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How to tune a paper cleaning process by means of modified gellan hydrogels☆

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ABSTRACT

Paper manuscripts are among the most valuable historical artifacts, but they are also very fragile. Furthermore, they were often strengthened by gluing external materials (like wooden or cardboard lining paper) to the original piece. The glues induced structural transformations that accelerate the degradation processes of the artwork itself. In this work, we present efficient and versatile multicomponent materials able to remove specific contaminants from paper artworks in a single, simple and not invasive approach. The materials are based on a rigid gellan hydrogel as a carrier for tuned cleaning agents, such as *proteinase K* enzyme, which is able to hydrolyze not easily removable glues into smaller fragments soluble into the gel, and suitable polimeric surfactants capable to remove hydrophobic contaminants. To assess the effectiveness and safety of the proposed cleaning method, we have employed a multitechnique approach, using several spectroscopic techniques, rheology characterization, scanning electron microscopy, high-performance liquid chromatography analysis, and pH measurements.

1. Introduction

Paper-based artworks are very complex materials, made up of different components as cellulose, binders (starch or animal glues), fillers (alum, kaolin), and inks, that during aging undergo degradation processes leading to a decrease in paper stability and color changes. Furthermore, they were often strengthened by gluing external materials (like wooden or cardboard lining paper) to the original piece, and the used glue underwent structural transformations that cause a loss in compactness, yellowing, and an acidity increase, thus accelerating the degradation processes of the artwork itself [1]. Due to its complexity and its fragile structure, paper is a very difficult medium to restore; moreover, wet cleaning (a necessary step during paper restoration process) is performed commonly by water bath that can cause swelling of fibers and dissolution of several components [2]. The use of hydrogels represents an alternative to overcome the disavantages above reported. Due to the water uptake capability and high viscosity values of gels, the penetration of the liquids into the paper artworks is significantly reduced, therefore minimizing damages [3–7]. However, to ensure a complete removal of the gel [8,9], procedures often unsafe for the artworks, like invasive mechanical action (i.e., removal with a brush) or solvents, are required. In this context, a smart possibility is represented by the use of rigid hydrogels [3,5,9–12] as they can be completely and easily removed in one step, as one body, without leaving residues after their application. The use of rigid gels based on the deacylated polysaccharide gellan gum and calcium acetate (Gg) has been recently assessed to be as a new efficient wet cleaning agent for paper samples [2,13–14]. Gellan gum is a linear anionic hetero-polysaccharide produced by *Pseudomonas elodea*, with a repeating unit of D-glucose(β 1,4)-D-glucuronic acid(β 1,4)-D-glucose(β 1,4)-L-rhamnose(α 1,3). In the presence of calcium ions, it forms hard and rigid hydrogels that are homogenous, transparent, and stable to pH variations [12–19]. The pH stability assures that the gel can be applied to every kind of paper samples whatever its acidity or in the presence of alkaline reservoir. In a recent paper [20], we have shown that Gg can be applied on a paper sheet and removed from it as one body, and it is able to release water in a controlled manner. Furthermore, a pressure of 10^3 Pa can be applied on the hydrogel, for about an hour, without causing gel brittleness or fragmentation, and the size of its meshes is sufficiently large to allow the diffusion of both enzymes and degradation products removed from the paper samples. However, it should be pointed out that given that the chemical–physical properties of paper contaminants and

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degradation products are very different (i.e., hydrophilic, hydrophobic, or, as for the glue, polymeric compounds), a multistep approach is often required to complete the cleaning procedure. In this context, Gg alone is not able to remove specific contaminants like starch paste or animal glue or greasy materials (see below). In literature, for example, it is reported that Gg alone is able to remove a lower amount of gelatin from paper with respect to a water bath [12]. Therefore, the idea of this research was to evaluate the possibility of using Gg as a carrier of tunable cleaning agent. In this case, Gg, loaded with specific enzymes or amphiphilic polymers, could be applied on several paper samples to remove glues or hydrophobic materials with a significant reduction in the cleaning application time. In this system, the enzyme (or surfactant) works as a selective cleaning agent, while the rigid gel plays the role of support and removal matrix for the enzymatic products. We have recently assessed that Gg, spiked with hydrolytic α-*amylase* enzyme, represents a valid system to clean paper artworks from starch paste with high yield in a reasonable time [20]; in this article, we have explored in more detail the hydrogel versatility, preparing two new Gg-based gels. The first, called EGg, is obtained entrapping in Gg the *proteinase K* enzyme (a protease active in a wide range of temperature and pH [21]) and has been proposed for the removal of animal glue from paper samples. The second system, called GgP, obtained by mixing Gg with the amphiphilic PLU gel, has been studied for the removal of greasy foxes and contaminants.

PLU gel is a system prepared by mixing solutions of α-cyclodextrin and Pluronic P-123 polymer in an appropriate ratio (see Section 2.8). It has been tested on new and aged paper samples soiled with linseed oil [7,22]. However, given that PLU gel is a thixotropic system, after the application on paper samples, it must be removed using a soft brush; this procedure could be invasive and harmful for very fragile artworks. In this case, a rigid hydrogel could be a more suitable choice; to this end, we have tested GgP, obtaining a mixed gel suitable to clean greasy paper samples.

In order to test the efficacy of these innovative systems (EGg and GgP), we have characterized soiled paper samples before and after the cleaning process by means of several techniques like Fourier transform infrared spectroscopy (FTIR), UV–Vis absorption and fluorescence spectroscopy, optical microscopy, scanning electron microscopy (SEM), pH measurements, and high-performance liquid chromatography (HPLC).

2. Materials and methods

2.1. Materials

Proteinase K from *Tritirachium album* [EC-3.4.21.64, ≥30 U/mg] α-cyclodextrin (α-CD), PEO_{20}-PPO_{70}-PEO_{20} (Pluronic P-123, MW ~ 5800 u.m.a.), calcium acetate, calcium chloride, the Bradford assay kit, and PIPES buffer were obtained from Sigma (Sigma-Aldrich, Mo, St. Louis, USA). Iodine was from Carlo Erba Reagenti (Carlo Erba Reagenti srl, Milano, Italy). Gellan gum was KELCOGEL® CG-LA product by CP Kelco (Atlanta Georgia, USA). Solvents such as methanol were of spectroscopic and chromatographic grade (Carlo Erba Reagenti srl, Milano, Italy). All reagents used were of analytical grade and used without further purification. In all cases, in the preparation of buffer solutions, bidistilled water (Millipore, Billerica, MA, USA) was used. Animal glue, obtained from waste (such as bones and skins) of rabbit was a gentle gift of restorers.

Paper samples were from ALBET (ALBET® LabScience, Hahnemühle FineArt, Germany). Its characteristics are as follows: density, 80 g/m^2; rate of filtration, 1196 s/100 mL.

2.2. Hydrogel preparation

The hydrogels were prepared following a general protocol already described in previous works, with slight modifications [2,15]. To prepare the hydrogel containing calcium ions, an aqueous solution containing deacylated gellan gum (20 g/L) and calcium acetate (0.40 g/L) was mixed for almost a minute in a microwaver at 600 W (Mars Microwave, CEM Corporation, Matthews, NC, USA) until the sample boiled and became transparent; then it was left to cool on a Petri dish. To prepare gels loaded with *proteinase K* (EGg), an aliquot of a concentrated enzyme solution (35 μM or 173 μM in PIPES buffer, pH 8 (PIPES 25 mM plus $CaCl_2$ 8 mM) was added to Gg at 60 °C during the cooling step in a Petri disk. The final enzyme concentration was 5.8 μM. For the preparation of the hydrogel containing α-CD and Pluronic P-123 (GgP), we initially prepared the hydrogel based on α-CD and PEO_{20}-PPO_{70}-PEO_{20} (PLU hydrogel), following the hydrogel preparation general protocol reported elsewhere with slight modifications [7,22]. Equal volumes of aqueous solutions of α-CD (0.20 g/mL) and PEO_{20}-PPO_{70}-PEO_{20} (0.56 g/mL) were mixed together, vortexed for several minutes, and then gently stirred for almost an hour, at room temperature. The final solution was added to Gg in a 7:1 (v:v) ratio Gg/PLU gel.

2.3. Hydrogel characterization: rheological measurements

Rheological experiments were carried out by means of a controlled stress Haake RheoStress 300 Rotational Rheometer, equipped with a Haake DC10 thermostat. The geometry used for the tests was a cross-hatch plate device (Haake PP35 TI: diameter = 35 mm) in order to reduce the extent of the wall slippage phenomena [23]. The hydrogel, with a thickness of about 1.0 mm, was laid with care on the lower plate of the rheometer. The upper plate was then lowered until it reached the hydrogel surface. The procedure followed for the sample characterizations was already described [24]. Gap setting optimizations have been undertaken according to the procedure described elsewhere [25]. Frequency sweep experiments were performed at 25 °C in the range 0.01–10 Hz, in the linear viscoelastic region, preliminary assessed by stress sweep experiments. The mechanical spectra were then recorded applying a constant deformation ($\gamma = 0.01$) in the linear regime. Stress sweep experiments were performed (25 °C and 1 Hz) in the range 10^1–10^4 Pa.

2.4. Paper samples preparation

Before carrying out the experiments on paper samples soiled with glue, we first prepared the glue by mixing 2 g of pellet in 14 mL of deionized water. The suspension was left for 24 h, at room temperature, and then stirred in a water bath at 50°–60 °C until it became a paste. Aliquots of 2.4 mL of animal glue were spread on circular samples of filter papers (diameter: 4.8 cm) and left to dry for almost 48 h. Samples has been used up to 2 years after their preparation.

Paper samples soiled with linseed oil were prepared by dipping paper samples for 20 s in linseed oil and left to dry for almost 48 h.

To perform tests on artificially aged samples, the soiled paper samples were left for 21 days at 80 °C and 65% relative humidity [20].

2.5. Hydrogel application and removal

Paper samples were fully covered with the gel; on the top, a PET film (Mylar®) was applied, and the whole system was uniformly pressed to ensure a close contact between the gel and the sample (usually, with gels of 6 cm in diameter and 1 cm height a weight of 500 g has been applied). The time of the gel application for cleaning was 1 h with the exception of some specific cases. Gg was also used as a reference sample to highlight possible problems related to the presence of the hydrogel itself in the tests. After the cleaning procedure, the gel was removed in one step only by taking it, without the use of spatula or liquid component.