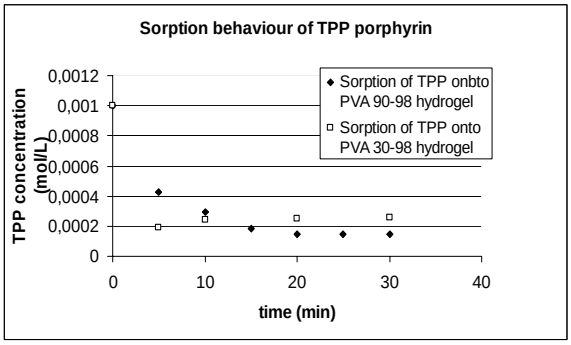


Fig. 2. Sorption curves of TpyP and TPP porphyrins on hydrogels of PVA 30-98 and 90-98.

Because DMSO is toxic, it has to be removed from the hydrogel structure. We eliminated the DMSO by extraction with distilled water. We have monitored the collapse of the DMSO-swollen gel after immersion into water and then into a phosphate-buffered salt solution (PBS, as used in cancer therapy clinical trials, provided by Victor Babes Institute in Bucharest) (Fig.4). In this manner, hydrogel tablets swollen in water and loaded with encapsulated porphyrins TPyP and TPP (only DMSO-soluble) can be prepared.

Water soluble porphyrin (TSPP) sorption on the two types of PVA hydrogels, as a time function is presented in Fig. 5.

For TSPP, the sorption on PVA 90-98 is much stronger than that on the PVA 30-98 hydrogel.

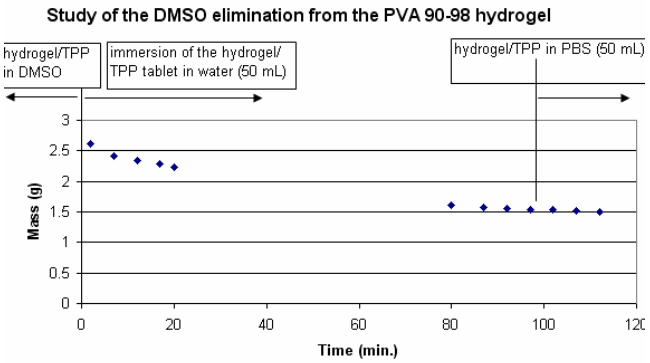


Fig. 4. Study of DMSO elimination from the PVA 90-98 hydrogel.

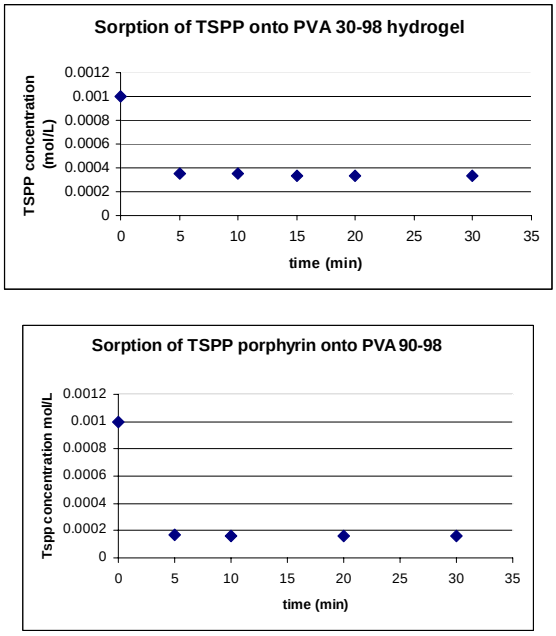


Fig. 5. Sorption curves of TSPP porphyrin on hydrogels of PVA 30-98 and 90-98.

4. Discussion

The experimental data reveal that all the studied porphyrins, even they are water soluble or not, have a high affinity for PVA hydrogels.

One can also observe from the sorption curves that the sorption equilibrium is reached fast (in 5 min in most cases).

If we compare the two gels, one prepared from PVA 90-98 and the other from PVA 30-98, we can conclude that the gel with the higher molecular weight (PVA 90-98) could be better loaded with porphyrins (Fig.6). This fact could be explained by the higher capacity of gels obtained by PVA with higher molecular mass to swell, comparatively with that obtained by low molecular mass, due to the mechanism of preparation [11].

This results in the increasing of the solvent flux into the PVA 90-98 gel, bringing a higher amount of porphyrin.

Thus, a more sustained release characteristic could be provided by PVA 90-98 when the resulting tablet (with encapsulated porphyrin) is placed in the appropriate environment. We can thus conclude that PVA 90-98 is more suitable for controlled release.

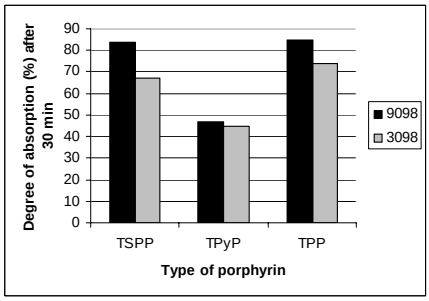


Fig. 6. Comparison between the sorption degree of porphyrins on the PVA hydrogel, as function of PVA molar mas and porphyrin type.

The sorption mechanism of the porphyrins onto the PVA hydrogel can be interpreted as having two components: physisorption and chemisorption. In physisorption, the porphyrin is encapsulated in the pores of the nanostructured hydrogels. This mechanism is mainly controlled by diffusion. The diffusion mechanism is not ideal (Fickian), but rather Stephan diffusion, because of the anisotropic porous structure of the gel [12]. The chemisorption mechanism consists of the hydrogen bonding between the –OH groups of the poly(vinyl alcohol) and the pyrrholic nitrogen of the porphyrin molecule. The nature and the intensity of the chemisorption depends largely on the conformation of the porphyrin molecule and the solvent used.

The hydrogel tablets loaded with TSPP, TPyP or TPP by sorption exhibit a porphyrin concentration gradient. The depth of porphyrin penetration into the gel is dependent on the porphyrin type and solvent.

The study of the DMSO elimination from the hydrogel shows an asymptotic elimination characteristic, with equilibrium reached after 2 hours. This method can serve for the elimination of toxic DMSO from the gel (used in pharmaceutical preparations for drug delivery of DMSO-soluble porphyrins) by the introduction of the DMSO-swollen gel into water or solutions of biocompatible electrolytes.

5.Conclusions

Nano porous hydrogels have been prepared by freezing – thawing technique, starting from PVA-s with different molar mass (PVA 90-98 and PVA 30-98) aiming to use them as vehicles for different porphyrins with application in the photodynamic cancer therapy.

By using sorption technique, two types of porphyrin-loaded PVA hydrogels have been obtained.

Three types of porphyrins have been encapsulated into PVA hydrogels: TSPP (water soluble) and TPP and TpyP (water insoluble). All the porphyrins showed a high affinity for PVA, due to their interactions by hydrogen bonds. The sorption degree on the PVA hydrogels was higher for TSPP and TPP, by comparison with TpyP and on hydrogels synthetized by PVA with higher molecular mass.

DMSO, as solvent involved in water insoluble porphyrins sorption step, could be removed from the hydrogel, by extraction with distilled water, avoiding the presence of a toxic substance in the final device.

We can conclude that PVA hydrogels represents an efficient encapsulation vehicle for porphyrins, both water soluble and non-water soluble. Their biocompatible, biodegradable, non-toxic and non-carcinogenic nature makes them especially effective for pharmaceutical applications, but also for environmental uses, such as advanced waste-water decontamination. Hydrogels prepared from high molecular mass PVA have a better sorption profile for porphyrins, and are better suited for the preparation of controlled-release vehicles.

Acknowledgements

We gratefully thank to Prof. Dr. Christian Friedrich from Albert Ludwigs University from Freiburg, Germany, for helping to realize this study and also to CNCSIS for funding our research through national grants CEEX 10/2005, CEEX 18/2005 and CEEX 148/2006

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