Electronic Supplementary Information (ESI)

Versatile hydrogels: an efficient way to clean paper artworks

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Reagents: α-Cyclodextrin (α-CD) was purchased from Fluka (Fluka Chemie, Buchs, Switzerland). The polymers, PEO (MW=100000 Da) and PEO_{20}-PPO_{70}-PEO_{20} (PLURONIC P123, MW~5800 Da), were Aldrich products while α-amylase [EC-232-560-9; 30500U/ml], fluorescein-isothiocyanate, (FITC) and FITC-dextrans of different molecular weights (MW=10000 and 70000) and wheat starch were obtained from Sigma (Sigma-Aldrich, Mo, St. Louis, USA). Solvents such as methanol and DMSO were of spectroscopic and chromatographic grade and were from Carlo Erba Reagenti (Carlo Erba Reagenti srl, Milano, Italy). All reagents used were of analytical grade and used without further purification. Real paper samples (RS), are paper sheets part of the printed volume "*Theatrum Veritatis and Justitiae*" Venezia, 1735.

Hydrogel preparation: The hydrogel were prepared according to literature[1-3]. To prepare the hydrogel based on α-CD and PEO (PEO hydrogel), an aqueous solution of α-CD (0.372 g/ml) was added to an aqueous solution of PEO (0.134 g/ml), while for the hydrogel based on α-CD and PEO_{20}-PPO_{70}-PEO_{20} (PLU hydrogel), the initial α-CD and PLU hydrogel concentrations were 0.372 g/ml and 0.556 g/ml, respectively. In both cases, cavitand and polymer solutions were mixed in a 1:1 (v/v), vortexed for several minutes and then gently stirred for almost an hour, at room temperature. Only the PLU hydrogel was subsequently kept at 4° C overnight. Hydrogels were stable at 4°C for several weeks.

For compatibility and removability studies, each hydrogel was applied on filter paper samples, with a spatula and left to act for a fixed time (15, 30 or 45 minutes); then, it was removed with a humid soft brush (roughly five brushstrokers).

For investigation of a real sample, the cleaning procedure was carried out by applying each hydrogel for 45 minutes on fragments (RS) from a printed volume "*Theatrum Veritatis and Justitiae*" Venezia, 1735, and then removing them as above described.

To evaluate the efficacy for removing hydrophobic patina, each hydrogel was applied for 45 minutes on various filter paper samples that had been soiled with linseed oil. In some cases, artificial ageing for 20 days at 80° C was carried out before starting the cleaning so as to give a sample mimicking actual

aged paper samples *(aged)*[11]. However, some samples were treated with hydrogels immediately after their preparation *(fresh).* In all cases, the hydrogels as previously described.

Two different dextran polymers were used to load the hydrogels, differing for the molecular weights (10000 Da and 70000 Da, respectively); in both cases the dextran were labeled with fluorescein isothiocyanate (FITC), as fluorescent dye. The samples were prepared according to the following procedure: a 1.0 mg/mL of dye aqueous solution was mixed with the polymer one (PLU or PEO at concentration 0,556 g/mL and 0.134 g/mL, respectively) before preparing the hydrogel. The final dye concentration into the hydrogels was 0.5 mg/ml. Hydrogels loaded with the α-amylase labeled with FITC were also prepared. In this case, an aliquot of a concentrated enzyme solution ([Enzyme]= 500 μM in PIPES buffer, [PIPES]=25mM, [$CaCl_2$]=8mM, pH=6.9) was mixed with the polymer solution (PLU or PEO at concentration 0,556 g/mL and 0.134 g/mL, respectively) before preparing the gels; the final enzyme concentration were 5μM. .

Hydrogel characterization: Hydrogel viscosities were measured at 25°C on a thermostated Brookfield DVII viscometer, using a SC4- 21 splinde.

The mobility of the incorporated macromolecules was investigated, by means of fluorescence recovery after photobleaching (FRAP). Experiments were performed on hydrogels loaded with different FITC labeled dextrans of 10 kDa (10M) and 70 kDa (70M), and on hydrogels loaded with fluorescein labeled-α-amylase enzymes.[4] Dextrans were used as models of macromolecules having different dimensions. FRAP experiments were performed on a Olympus Fluoroview 1000 Confocal Laser Scanning System equipped with an inverted microscope, Olympus IX-81. Experiments were carried out using a 60x /1.35 oil immersion objective. Bleaching experiments were performed using the 488 nm line of a 30 mW argon ion laser at a maximum of its intensity. Experimentally, 20 pre-bleach images (resolution: 128x128 pixels) obtained at 13% of full laser intensity were collected; then a uniform region of interest (ROI) 40 μm in diameter was bleached (bleaching time= 2s). After this bleaching, 500 images were collected with lower than 13% intensity in order to follow the fluorescence recovery inside the bleached ROI. The time interval between consecutive images was set to 0.2 s.[5]

Data analysis was performed according to the FRAP model described by Soumpasiss and coworkers.[6,7] The experimental fluorescence recovery curves were fitted using the following expression:

$$F(t) = ke^{-\frac{\tau_D}{2t}}\left[I_0\left(\frac{\tau_D}{2t}\right) + I_1\left(\frac{\tau_D}{2t}\right)\right] \qquad (1)$$

where F(t) is the normalized fluorescence intensity in the bleached region, I_0 and I_1 are the modified Bessel functions of the first kind, of the zero and first order, respectively, k is the mobile fraction and τ_D is the characteristic diffusion time of the dye. The diffusion coefficient of the dye can be obtained by $D= w^2/\tau_D$, where w is the radius of the bleached spot.

FITC-labeled-α-amylase was prepared according to a procedure reported elsewhere.[8] To prepare the loaded hydrogel the solution containing FITC-labeled enzyme was diluted 1/10 (v/v) with polymer to give a final enzyme concentration in the hydrogel of 5μM.

Starch paste preparation and removal*:* The starch paste was prepared mixing 6.0 g of wheat starch in 15 ml of deionized water. The suspension was stirred for several hours until it became a paste. It was used within a few days.

The preliminary tests was carried out on starch paste deposited on Petri dishes, and left to dry for at least 24 hours; then each dish was covered with a layer of hydrogel (PEO or PLU hydrogel) loaded with the enzyme, and left at room temperature for different interval time (from 10 to 45 minutes). Hydrogel without enzyme was also used as control. The enzymatic activity was tested, by using an iodometric procedure reported elsewhere[9,10], monitoring the absorbance of the iodine-soluble starch complexes. Starch paste treated with hydrogel not containing the enzyme does not give evaluable absorbance data.

In the tests on paper samples, 1.6 g of starch paste was spread on circular samples of filter papers (4.8 cm of diameter) by using a spatula,; such samples were aged for 8 days at 80°C and 65% relative humidity [11] and then cleaned with PEO or PLU hydrogels spiked with α-amylase enzyme at room temperature (final enzyme concentration into the gel=5μM),using a treatment time of 45 minutes. The removal was carried out as previously described.

Spectroscopic analysis: Mid-FTIR spectra were acquired on a Thermo-Nicolet (mod. Nexus) instrument (Thermo Scientific Inc., Madison WI), equipped with an attenuated total reflectance (ATR) ZnSe cell for measurement in the 4000-700 cm^{-1} region, at a resolution of 4 cm^{-1}. Spectra were collected by placing the paper samples directly on the ATR cell. A total of 256 scans were collected for each sample.

UV-Vis experiments were carried out on a Cary 100 spectrometer (Varian, Palo alto, CA, USA) using a 1mm cuvette path length.

Chromatographic analysis and pH measurements. HPLC analyses were performed with a THERMOQUEST instrument (Shimadzu, Kyoto, Japan), equipped with two pumps and an UV/Vis detector LCGA SPD-10A (Shimadzu, Kyoto, Japan). The apparatus is equipped with a controller SN 4000 (Shimadzu, Kyoto, Japan) that can process data in real time through the CHROMQUEST software. The chromatographic analysis was performed on extracts obtained by treating 1 cm^2 of every sample (paper or hydrogel) with 1 mL of distilled water, stirring overnight at room temperature. An anion exchange column (STRATA SAX column 55 μm, 70 Å, 100 mg of sorbent mass and 1mL of volume, 2.5 cm x 0.6 cm., Phenomenex, Torrance, CA, USA -) was used for the isolation of the acid component of each sample analyzed.

The composition of the mobile phase was 25 mM phosphate buffer of aqueous solution at pH 2.4 and 1% (v/v) methanol. The chromatographic column used was a C18 column (5 μm 150 x 4.6 mm ID - VYDACTM, WR Grace & Co, USA) with a flow rate of 0.7 mL/min and using a detection wavelength equal to λ= 210 nm.[12] Each chromatographic analysis was repeated three times in the same day (reproducibility intra-day RSD%= 2%) and on different days (reproducibility inter-day RSD% ==1%) for all the samples. Also the measurements were carried out three times on the same sample extract (RSD% = 2%) and on three different extractions of the same paper sample (RSD % = 4%)

Measurements of pH were carried out on the aqueous extract, obtained as described in previously, before and after the water and/or hydrogel treatments[13,14] by using an Amel Instrument 334-B pHmeter with a combined glass electrode Ag/AgCl 6mm (Amel Instrument, Italy)

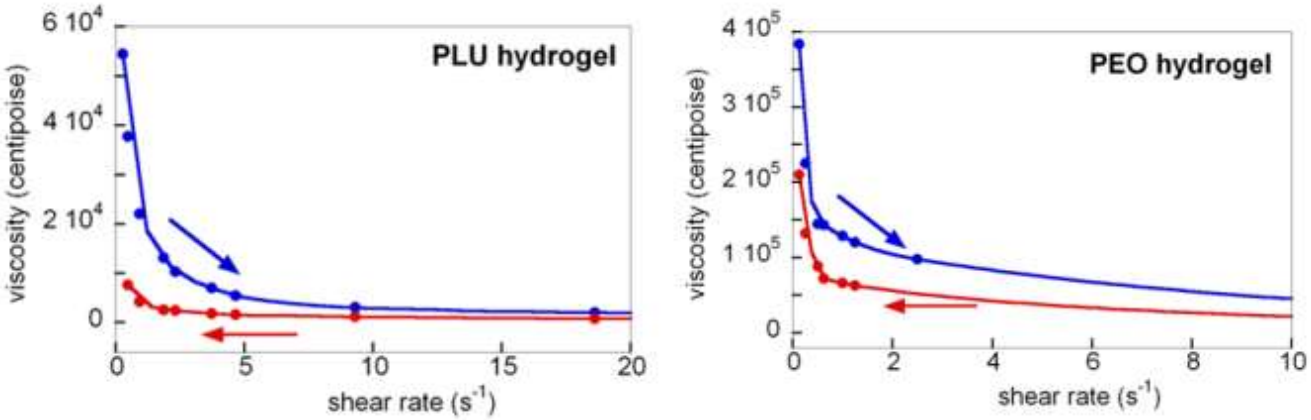

Fig.S1: Viscosity curves of PLU (on the left) and PEO (on the right) hydrogels as a function of a ramp up (blue) and a ramp down (red) of shear rates. The observed differences between the ramp measurements are typical of a thixotropic behavior[15]

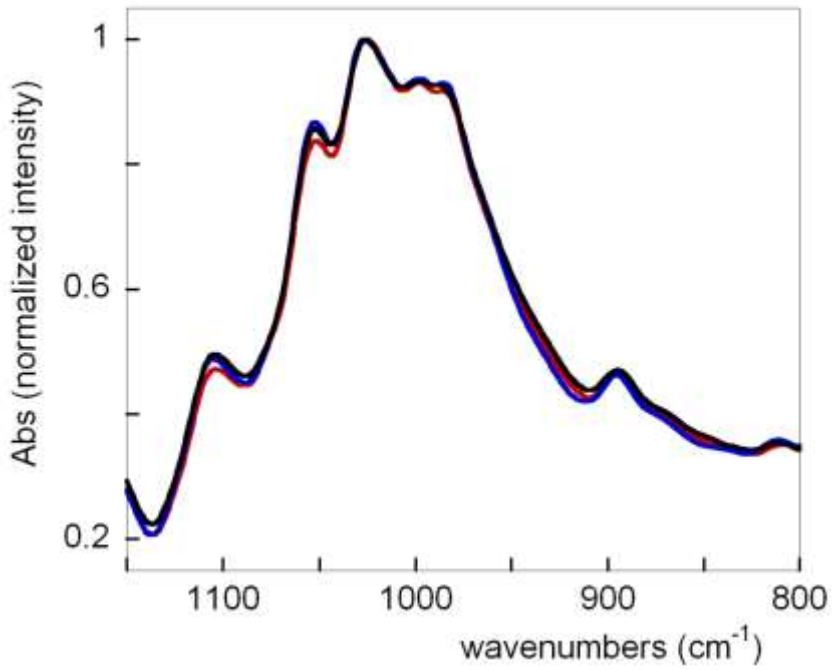

Fig. S2 FTIR ATR spectra of paper. Paper non treated (red line), paper after PEO hydrogel (black line), or PLU hydrogel (blue line) treatment for 45 minutes. The overlap between the spectra suggest that , in both cases, the hydrogels are removed.

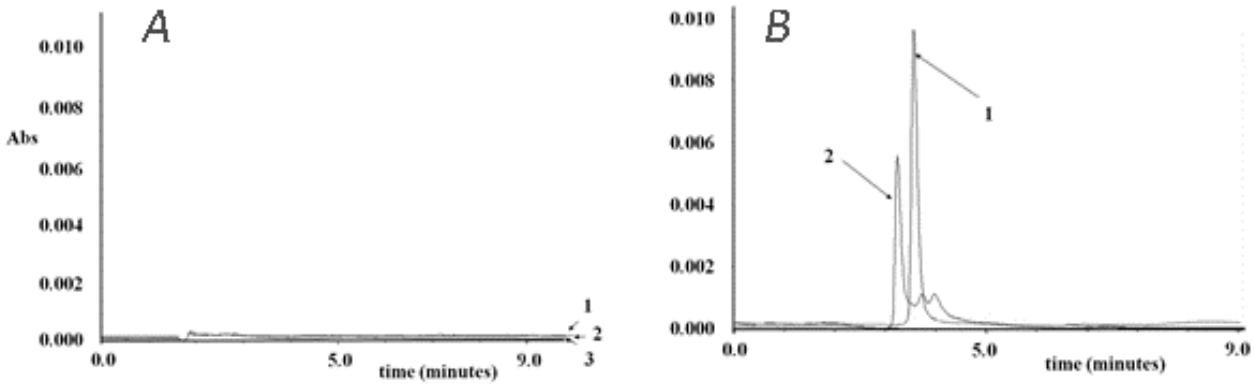

Figure S3 A) HPLC analysis of filter paper (line 1), filter paper treated with PEO gel (line 2), and filter paper treated with PLU gel (line 3) for 45 minutes. B) HPLC analysis of PLU gel (line 1) and PEO gel (line 2)

TABLE S1 Hydrodynamic radii (R_H), diffusion coefficients in free water (D_0), the obtained mobile fraction (k) and the diffusion coefficients in hydrogels of (D_E) of FITC-dextrans and FITC-α-amylase.

probe	R_H (nm)	D_0 ($\mu m^2/s$)	D_E[a] (PEO) ($\mu m^2/s$)	k[a](PEO)	R[b] (PEO)	D_E[a] (PLU) ($\mu m^2/s$)	k[a] (PLU)	R[b] (PLU)
10M dextran	2.0[c]	98[e]	52	0.95	0.992	92	1.0	0.966
70M dextran	6.6[c]	42[f]	21	0.73	0.980	22	0.60	0.980
FITC-α-amylase	3.2[d]	60[g]	44	0.70	0.982	17	0.66	0.984

[a] obtained from analysis fitting of FRAP release curves, according to eq 1 *(see paper)*
[b] coefficient of determination for least square fits, on the basis of eq.1 *(see paper)*, of the experimental recovery profile.
[c]: ref. 16
[d]: ref. 17
[e]: ref. 18
[f]: ref. 19

[g]: ref. 20

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