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High-performance liquid chromatography and UV-visible measurements to optimize the storage of volume holograms in hydrogels

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ABSTRACT

The storage of time-stable holographic gratings in photohydrogels when the material is immersed in liquid media represents a great challenge at present. A very important stage in the process of storing holograms in photohydrogels are the washing stages to eliminate the remains of the components that have not reacted in the photochemical reaction. The main goal of this work is focusing on the study of the optimization of the washing stages of the photohydrogels based on acrylamide and *N*,*N*'-methylenebis(acrylamide) once unslanted transmission holograms have been stored. For the purpose of determining the compositions of the wash solutions, High-Performance Liquid Chromatography and UV-visible measurements have been employed in our system. PBST and DMSO:H_2O 6:4 (v/v) are used as solvents in the washing stages. The diffraction efficiencies are measured during the washing stages and after the storing of the holograms during several days in PBST. Maximum diffraction efficiencies of 38.0 and 27.6% are reached when PBST and DMSO:H_2O 6:4 are employed, respectively.

Keywords: Photohydrogel, unslanted holographic transmission gratings, washing stages, maximum diffraction efficiencies, high-performance liquid chromatography, UV-Visible spectroscopy

1. INTRODUCTION

Hydrogels are 3D polymeric networks with high swelling capacity in water and appropriate chemical, mechanical and biological features, which allow the storing of bioactive substances. Conventional hydrogels are prepared using synthetic polymers including polyacrylamide, poly(N-isopropylacrylamide) (PNIPAM), poly (vinyl alcohol), alkenes, alginates, synthetic or polysaccharide-based natural polymers, and several reaction types (covalent crosslinking, self-assembly, gelation) and activation modes (thermal, photochemical, and chemical) are employed for their synthesis[1]. Outstanding hydrogel features have been studied considerably in tissue engineering[2], drug release[3,4] and biosensors[5]. These hydrogels can be chemically modified to become photohydrogels in order to store holographic gratings[6,7]. The diffraction efficiencies (DE) of the holograms stored in photomaterials such as photohydrogels and photopolymers decreases over time due to the concentration gradient generated in the recording process. Molecular diffusion processes take place inside the material matrix. The generated polymer chains tend to diffuse towards non-exposed zones. Furthermore, the components that do not react in the non-exposed zones diffuse towards the exposed zones. These diffusion processes depend on the characteristics of the molecular components and the composition of the medium. A process to remove the concentration gradient is necessary to provide temporal stability to the stored holograms. Different techniques have been used with the aim to increase the stability of the holograms stored in photopolymeric materials such as exposure to ultraviolet light, dehydration of the photopolymer layers under controlled temperature conditions and incoherent light (LED lamp)[8,10]. LED lamp exposure post-recording is cheaper and simpler compared with other methods for photopolymer-based holograms. However, when these holograms are stored in stable photohydrogel in aqueous media, a method based on continuous

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washing of the post-recording material is more convenient. When an LED lamp is used, polymerization is produced in non-exposed zones. This polymerization is avoided when non-reacted components are removed by washing. In this work, the number of washing stages that need to be performed to remove non-reacted components in the hologram recording process was optimized by High-Performance Liquid Chromatography (HPLC) and UV-Visible studies when PBST and DMSO:H_2O 6:4 (v/v) are used as solvents. The performance of the hologram during the washing process and their temporal stability was measured.

2. EXPERIMENTAL PROCEDURE

The experimental steps necessary to carry out this work are shown in Figure 1. Initially, the photohydrogel layers were prepared. For this, the hydrogel matrix was synthesized and subsequently the necessary compounds to obtain the photomaterial were incorporated. In a second part, unslanted transmission volume phase holographic gratings were stored in the photohydrogels through a recording process. In order to remove the non-reacted compounds in the recording process, several washing stages were carried out using two different solvents, PBST and DMSO:H_2O 6:4 (v/v). Finally, the washing solutions were analyzed by HPLC and UV-visible measurements. The following sections describe the details of each procedure.

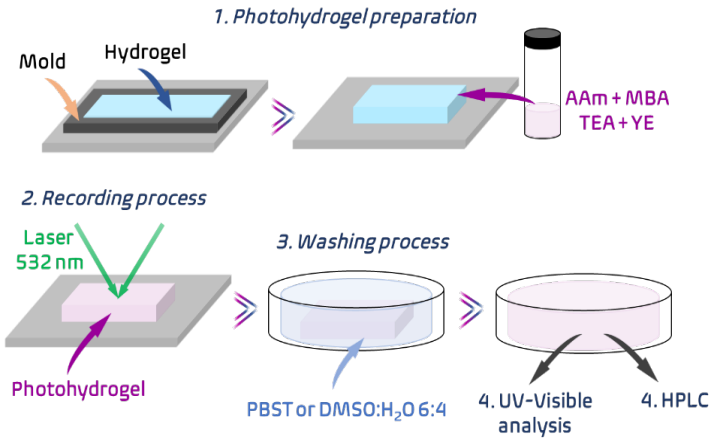

Figure 1. Procedures carried out for the storage of the transmission holographic gratings and the analysis of the washing solutions.

2.1 Photohydrogel preparation

In order to synthesize the hydrogel matrices, AAm (78.2 mg) and MBA (7.5 mg) were dissolved in 0.5 mL of distilled water. The solution was homogenized by stirring during 1 h. KPS (5.0 mg) was then added, and the solution was sonicated for 2 min until the KPS was completely dissolved. 500 µL of the resulting solution were quickly deposited onto a levelled glass slide (7.5 cm × 2.5 cm). The system was fastly sealed with another glass slide and tightened with two clamps. The hydrogel was allowed to polymerize at room temperature for 2 h. After the polymerization time, the hydrogel was removed from the mold, washed with distilled water and stored in water at 4 ◦C. The thickness of the hydrogel matrices was 340 ± 10 µm. Subsequently, an solution in DMSO:H_2O 6:4 (v/v) containing the next molar fraction was prepared: 0.0475 AAm (polymerizable monomer), 0.0121 MBA (crosslinker), 0.0030 TEA (co-initiator and plasticizer), $7.91 \cdot 10^{-6}$ EY (photosensitizer dye), 0.2582 DMSO and 0.6791 H2O. Then, 700 µL of this solution were deposited over pieces of hydrogels with sizes of 2.6 × 1.5 cm for 20 min at 22 °C for absorbing the compounds within the hydrogel matrix. After this time, the photohydrogel ($n \sim 1.43$ at $\lambda = 589$ nm) were placed onto flat glass slides ($n = 1.4699$ at $\lambda = 632.8$ nm, SLIB-G10-050, Labbox) for the holographic recording. All compounds were purchased from Sigma-Aldrich (Madrid, Spain).

2.2 Holographic transmission set-up

Figure 2 shows the experimental holographic set-up used for the recording process of the holographic gratings. The process was carried out under controlled light conditions, to which the material was not sensitive. A continuous (CW) Nd:YVO_4

laser (Verdi-2W, Coherent, Santa Clara, CA, USA) emitting at λ = 532 nm was used. The laser beam was split into two secondary beams, object and reference beams, using a beam splitter (Newport, Irvine, CA, USA). The ratio of intensities between both beams was 1:1. Then, the beams were spatially filtered and collimated. The diameter of both beams was 0.35 cm. The object and reference beams were spatially overlapped at the sample with the recording angles $\theta_o = \theta_r = 18.7°$, with respect to the normal incidence. The working total intensity (sum of both intensity beams measures in the hologram plane) was 19.3 ± 0.6 mW/cm^2 and the exposure time was 8.0 ± 0.1 s. The laser beams were linear polarization perpendicular to the plane of incidence that allows optimal interference. The holograms were recorded at a theoretical spatial frequency of 1205 lines/mm (period $\Lambda_{th} = 0.830$ μm).

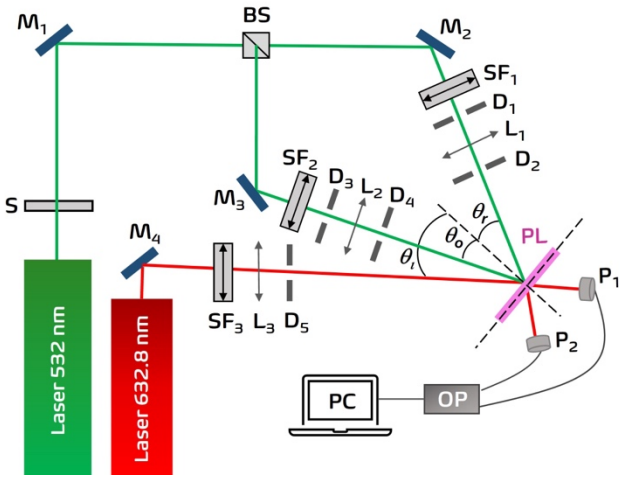

Figure 2. Holographic setup for transmission gratings. S: shutter; BS: beam splitter; SFi: spatial filters (microscope objective and pinhole); Mi: mirrors; Li: lens; Di: diaphragms; θ_o, θ_r: object and reference recording angles, θi: incident reconstruction angle; PL: photohydrogel layer; Pi: photodetectors; OP: optical power meter; PC: data recorder.

2.3 Photohydrogel washing process

The photohydrogels with the stored holograms were frequently immersed for 5 min in 5 mL of solvent. Each washing stage was denoted as Wi, where i = 0, 1,… indicates the number of washes that have been performed. Washing solutions were recovered and stored for analysis by HPLC and UV-visible. The DE of the unslanted transmission holograms were measured immediately after the recording process and after several washing stages. After that, the hydrogels were immersed in PBST for 1 h and they were reconstructed again. Finally, the hydrogels were immersed in PBST and stored at 4 °C in a fridge inside of a sealed container to preserve the material. The DE was obtained after 96 h to check its temporal stability.

2.4 High-Performance Liquid Chromatography (HPLC) and UV-Visible Analysis

An uHPLC 1260 Infinity Binary LC System (Agilent Technologies, Inc. Santa Clara, CA, USA) was employed for the chromatographic studies of the washing solutions. Separation was performed on an Agilent zorbax eclipse XDB-C8 column (4.6 mm × 150 mm, 5 μm particle size). The column temperature was controlled at 30 °C. The mobile phase was 5.0% v/v acetonitrile in water with formic acid (0.1% v/v). The elution flow rate was 1.0 mL min 1. A diode array detector (DAD) at two wavelengths, 210 and 248 nm, was used. The injection volume was 5 μL. The UV-visible analysis of the washing solutions was carried out in a double beam spectrophotometer (Jasco, V-650, Madrid, Spain).

3. RESULTS AND DISCUSSION

3.1 Optimization of the washing stages number by HPLC and UV-Visible Analysis

Standard solutions in PBST of all compounds were prepared and analyzed by HPLC to identify their retention times. AAm and MBA were detected at retention times of 2.10 and 4.80 min, respectively. EY presented two bands with retention times of 3.89 and 4.80 min. TEA, TEMED and KPS were not identified under the conditions of the chromatographic method used while that the signal for PBST appeared a time of 2.06 min. Once all retention times were identified, the chromatograms of the washing solutions were obtained. Figure 3a and inset show that AAm was detected in the washing samples from W1 to W4. AAm was not detected in W5 and W6. MBA was detected in all wash samples but the signal for W6 was weak and could be confused with noise signal. Figure 3b shows that the areas of the chromatograms for AAm and MBA signals present a decreasing exponential trend. Since EY showed weak absorption at 210 nm, UV-Visible analysis of all washing sample was carried out to detect its presence. The absorption of YE at 517 nm as a function of the washing stage is shown in the inset of Figure 3b. The absorption values of YE follows a decreasing exponential trend and the largest amount of YE that not reacted in the recording process is removed in W1 and W2.

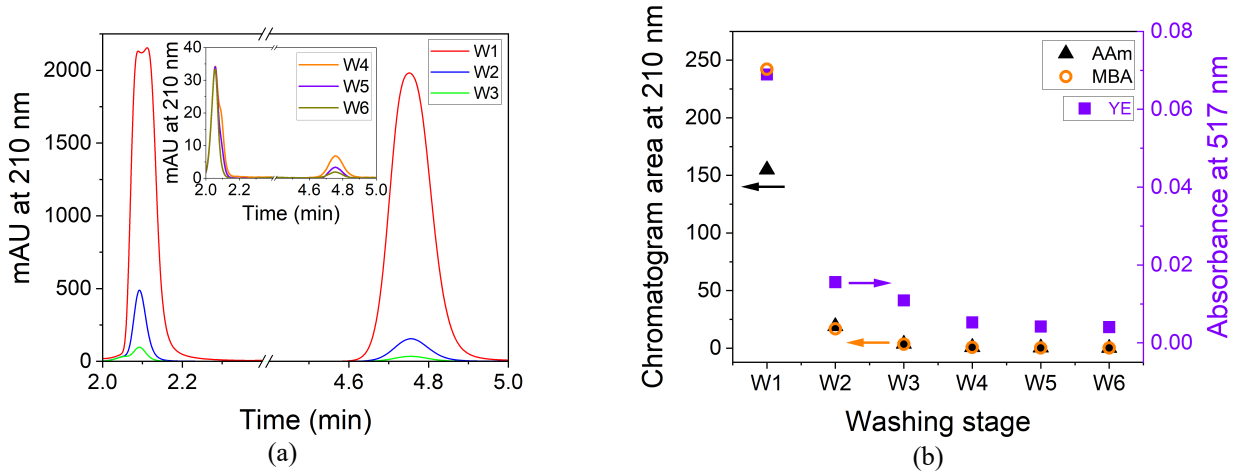

Figure 3. Chromatogram (Mau = mili OD at 210 nm) of washing solutions: (a) W1, W2, W3 and (Inset) W4, W5, W6. Detection was carried out by absorption measurements at 210 nm; (b) Chromatogram area for AAm (black filled triangles) and MBA (empty orange circles) at 210 nm; Absorbance of YE (purple filled squares) at 517 nm in the washing solutions. The solvent used was PBST.

Since that DMSO exhibits an absorption band at 210 nm, the detection conditions in HPLC were changed when the DMSO:H_2O 6:4 were employed. Standard solutions of all compounds in this solvent were prepared and analyzed by HPLC. A wavelength of 248 nm was selected to identify the AAm signal. AAm was detected at a retention time of 2.13 min. The MBA signal was identified at 210 nm where its intensity is greater compared with to that obtained when a wavelength of 248 nm was used. MBA was detected at a retention time of 4.77 min. EY and TEA signals appear at the same retention time as MBA but its intensities were weak and they did not represent a problem for the detection of MBA. TEMED and KPS are not identified at both wavelengths, 248 and 210 nm. When the HPLC conditions were set and the retention times of all compounds were measured, the chromatograms of the washing samples with DMSO:H_2O 6:4 were obtained. The results are shown in the Figure 4a and inset. AAm and MBA were detected in W6, but its signal was very weak and close to the detection limit. The areas of the chromatograms for AAm and MBA show the same decreasing exponential behavior compared to that observed when the PBST solvent was used (Figure 4b). Most of the non-reacted AAm and MBA amount are removed in W1 and W2. On the other hand, the area values for MBA are 242 and 198 when PBST and DMSO:H_2O 6:4, respectively, are used for washing. This means that more MBA is removed in W1 when PBST is used. In the inset of Figure 4 can be observed an exponential decrease in the UV-Visible absorption for EY at 527 nm. The EY signal was close to zero from W5 onwards indicating that it was practically removed.

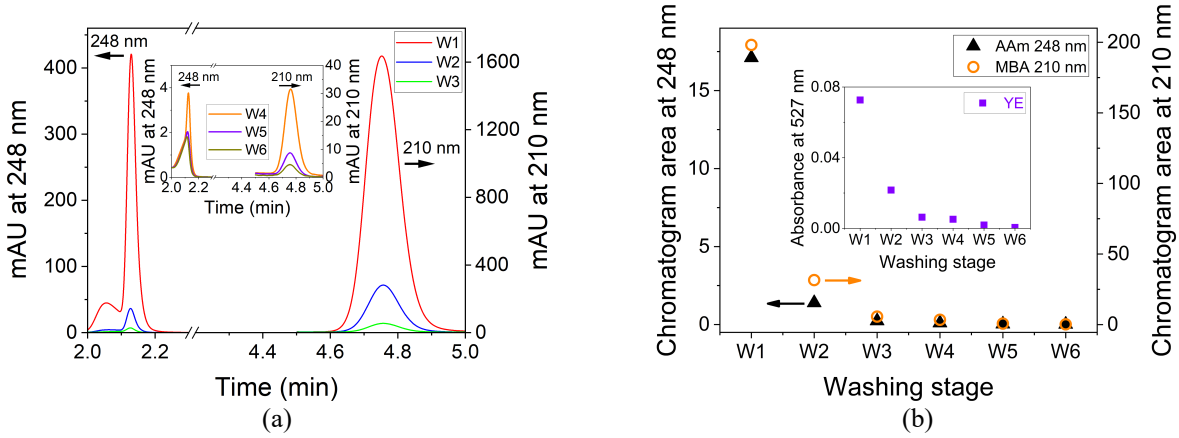

Figure 4. Chromatograms (mAU = mili OD at 210 and 248 nm) of washing solutions: (a) W1, W2, W3 and (Inset) W4, W5, W6. Detection is carried out by absorption measurements at 248 nm for AAm and 210 nm for MBA; (b) HPLC area for AAm at 248 nm (black filled triangles) and MBA at 210 nm (empty orange circles); Absorbance of YE at 527 nm (purple filled squares) of the washing solutions. The solvent used was DMSO:H_2O 6:4 (v/v).

3.2 Measure of diffraction efficiency as a function of the washing stages and temporal stability

The angular scans of unslanted transmission gratings stored in the photohydrogel for the two solvents used in the washing stages are shown in Figure 5. If PBST is employed (Figure 5a), the maximum diffraction efficiencies (DE_{max}) drop slightly from 27.3% in W0 to 25.6% in W2. An increase in DE_{max} takes place from W2 to W4. From this stage, the value for DE_{max} practically remains constant until W6 in which a DE_{max} value of 38% was obtained. The photohydrogel was immersed in PBST for one hour and the DE_{max} was practically the same value as that obtained in W6. After of 96 h immersed in PBST, a DE_{max} of 35.0% were measurement, indicating that the holograms were time-stable in PBST. In the inset of Figure 5a is shown a transmission grating stored in a photohydrogel after four days immersed in a PBST. When the DMSO:H_2O 6:4 was employed, DE_{max} decreases from 25.6% in W0 until 12.7% in W2. DE_{max} values for W4 and W6 are 19%. After the photohydrogel was immersed in PBST for one hour, the DE_{max} reached a value of 27.6%. The bending of the holographic gratings produced the asymmetries in the angular scans and affected the DE_{max} values achieved. This effect was more pronounced when DMSO:H_2O 6:4 is used.

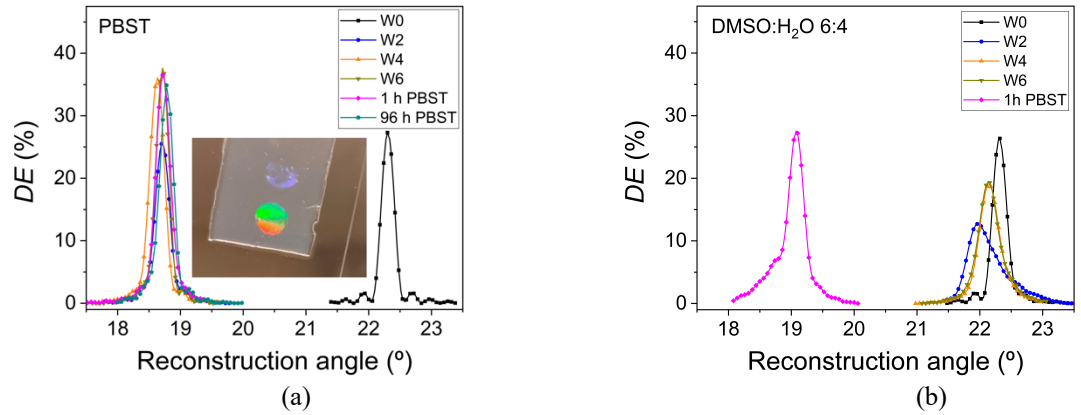

Figure 5. Diffraction efficiency as a function of the reconstruction angle for the two solvents used in the washing stage: (a) PBST; (b) DMSO:H_2O 6:4. After exposure (W0, black square), after two washing steps (W2, blue circle), after four washing steps (W4, orange triangle), after six washing steps (W6, dark yellow triangle) and after one (1 h PBST, magenta rhombus) and 96 hours (96 h PBST, dark cyan pentagon) immersed in PBST. Inset in (a) is an image of the unslanted transmission gratings daylight illuminated.

4. CONCLUSIONS

The number of washing stages required for the complete elimination of compounds that non-reacted in the recording process were studied by means of HPLC and UV-Visible spectroscopy when the dimensions of the photohydrogel were fixed. Six washing stages were required when both PBST and DMSO:H_2O 6:4 (v/v) solvents were used in our system. A higher DE_{max} value was reached at the end of the washing process when PBST was used. A greater bending is obtained in the angular scan measurements when DMSO:H_2O 6:4 (v/v) is employed. Stored unslanted transmission gratings were stable time-stable when hydrogels were immersed in PBST. The different response observed in the holograms opens the possibility of their use for the sensing of organic solvents.

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