

# Gellan gum hydrogels as such and ionic-liquid doped as modulable micro-invasive tools for Cultural Heritage studies

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**Abstract** - Rigid Gellan gel, composed of Gellan gum and calcium acetate, is known to be biocompatible and capable of absorbing water-soluble compounds present on fragile artworks; moreover, when implemented on screen-printed electrodes, it can result in a device providing both absorption and *in situ* electroanalytical detection of species of interest for Cultural Heritage researchers. In this application, its properties and performances could be modulated by doping with ionic liquids, innovative media consisting of molecular salts with low melting points. In this preliminary investigation, a series of absorbing conductive materials based on Gellan hydrogel, used as such and doped with 1-ethyl-3-methylimidazolium methanesulfonate in various amounts, are paired with screen-printed sensors and tested as tools for the voltammetric detection of lawsone, a red-orange dye found in the leaves of the henna plant (*Lawsonia Inermis*) and used since the time of Ancient Egypt to dye textiles.

## I. INTRODUCTION

Cultural Heritages (CH) are an integral element of human history and culture; consequently, their preservation is a primary objective of modern science. In recent decades, science applied to CH has expanded its applications with innovative methodologies and procedures, enhancing the relationship between science, art, and conservation. In this regard, the scientific approach has been appropriately applied from two perspectives: conservation and archaeological study. The first objective is to develop new methods and products for preserving various types of materials, while the second objective is to increase our understanding of production technology and ancient cultures. In both cases, non-invasive techniques are preferred because they reduce

material consumption and, as a result, do not alter the physical and chemical structures of the artefact, thereby preserving the integrity of the analyzed artwork without causing damage. A significant amount of current scientific research is devoted to the development of new strategies, methodologies, sampling techniques, and data analysis techniques to improve the results obtained by non-invasive methodologies.

Since the late eighteenth century, when the first documented technical examinations of CH objects, such as artworks and archaeological remains, were conducted, the application of analytical techniques in this sector has expanded steadily. Indeed, due to their ability to undergo morphological and chemical changes during ageing, inorganic and organic compounds used as coloring agents in paint layers or as dyes have attracted the attention of archaeologists, who recognize the importance of understanding the chemical composition of cultural artefacts. As a result, chemistry, particularly analytical chemistry, has been gradually incorporated into research and other activities related to the humanities and art [1-5]. In this context, innovative and non-invasive cleaning and diagnostic tools, based on Gellan gum and calcium acetate hydrogels combined with an electrochemical sensor, have been developed [6,7]. This system combines the efficiency of Gellan gum hydrogel (GG) as a paper cleaning agent, with the sensitivity of electrochemical sensors evaluating the amount of glucose produced by the degradation of cellulose, thus indicating when the cleanup process is complete. Indeed, this hydrogel can gradually release controlled amounts of water and also absorb the water-soluble degradation products present on the paper, by capillary action. In this way, it is possible to slow down the paper ageing process. Thanks to the hydrogel mesh size, about 1  $\mu\text{m}$ , GG could be easily loaded with hydrolytic enzymes or poloxamers to facilitate the removal from paper artwork of unwanted and/or dangerous materials like

old and deteriorated glues or greasy materials [8,9]. Regarding the use of GG as a paper cleaning agent, all the fundamental requirements are satisfied [10,11]. GG is rigid enough to be easily handled by operators without leaving residues and exhibits an excellent degree of transparency. This last feature allows monitoring of the removal process visually. Removal of material from the artworks occurs according to a process of concentration gradient, without causing any morphological changes to sample morphology and guaranteeing the structural and aesthetic preservation of paper supports.

Concerning the performances of gellan patches coupled with screen-printed electrodes for *in situ* electroanalysis, an attractive perspective is the modulation of hydrogel performances by doping with ionic liquids. Ionic liquids (ILs) are organic salts that remain liquid beneath 100°C. Some of them are still liquid at room temperature (RTILs). Because of their composition, they can act at the same time as solvents and as proper conductive media for electrochemistry and electroanalysis. In the last decades, ILs have become of great interest as innovative solvents, and they have been involved in many applications like sensors, batteries and green extractions [12]. Some of their advantageous features are high viscosity, low volatility, and non-flammability. It is possible to design ILs to obtain specific physical and chemical properties such as acidity/basicity or chirality [13,14]. ILs are particularly up to date as advanced media for electroanalysis.

In this study, a sensitive, low-cost and fast electrochemical sensor is preliminarily tested by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). It is based on screen-printed electrode (SPE) supports modified with Gellan gel, either as such or doped with 1-ethyl-3-methylimidazolium methanesulfonate ionic liquid in different proportions, modulating the device performances in the detection of a red-orange dye, lawsone, used as tissue colorant in the past, chosen as a first case study.

## II. MATERIALS AND METHODS

### A. Hydrogel preparation

Gellan gum hydrogels (GG) were prepared as follows [15]. Gellan gum powder Kelcogel (CP Kelco, San Diego, California) and calcium acetate (Merck KGaA, Darmstadt, Germany) were dissolved in double-distilled water, under stirring at room temperature, with final concentrations of 2 % and 0.04 % (w/v) respectively. The resulting mixture was put for almost a minute in a microwave at 600 W (Mars Microwave, CEM Corporation, Matthews, NC, USA) until boiling and becoming transparent. Then the homogeneous solution was poured and cooled to room temperature in a Petri dish of proper dimensions for 1 h. Hereafter this sample will be called A.

### B. Ionic liquid-incorporating hydrogel preparation

GG doped with ionic liquid was prepared by adding proper amounts of 1-ethyl-3-methylimidazolium methanesulfonate (Sigma-Aldrich Corporation, Saint

Louis, USA) to the Gellan gum and calcium acetate solution. The mixing was done during cooling in a Petri dish and the thickness of the resulting gels was about 1 mm. Different proportions of 1-ethyl-3-methylimidazolium methanesulfonate ( $\text{Emim}^+ \text{MeSO}_3^-$ ) have been incorporated into the GG mixture.  $\text{Emim}^+ \text{MeSO}_3^-$  / solution v/v ratios of 1:100, 1:50, 1:10, 1:5, and 1:2 were chosen. Hereafter these samples will be called B, C, D, E, and F respectively. The resulting hydrogels were rounded using a hollow cutter of 9 mm diameter. In this way, 6 little “lenses” were made as shown in Figure 1.

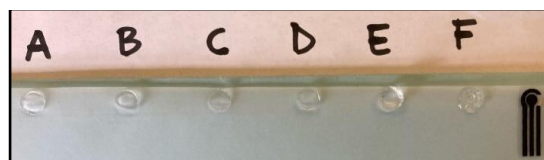


Fig. 1. Photos of resulting hydrogels with IL / solution volume ratio of A (0:1), B (1:100), C (1:50), D (1:10), E (1:5), F (1:2).

### C. Apparatus

#### 1) Cyclic voltammetry and differential pulse voltammetry

Cyclic voltammetry (CV) and Differential Pulse Voltammetry (DPV) analyses were performed using an Autolab PGSTAT potentiostat (Metrohm) controlled by GPES software. Concerning CV, the scan rate was  $0.025 \text{ V s}^{-1}$ , if not otherwise specified, whereas the step potential was 0.002 V. For DPV the step potential was 0.001 V, and amplitude was 0.05 V.

Screen-printed electrodes were produced in-house in the University of Rome Tor Vergata. Working and counter electrodes (WE, CE) were made of graphite ink, and pseudo-reference electrode (RE) of  $\text{Ag|AgCl}$ -based ink. The electrodes were printed on plastic support.

#### 2) ATR-FTIR spectroscopy

FTIR absorption spectra of dried samples were acquired with alS50 Thermo-Scientific instrument (Thermo Scientific Inc., Madison WI USA) in Attenuated Total Reflectance (ATR) mode using a single reflection diamond cell. Spectra were recorded from 4000 to  $525 \text{ cm}^{-1}$ , averaging over 32 scans with of  $2 \text{ cm}^{-1}$  resolution. All the experiments were performed in triplicate, yielding consistent and reproducible results.

## III. RESULTS AND DISCUSSION

### A. Hydrogel characterization

Increasing the percentage of incorporated ionic liquid, a sol-gel transition occurs faster, and from the ratio 1:10 onwards, a phase separation is visible. Moreover, increasing the percentage of incorporated ionic liquid makes the gel more brittle and irregular.

The incorporation of the ionic liquid into the hydrogel was assessed via ATR-FTIR spectroscopy. First, the 1-ethyl-3-methylimidazolium methanesulfonate structure was investigated utilizing ATR-FTIR analysis (Fig. 2, green profile). As shown in Figure 2, in the region from 860 to 500  $\text{cm}^{-1}$  several strong bending bands are observed, namely, a ring in-plane symmetric bending (525 and 550  $\text{cm}^{-1}$ ), a ring out-of-plane antisymmetric bending (600, 620, and 648  $\text{cm}^{-1}$ ), a C–O–S bending (770  $\text{cm}^{-1}$ ). Moreover, C–O–SO<sub>3</sub> symmetric and antisymmetric stretching vibrations are located at 1040  $\text{cm}^{-1}$  and 1170  $\text{cm}^{-1}$ , respectively. Ring in-plane symmetric and antisymmetric stretching vibrations of CH<sub>2</sub>(N) and CH<sub>3</sub>(N) are visible at 1330  $\text{cm}^{-1}$ , 1360  $\text{cm}^{-1}$ , 1390  $\text{cm}^{-1}$ , 1430  $\text{cm}^{-1}$ . Finally, a sharp peak at 1570  $\text{cm}^{-1}$  attributable to ring in-plane s symmetric/antisymmetric stretching and CH<sub>3</sub>(N), CH<sub>2</sub>(N) stretching vibrations were observed [16]. Not-doped GG was characterized using the same technique (Fig. 2, black profile). A characteristic carbohydrate fingerprint is shown between 900 and 1200  $\text{cm}^{-1}$ . Additionally, the spectrum displayed a peak at 1600  $\text{cm}^{-1}$  related to the presence of carboxylate moieties. As expected, GG doped with ionic liquid displayed peaks characteristic for both Gellan gum and Emim<sup>+</sup> MeSO<sub>3</sub><sup>-</sup>, indicating the successful incorporation of both components (Fig. 2, blue and red profiles). In detail, focusing on the band attributable to the carboxylate (1600  $\text{cm}^{-1}$ ), a slight shift is visible concerning that of not-doped GG, demonstrating the interaction with the ionic liquid. Furthermore, this band decreases in intensity as the percentage of ionic liquid present in the mixture increases, indicating the increase of the incorporated Emim<sup>+</sup> MeSO<sub>3</sub><sup>-</sup> amount.

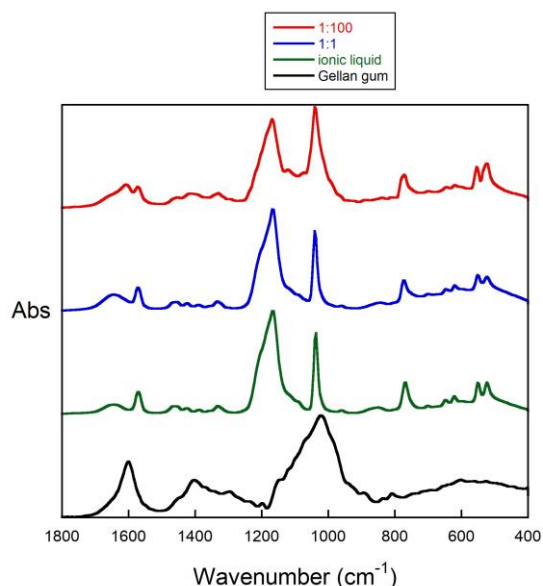


Fig. 2. ATR-FTIR spectra of dried undoped Gellan gum hydrogel (BLACK), ionic liquid (GREEN) and Gellan gum hydrogels, doped with ionic liquid 1:1 (BLUE) and 1:100 (RED).

### B. Hydrogel application effects

A, B, C, and D hydrogel samples, with a thickness of about 1 mm, were placed in contact with Whatman filter paper soaked in lawsone (2-hydroxy-1,4-naphthoquinone) solution (about 0.5 g/mL), for about 30 minutes (Fig. 3A); the conditions under which the experiment was carried out were identical to those used during a standard gel cleaning process [17,18]. During the treatment, a uniformly pressed polymethylmethacrylate film was applied over each hydrogel to minimize the evaporation of water and to ensure close contact between the gel and the sample. It is interesting to note the gel absorption efficacy on the colored paper during the treatment. As shown in Fig. 3B as evidence of the gel ability to absorb products present on the paper by osmosis, Whatman paper resulted in being very discolored at the end of the cleaning procedure.

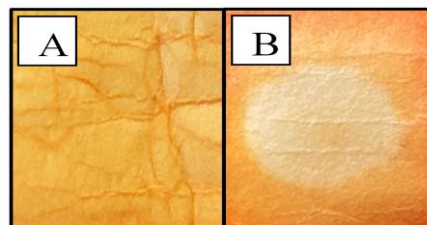


Fig. 3. Photos of Whatman paper (A) before and (B) after 30 minutes of cleaning procedure.

### C. Preliminary electrochemical detection tests

#### 1) Electrochemical windows

SPE supports modified with GG or IL-doped-GG lenses, tested by CV and DPV, show available potential windows of about 2 V (from  $\sim -1.0$  to  $\sim +1.1$  V vs Ag|AgCl pseudoreference; a synopsis of CV patterns is provided in Figure 4; the reduction peak at  $\sim -1.0$  V is possibly linked to oxygen reduction since it was significantly reduced performing the experiment in a vial under nitrogen flux). Such windows can enable detection of a variety of electroactive dyes of interest in the CH fields.

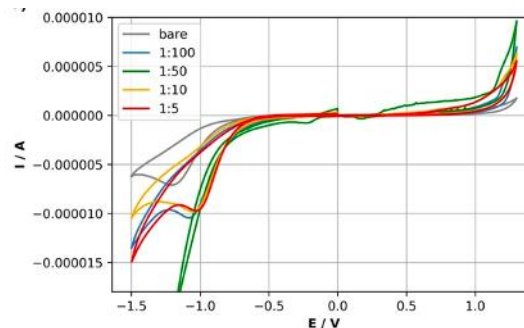


Fig. 4. A comparison of CV potential windows obtained with various GG- or IL-doped-GG- modified sensors.

## 2) Detection tests with lawsone as a model dye

For our preliminary tests we have selected as a case study lawsone, used as tissue colorant in the past, featuring a neat oxidation peak within the available potential window, at  $\sim 0.9$  V vs Ag|AgCl pseudoreference.

Two protocols have been applied.

“*Ex situ*, saturation” detection tests have been performed on GG lenses, as such and IL-doped, after equilibration with the dyed paper according to the 30’ application protocol described in paragraph B). After the absorption time the lenses were transferred on the SPE supports to observe in CV and/or DPV scans the lawsone oxidation peak, the current of the latter accounting for equilibrium concentration of the dye in the GG lens.

“*In situ*, fast/microinvasive” revelation tests have also been performed in order to evaluate the diffusion rate of the dye within the gel phase. The minimum contact time necessary to detect the dye presence in the paper (an interesting feature in the perspective of minimizing the sensor impact on the manifold) was also evidenced. In this case, the whole GG- or IL-doped GG- modified SPE sensor was directly applied on the dyed paper, and CV or DPV patterns were recorded at different times.

The GG-modified SPE sensors were successful in both kinds of tests. The rapidity of the dye revelation is particularly interesting, with a sharp peak observed for lawsone just 1 minute after application (Figure 5; the subsequent decrease might be ascribed to progressive sensor fouling after repeated scans, considering that the lawsone first oxidation is chemically irreversible).

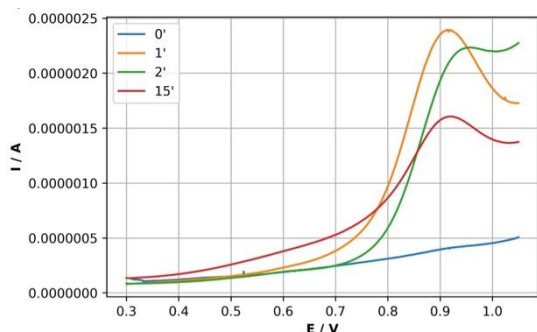


Fig. 5. Subsequent DPV scans on a GG-modified SPE sensor contacted with lawsone-dyed paper.

## IV. CONCLUSIONS

Gellan hydrogels as such or doped with different percentages of Emim<sup>+</sup> MeSO<sub>3</sub><sup>-</sup> were prepared. High IL concentrations interfere with the gel formation process and alter the gel properties, while for low IL / solution v/v ratios, the incorporation of IL into the gel is confirmed.

Undoped and doped gels were tested for absorption of lawsone from dyed paper, both after “*ex situ*” equilibration of gel lenses with the sample followed by implementation of the absorbing phase on the SPE sensor, and in fast detection mode, by directly contacting the dyed sample with the sensor. The GG-modified SPE sensors were successful in both kind of tests, and remarkably the

lawsone presence was clearly detectable after about 1’ of application. Successful tests have also been obtained with different IL-doped-GG-modified SPE sensors; we are now focusing on the issues of reproducibility and possible rationalization of the GG-doping effects as a function of IL vs water proportions.

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