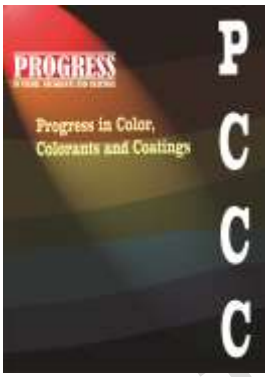
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Gellan Gum-Based Hydrogels Functionalized with NSF, Enzyme, and ZnO Nanoparticles for Targeted Removal of Hybrid Stains from Paper Artifacts

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Abstract

The conservation of paper artifacts is challenging due to the sensitivity of cellulose and complex multicomponent stains. Conventional cleaning methods can cause mechanical damage, leave chemical residues, and introduce excessive moisture. This study evaluates gellan gum hydrogels modified with nanostructured fluid microemulsion (NSF), lipase and protease enzymes, and zinc oxide nanoparticles for removing complex soiling from paper. Eight formulations were tested on artificially soiled, aged paper samples, with contact times of 30 and 60 minutes. Cleaning efficacy was assessed using colorimetry, stereo microscopy, and FTIR spectroscopy. Results showed that NSF-containing formulations achieved the highest initial cleaning efficiency, which was further enhanced by combining NSF with ZnO. Enzymes alone had limited effectiveness but became more effective when combined with NSF. Secondary aging revealed that some formulations, especially those containing surfactants and applied for longer periods, caused yellowing or the appearance of new FTIR absorption bands linked to organic-inorganic complexes. By contrast, the combination of NSF with ZnO ensured better color stability. These findings highlight the considerable potential of gellan gum hydrogels for targeted cleaning of complex stains and underscore the importance of optimizing formulation and

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contact time to preserve the long-term stability of paper artifacts.

Keywords: Gellan gum hydrogel; Cleaning; Paper conservation; Surfactants; Enzyme; Zinc oxide nanoparticles.

1. Introduction

Historical paper artifacts, including manuscripts, rare documents, and artworks, represent a cornerstone of cultural heritage, preserving the intellectual, artistic, and scientific achievements of past civilizations. However, the cellulose-based structure of paper is inherently susceptible to deterioration. Degradation occurs through hydrolysis, oxidation, microbial activity, and the cumulative effects of environmental pollutants, light exposure, and improper handling and conservation [1-5]. Among the most challenging forms of contamination are hybrid organic-inorganic stains, complex assemblages of lipids, such as oils and greases, interwoven with particulate matter including soot, dust, and metal oxides [6, 7].

These hybrid stains arise from multiple, often overlapping sources. Soot from incomplete combustion deposits carbonaceous particles that can form covalent interactions with cellulose fibers, intensifying structural weakening [6]. Fungal metabolites contribute pigmented residues, which in combination with iron oxides produce the characteristic discoloration known as “foxing” [8, 9]. Routine handling or Improper storage introduces additional complexity, resulting in a combination of lipids and environmental dust on paper surfaces. The interactions between these components are often synergistic, accelerating degradation through fiber weakening, translucency, and irreversible loss of

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historical information [10, 11]. Such multifactorial deterioration presents a conservation challenge, as conventional cleaning methods frequently fail to address the composite nature of these stains.

Traditional approaches, including aqueous immersion, organic solvent treatments, chemical surfactants, and mechanical removal, each carry significant limitations. Immersion in water-based solutions, while effective for soluble contaminants, can cause excessive fiber swelling, hydrolyze sizing agents such as gelatin, and trigger undesirable ink migration. Classical chemical cleaning methods may leave residues that remain in the fiber network or catalyze the degradation of cellulose and can sometimes be hazardous to the health of the restorer [12-15]. Mechanical cleaning methods, including surface abrasion, often compromise the integrity of fragile materials [16-18]. Advanced techniques such as laser ablation provide precise energy delivery but carry thermal risks that may damage delicate organic substrates [19]. Collectively, these limitations highlight the need for controlled, localized cleaning strategies that can selectively remove complex stains without introducing further damage.

Hydrogels, three-dimensional, hydrophilic polymer networks, have emerged as versatile tools in heritage conservation. Because they confine solvents and reagents at the contaminant–paper interface rather than allowing free diffusion into the bulk substrate, appropriately formulated hydrogels can reduce bulk wetting and limit collateral mechanical damage relative to immersion or free-liquid application [20-24]. It should be emphasized that this advantage is not an intrinsic property of hydrogels as a class; its magnitude depends strongly on gel composition, the water activity of the system, the solvent or additive incorporated, contact time, and the sizing and porosity of the paper

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substrate itself. Under unfavorable combinations of these parameters, moisture-related swelling and damage can still occur. Their rheological properties allow conformal contact with irregular or delicate surfaces, while their generally favorable biocompatibility profile mitigates some of the toxicity concerns associated with traditional chemical treatments [25-27].

A variety of hydrogel systems, in addition to gellan gum, have been explored for cleaning paper and related heritage substrates, each offering a different balance of mechanical behavior, retentiveness, and compatibility with the substrate. Semi-interpenetrated p(HEMA)/PVP hydrogels have been used as carriers for hydrolytic enzymes in the removal of glues and starch pastes from paper artworks [14]. Physically strong PVA/poly(N-(2-hydroxyethyl)acrylamide) hydrogels have been developed to address interfacial adhesion issues in cleaning Xuan paper [28], while agar-based solvent-gels have been formulated for the localized removal of pressure-sensitive-adhesive residues [29]. More recently, polyamidoamine hydrogels have been reported for the selective deacidification and wax removal of cellulosic heritage materials [30]. Carbopol (carbomer)-based hybrid nanogels incorporating ZnO, TiO_2, or Fe_3O_4 nanoparticles have likewise been applied to remove dust and soot stains from historical paper manuscripts [31, 32]; and cellulose-derivative poultices based on carboxymethyl cellulose and arbocel, combined with nanoparticle additives, have been used to remove calcifications and surface dirt from manuscripts [33].

Among hydrogels, gellan gum, an anionic exopolysaccharide derived from Sphingomonas elodea, offers particular advantages for conservation applications [24, 34, 35]. High-acyl gellan forms soft, elastic gels suitable for fragile or brittle papers, whereas

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deacylated variants yield rigid, transparent gels suitable for more robust materials [36, 37]. The shear-thinning nature of gellan facilitates precise application and complete removal, while its porous network promotes capillary-driven uptake of contaminants [35].

Lipases, for example, catalyze the hydrolysis of triglycerides into soluble fatty acids and glycerol, thereby reducing oil-induced hydrophobicity and restoring fiber transparency [38]. Surfactants such as sodium dodecyl sulfate (SDS) facilitate the emulsification of lipid residues and promote the dispersion of soot particles via micelle formation, effectively lowering interfacial tension [39].

Nanoparticles, particularly zinc oxide (ZnO), have been reported to contribute to cleaning performance not only through their antibacterial properties [40] but also through photocatalytic degradation of organic compounds under visible-light irradiation [41, 42], and through surface adsorption of metal ions, as demonstrated for Ni^{2+} and Cu^{2+} [43]. It should be noted that photocatalytic activity requires a light source of appropriate wavelength and intensity; in its absence, this mechanism is expected to contribute only marginally, and its role should be distinguished from ZnO's other, irradiation-independent effects, such as mild abrasive action and surface-mediated adsorption of contaminants. ZnO nanoparticles have also been incorporated into hybrid nanogel systems for cleaning dust and soil deposits from paper manuscripts [31]. In addition, nanostructured fluids (NSFs) offer the capacity to solubilize hydrophobic contaminants while reducing reliance on potentially harmful organic solvents [44]. Through the strategic integration of these agents, gellan hydrogels can be tailored to address both the organic and inorganic components of hybrid stains with enhanced precision and minimal risk to the paper

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substrate. Accordingly, the objective of this study is to assess the effectiveness of gellan gum-based hydrogel in removing hard and hybrid stains that contain both inorganic and organic compounds from the surfaces of paper artifacts. To this end, the optimization of the hydrogel with various additives, including nanoparticles, surfactant, and enzyme, has also been examined.

2. Experimental

2.1. Preparation of artificial soiling and sample preparation

To simulate hybrid soiling, an artificial soil mixture was prepared. This mixture consisted of 10 g of fine household dust (sieved to <150μm), 2 g of Iranian industrial soot, 40 mL of Iranian olive oil (Tarom Company, Iran), and 150 mL of white spirit (Vesta Company, Iran). The components were thoroughly mixed and stirred for 15 minutes using a magnetic stirrer, following a method adapted from Macken and Smith [45]. This final mixture was then applied as a uniform layer onto paper samples. All preparation steps were conducted under standard laboratory temperature and humidity conditions.

The composition of the artificial soil mixture was designed to reproduce the principal categories of contaminants most frequently reported on historical paper artifacts: particulate matter and lipidic/oily residues, which together represent the hybrid organic–inorganic soiling most commonly targeted in paper-cleaning studies. The relative proportions were adapted from the protocol of Macken and Smith [45] and adjusted to yield a visually uniform, reproducible soiling layer with a sufficiently high initial ΔE to allow meaningful discrimination between the cleaning efficacies of the tested formulations.

For the paper samples, 60 gsm laboratory-grade filter paper from Germany was cut into

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12×17 cm sheets. The paper samples were first sized with a starch solution and then burnished. The prepared artificial soil mixture was applied uniformly to the surface of each sample using a brush. The soiled samples were then allowed to equilibrate at ambient temperature for 48 hours before further analysis.

2.2. Hydrogel preparation and formulation

Gellan gum-based hydrogels were prepared using low-acyl gellan gum (Sitak Company, Iran) and calcium acetate (Merck, Germany). A solution containing 20 g/L gellan gum and 0.4 g/L calcium acetate in deionized water was prepared. This solution was exposed to 600 W microwave irradiation for approximately one minute (LG appliance) to obtain a transparent, homogeneous solution. The solution was then transferred to a Petri dish and allowed to gel at room temperature.

To prepare the additives, an NSF microemulsion was first formulated according to a previously reported method [44, 46, 47]. Briefly, 3.7 g of sodium dodecyl sulfate (SDS) was dissolved in 73.3 mL of deionized water. Subsequently, 7 mL of 2-butanol, 8 mL of propylene carbonate, and 8 mL of ethyl acetate (all from Merck, Germany) were added sequentially, with gentle stirring after each addition to ensure homogeneity.

A 3% enzyme solution was prepared by dissolving 3 g of commercial Racanzyme LTO-4950 (Asan Chem Company, Iran), which contains a 1:1 mixture of lipase and protease, in 100 mL of deionized water. The solution was stirred at a controlled temperature until a clear solution free of suspended particles was obtained. Similarly, a nanoparticle suspension was prepared by gradually adding 0.4 g of zinc oxide (ZnO) nanoparticles to 100 mL of deionized water, with gentle stirring to ensure complete dispersion and

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prevent agglomeration.

Based on these components, a two-factor experimental design was adopted, with gel formulation (eight compositions) and contact time (30 min and 60 min, applied as two consecutive 30-minute intervals) as the independent experimental factors, yielding a total of sixteen experimental conditions (S1-S16). For each condition, one dedicated paper specimen was prepared and treated; no independently soiled and treated replicate specimens were prepared per condition. The formulations included pure gellan gum hydrogel, hydrogels with enzyme, NSF microemulsion, a combination of enzyme and NSF, and analogous samples with the addition of ZnO nanoparticles. The ZnO nanoparticles were incorporated to increase the contact area and improve cleaning efficiency. The specific composition and coding for each sample are provided in Table 1. All hydrogel applications were carried out under ambient laboratory lighting conditions, without the use of a dedicated UV or visible-light irradiation source.

Table 1. Composition and contact time of the sixteen experimental conditions (S1-S16), representing the eight gel formulations tested at two contact times, 30 min and 60 min.

Sample Code	Contact Time (min)	Gel Composition
S1, S2	30, 2×30	Gellan 2% (100 cc)
S3, S4	30, 2×30	Gellan 2% (100 cc) + Enzyme 3% (16 cc)
S5, S6	30, 2×30	Gellan 2% (100 cc) + NSF (12 cc)
S7, S8	30, 2×30	Gellan 2% (100 cc) + NSF (12 cc) + Enzyme 3% (16 cc)
S9, S10	30, 2×30	Gellan 2% in nanoparticle suspension (100 cc)
S11, S12	30, 2×30	Gellan 2% in nanoparticle suspension (100 cc) + Enzyme 3% (16 cc)
S13, S14	30, 2×30	Gellan 2% in nanoparticle suspension (100 cc) + NSF (12 cc)
S15, S16	30, 2×30	Gellan 2% in nanoparticle suspension (100 cc) + NSF (12 cc) + Enzyme 3% (16 cc)

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2.3. Accelerated aging

To simulate the environmental conditions affecting historical documents and to accurately evaluate the degradation of the paper, an accelerated aging method was used [48]. The prepared paper samples, including the soiling layer, were placed in a controlled-temperature and humidity oven (Pars Azma, Iran). Aging was conducted at 80°C and 65% relative humidity for 72 hours, conditions widely used in accelerated aging protocols for cellulosic materials [49-52] and selected to promote hydrolytic and oxidative degradation pathways broadly representative of those implicated in natural paper aging, within a laboratory-practicable timeframe. It should be noted that accelerated thermal-humidity aging primarily drives hydrolytic and thermo-oxidative reactions, which may not fully reproduce the slower, photo-oxidative and pollutant-mediated degradation pathways that dominate natural aging over decades to centuries; the aging step in this study is therefore intended to provide a comparative stress test of formulation stability rather than a precise simulation of long-term natural aging. This process was carried out in two stages: an initial stage to fix the soiling onto the paper and a second stage after the hydrogel treatment to evaluate the stability of the cleaned areas.

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2.4. Colorimetry

Quantitative evaluation of color changes was performed using spot colorimetry. Measurements were taken with a 3NH NH310 portable colorimeter and its dedicated CQCS3 software. Before measurement, the device was calibrated using a standard PTFE plate to ensure accuracy and reproducibility. For each specimen, L*, a*, and b* values were measured at ten locations distributed across the treated area. These measurements

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were used to characterize within-specimen spatial homogeneity and measurement precision and were not considered independent experimental replicates, as only one specimen was prepared for each condition. The mean L*, a*, and b* values were then used to calculate a single representative ΔE value for each specimen. Because ΔE was calculated from the averaged L*, a*, and b* values rather than as the mean of ten independently calculated ΔE values, a standard deviation cannot be reported for the ΔE values themselves, nor can inferential statistical comparisons be performed. Accordingly, because only one specimen was prepared per experimental condition, differences between conditions are reported descriptively rather than interpreted using inferential statistics. Color changes for each specimen — including the soiled, uncleaned reference and all cleaned samples — were calculated relative to the Lab* values of the clean, unsoiled filter paper, using the ΔEab parameter in the CIE Lab* color space, where $L*$ represents lightness, $a*$ represents the red-green coordinate, and $b*$ represents the yellow-blue coordinate. Accordingly, a lower ΔE indicates a color closer to that of the original clean paper, while the ΔE of the untreated soiled reference represents the baseline color deviation prior to any cleaning treatment. This color space was used because its reduced dependence on observer and light source characteristics allows for the precise evaluation of even small color changes.

2.5. FTIR Spectroscopy

To investigate the chemical changes resulting from the cleaning treatments, Fourier-transform infrared (FTIR) spectroscopy was performed using a Jasco FTIR-680plus instrument (Japan). For each condition, a single sample was taken from the central,

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treated area of the specimen and analyzed as a KBr pellet, with 64 scans and a resolution of 4 cm^{-1}. Spectra were recorded before and after the cleaning procedure, and after secondary accelerated aging, to identify potential changes in the paper's chemical structure. A background spectrum of air was acquired before each analysis. Each spectrum was normalized independently to a 0–1 absorbance scale (min–max normalization).

2.6. Microscopic analysis

Morphological changes on the paper surface were evaluated using a ZSM 1001 stereo microscope (SaIran Company) at 40x magnification. Images of soiled, cleaned, and aged samples were recorded and analyzed to assess changes in texture, surface uniformity, and the presence or removal of pollutant particles.

3. Results and Discussion

The evaluation of the cleaning gels on the soiled paper samples demonstrated that all formulations could remove some degree of surface contamination. However, the extent and quality of the cleaning were highly dependent on the gel's composition and contact time.

In the nanoparticle-free group, formulations containing gellan gum with the NSF surfactant, particularly with a one-hour contact time, produced the most significant visual improvement. The role of the anionic NSF surfactant was notable, as it facilitated the dissolution and removal of dirt particles and reduced their adhesion to the cellulose fibers. In contrast, simpler formulations, such as pure gellan gum, only provided partial

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cleaning. In the group containing ZnO nanoparticles, formulations combining ZnO with NSF or NSF plus enzyme performed better than other compositions. Since the hydrogels were applied under ambient laboratory lighting rather than controlled UV/visible irradiation, a substantial photocatalytic contribution from ZnO is unlikely; possible non-photocatalytic mechanisms are discussed further below.

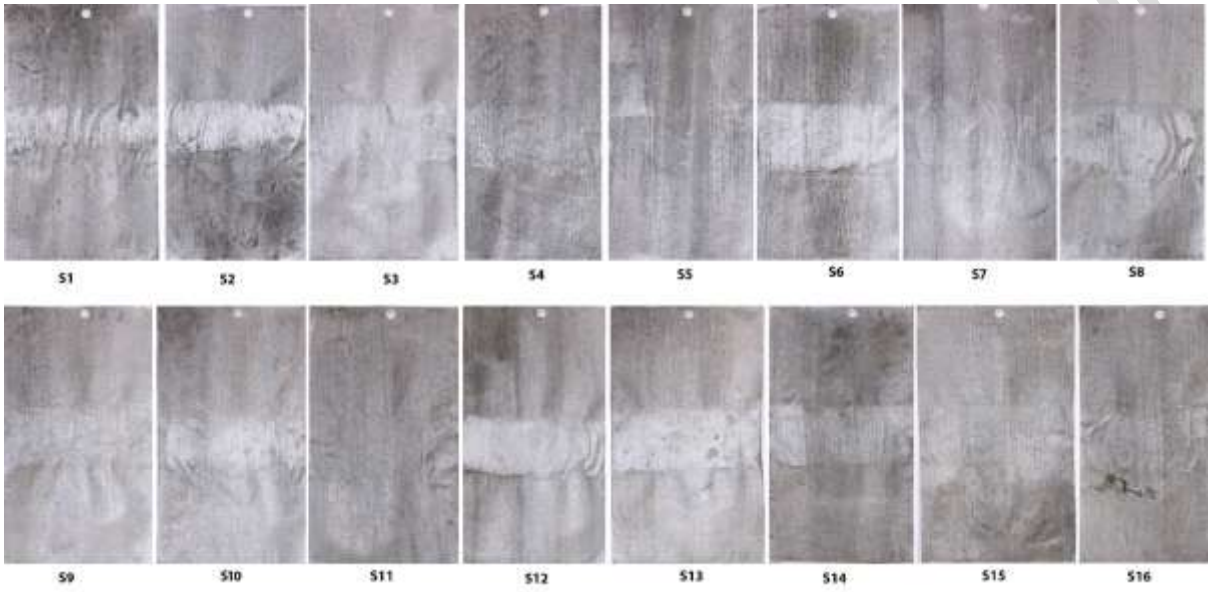


Figure 1. Images of paper samples after hydrogel application. The central stripe represents the area cleaned with the hydrogel.

Microscopic analysis of the paper surface before and after cleaning, and following accelerated aging, provided clear evidence of reduced foreign particles, improved surface uniformity, and the removal of adherent materials. Samples cleaned with NSF-containing formulations, especially in combination with ZnO, showed more thorough and uniform surface cleaning. However, in the aged samples, a yellowing was often evident, which may be attributed to the re-oxidation of cellulose or residual cleaning agents within the paper structure. This discoloration was more pronounced in samples with longer contact

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times and those containing surfactants, likely due to increased moisture absorption and the acceleration of oxidation reactions.

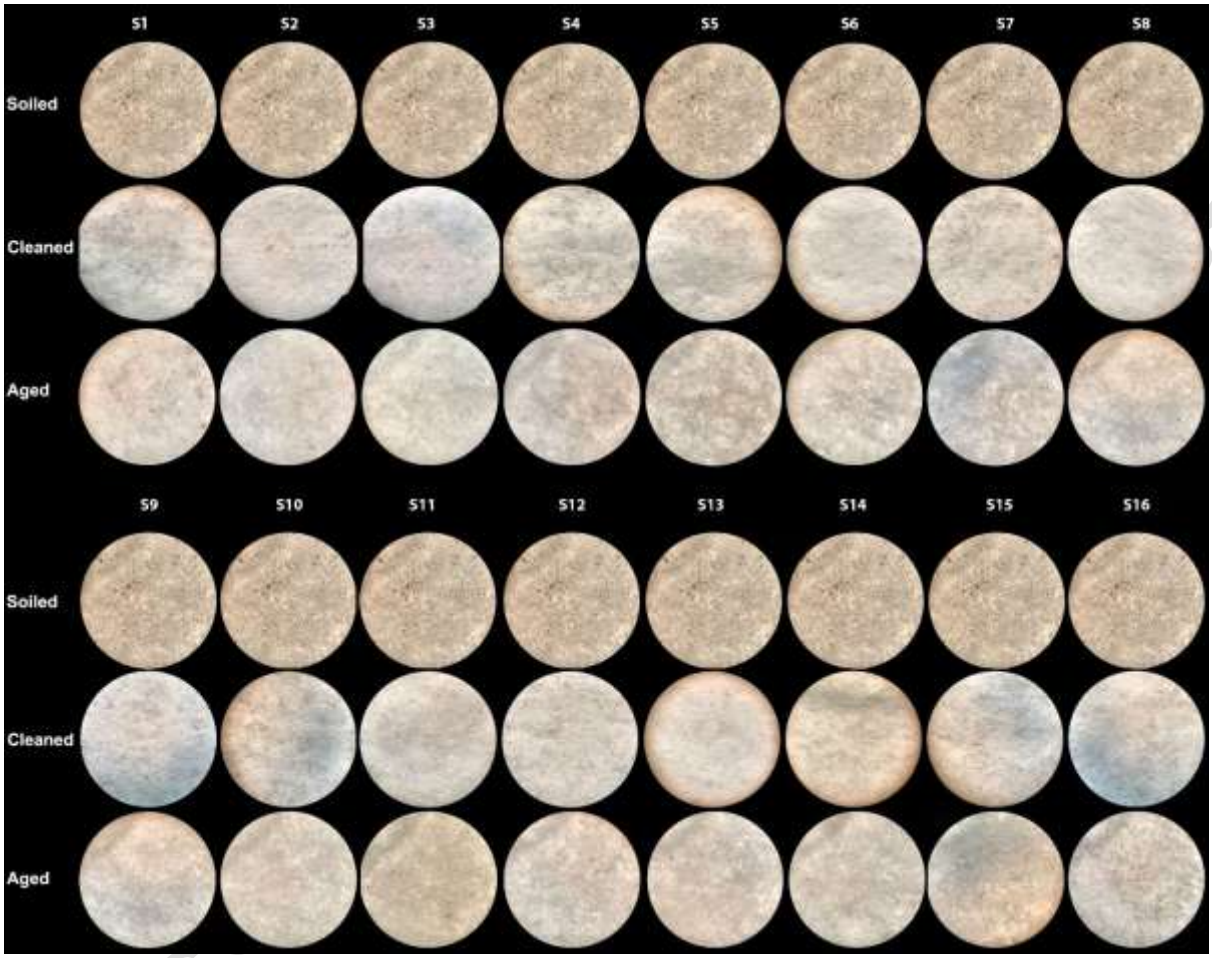


Figure 2. Stereomicroscopic images at 40× magnification of the paper surface at three stages: pre-cleaning, post-cleaning, and after secondary aging of the cleaned paper.

Colorimetric analysis revealed that the use of gellan gum-based hydrogels with different additives resulted in considerable changes in the ΔE parameter, and thus, a visual improvement of the soiled paper samples. The soiled reference sample had a ΔE of 20.35, indicating the largest color difference from the clean paper. Among the cleaned samples, the Gellan/NSF formulation with a one-hour contact time (S6) showed the best performance, reducing the ΔE to 4.76. This marked reduction highlights the high capacity

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of NSF as a surfactant for removing surface contamination. Other NSF-containing samples (S5, S13, S14) also showed considerable improvement compared to the reference, although their efficiency was somewhat impacted by the co-presence of ZnO nanoparticles.

The analysis of enzymatic formulations revealed a more complex pattern than a simple limited effect. The enzyme alone showed contact-time-dependent performance, from a moderate ΔE of 6.84 at 30 min (S3) to a higher ΔE of 10.40 at 60 min (S4); the ZnO-containing enzyme formulation without NSF (S11) performed poorly at both intervals (ΔE=14.63). Contrary to an expectation of synergy, combining the enzyme with NSF (S7, S8) did not improve on the best-performing individual component at either contact time: S7 (ΔE=9.52, 30 min) exceeded the enzyme-alone result (S3, ΔE=6.84), and S8 (ΔE=9.77, 60 min) exceeded the NSF-alone result (S6, ΔE=4.76). A consistent pattern was observed in the ZnO-containing group, where adding the enzyme to the ZnO/NSF formulation increased ΔE at both contact times (S13→S15: 6.15→7.93 at 30 min; S14→S16: 8.86→12.50 at 60 min). Given that this trend was reproduced across both the ZnO-free and ZnO-containing formulations and at both contact times, the data do not support a synergistic interaction between enzymatic hydrolysis and NSF-mediated emulsification; rather, they suggest a possible antagonistic interaction, which may arise from partial enzyme inactivation or denaturation by the anionic surfactant SDS present in the NSF microemulsion, a plausible mechanism given the well-documented protein-denaturing activity of SDS, though this was not directly tested in the present study. We note that this interpretation remains descriptive, as the present single-specimen design does not permit a formal, replicate-based interaction analysis. The effect of contact time

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was, however, clearly positive for the simpler Gellan/NSF formulation, with an increase from 30 minutes (S5: ΔE=10.88) to one hour (S6: ΔE=4.76) yielding a marked improvement. In more complex formulations containing both ZnO and enzyme, extending the contact time from 30 to 60 minutes was instead associated with a higher (worse) ΔE, as seen for S16 (ΔE=12.50), possibly related to excessive penetration of active compounds or the formation of undesirable secondary products within the paper structure. As only two contact-time points were examined in this study, these results should be interpreted as showing that the direction of the time-dependent effect differs between formulation types within this specific range, rather than as identifying an optimum contact time; a broader, multi-point time-course study would be needed to establish whether an optimal contact time exists for each formulation.

ZnO nanoparticles alone in combination with gellan gum (S9 and S10) resulted in relatively low ΔE values (7.42 and 9.42). However, in the presence of the enzyme and absence of NSF (S11), they performed poorly, yielding a ΔE of 14.63. The best results for the ZnO group were obtained when the nanoparticles were combined with NSF, as seen in S13 (ΔE=6.15), indicating that a proper balance between the antibacterial and whitening properties of ZnO and the detergent action of NSF is crucial.

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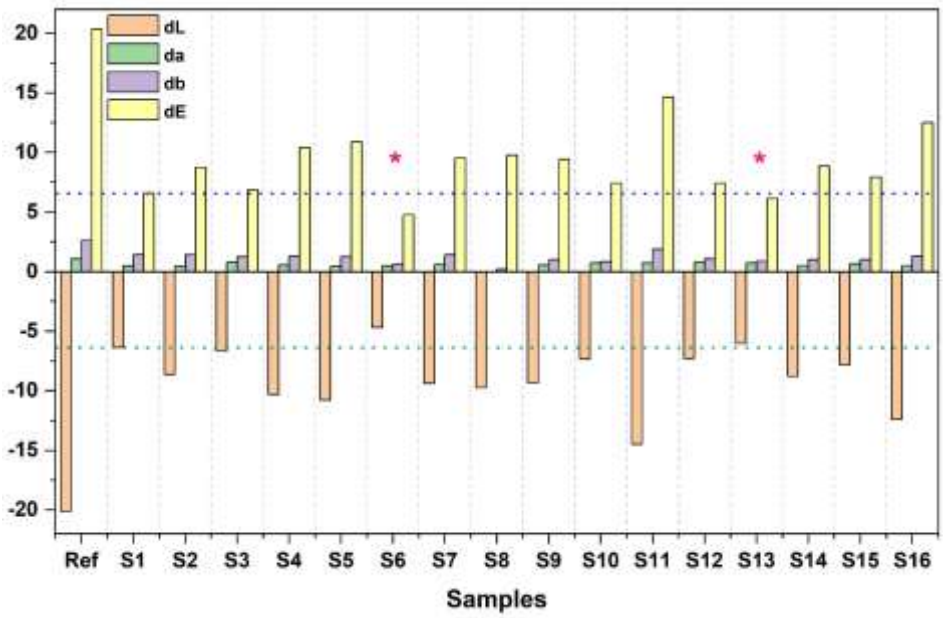


Figure 3. Comparison of ΔE values (relative to the clean, unsoiled filter paper) for samples cleaned with hydrogels, shown alongside the ΔE of the untreated soiled paper as a baseline reference.

Following temperature-humidity aging, the color stability of the samples varied considerably across formulations. Sample A-S3, containing enzyme and gellan gum with a 30-minute application time, showed the highest color stability with a ΔE of 3.19. In contrast, sample A-S14 (Gellan/ZnO/NSF) with a one-hour treatment time experienced the largest change, with a ΔE of 8.95. Samples containing NSF sometimes showed an increase in ΔE after aging, for example, A-S6 from 4.76 to 10.60. This increase is likely due to moisture absorption by residual SDS or other chemical changes during the aging process. Conversely, sample A-S12, with a ΔE of 4.11, exhibited good relative stability, which can be attributed to the protective role of ZnO. These results indicate that achieving an optimal balance between cleaning efficiency and long-term stability requires careful control of both the formulation and the contact time.

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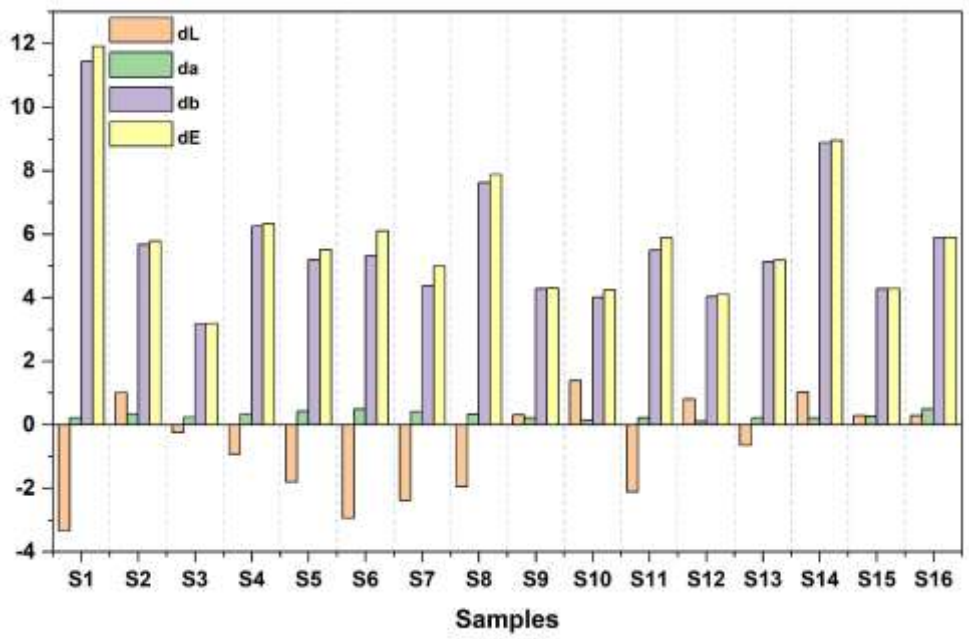


Figure 4. Comparison of ΔE values, calculated relative to each sample's own color before accelerated aging, for hydrogel-cleaned samples after accelerated aging.

FTIR spectroscopy of the paper samples after cleaning and accelerated aging provided evidence of chemical changes related to the removal or persistence of soiling compounds and gel residues on the paper surface. A characteristic peak at approximately 1740 cm^{-1}, attributed to the C=O stretching vibration of carbonyl groups in triglycerides and oils, showed a relatively high normalized intensity of around 0.44 in the soiled reference paper. After cleaning, most formulations showed a decrease in the intensity of this peak, consistent with removal of a portion of the oily contaminants. Specifically, sample S6 (Gellan + NSF) with a one-hour contact time showed a decrease in peak intensity to 0.25, corresponding to a 43.2% reduction. This finding is consistent with its colorimetric result (ΔE=4.76) and is compatible with, though not sole proof of, effective removal of fatty compounds by the NSF-containing formulation; a decrease in a single carbonyl-region band cannot by itself distinguish between removal by surfactant-mediated dissolution,

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enzymatic hydrolysis, or other physical transfer processes, and should be interpreted alongside the colorimetric and microscopic evidence rather than as independent mechanistic proof. Sample S3 (Gellan + enzyme) showed an even greater reduction, to a peak intensity of 0.20 (54.5%), which may be related to the enzyme's activity in hydrolyzing fatty esters.

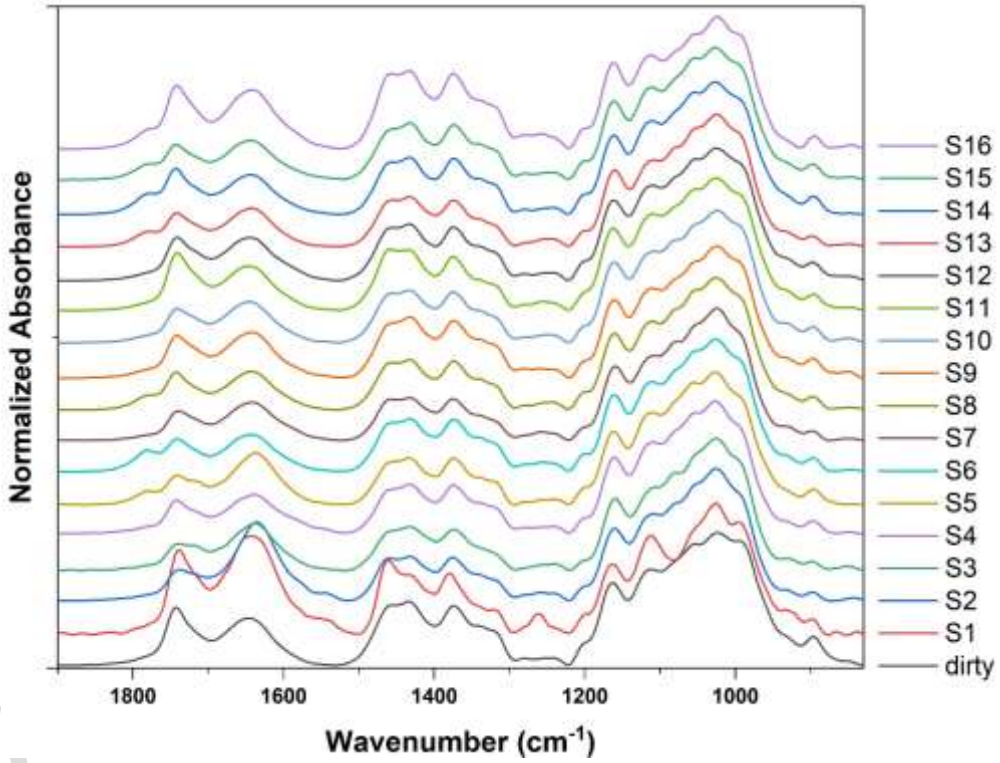


Figure 5. FTIR spectra of paper sample sections cleaned with hydrogels, alongside the spectrum of the uncleaned soiled paper.

However, post-aging data revealed that this reduction was not stable in all cases. Sample A-S3 experienced an increase in the 1740 cm^{-1} peak intensity back to 0.40, which could be due to the re-oxidation of organic residues or the formation of secondary products under high temperature and humidity conditions. In contrast, sample S16 (Gellan + ZnO

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+ NSF + enzyme) showed a different pattern: the 1740 cm^{-1} peak intensity increased after initial cleaning but decreased after aging. A plausible, though unconfirmed, explanation is a two-stage process: initial redistribution or incomplete removal of lipidic/gel residues in this multi-component, longer-contact-time formulation, consistent with its comparatively poor initial ΔE of 12.50, followed by degradation of these residual carbonyl-containing compounds during accelerated aging — potentially promoted by the combined presence of ZnO and residual SDS, both of which can facilitate oxidative breakdown of organic matter under thermo-oxidative stress. Confirming this mechanism would require targeted analyses.

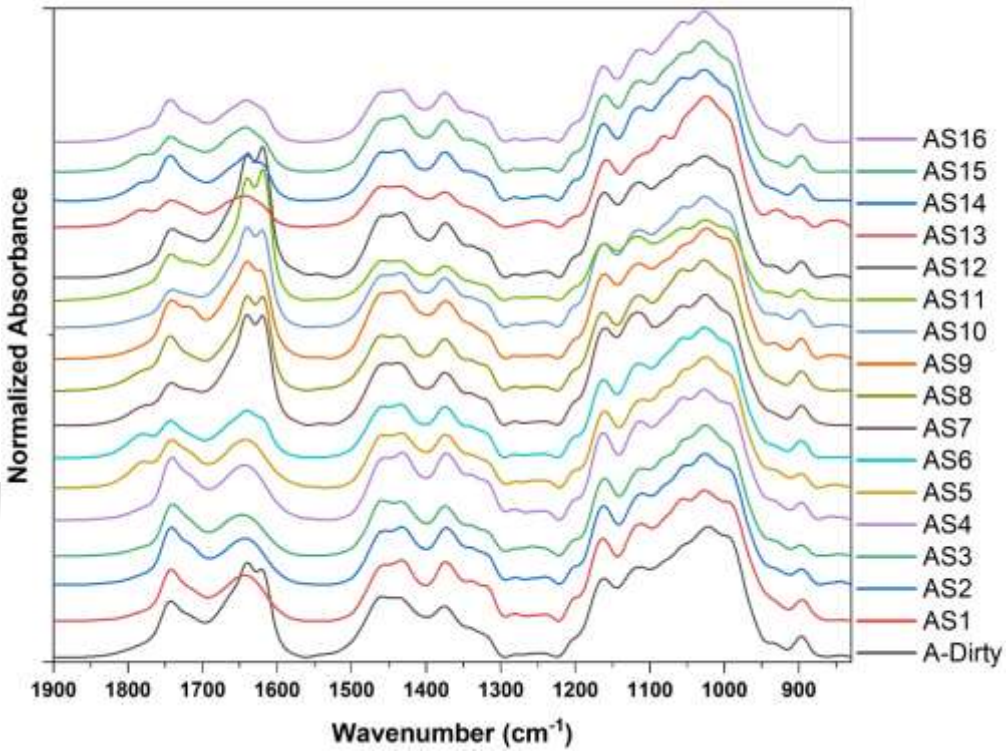


Figure 6. FTIR spectra of paper sample sections cleaned with hydrogels, alongside the spectrum of the uncleaned soiled paper after a secondary accelerated aging process.

Furthermore, a new peak was observed in some cleaned samples, particularly those containing NSF, at approximately 1780 cm^{-1}, which was absent in sample S10 (lacking

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NSF). Based on its association with NSF-containing formulations, we hypothesize that this band may originate from residual surfactant-related components or gel byproducts; confirming this would require reference spectra of the individual components, which were not acquired here. The intensity of this peak was comparatively weak in sample S15, consistent with more effective removal of such residues with this formulation.

Another peak at approximately 1620 cm^{-1} was observed in samples containing ZnO and also in the soiled paper; a plausible tentative assignment is to inorganic–organic complexes arising from interaction of Zn^{2+} ions with carbonyl or hydroxyl groups. This peak intensified after aging in most ZnO-containing samples, which is consistent with, though not proof of, the accumulation of residual ZnO particles or their oxidation products under accelerated aging conditions. Among these, sample S15 had the lowest intensity in this region, which aligns with its colorimetric results. Confirming this assignment would require reference spectra of the individual components, which were not acquired in the present study.

In summary, the FTIR results confirm that formulations containing NSF and enzyme are most effective at removing oily compounds. However, the long-term stability of this effect is dependent on the specific formulation, the presence or absence of ZnO, and the contact time. While a longer contact time may be more effective for initial cleaning, it also increases the probability of residual gel components and the formation of undesirable products during aging. These findings emphasize the importance of simultaneously optimizing cleaning efficiency and long-term stability when designing cleaning gels for paper-based artifacts.

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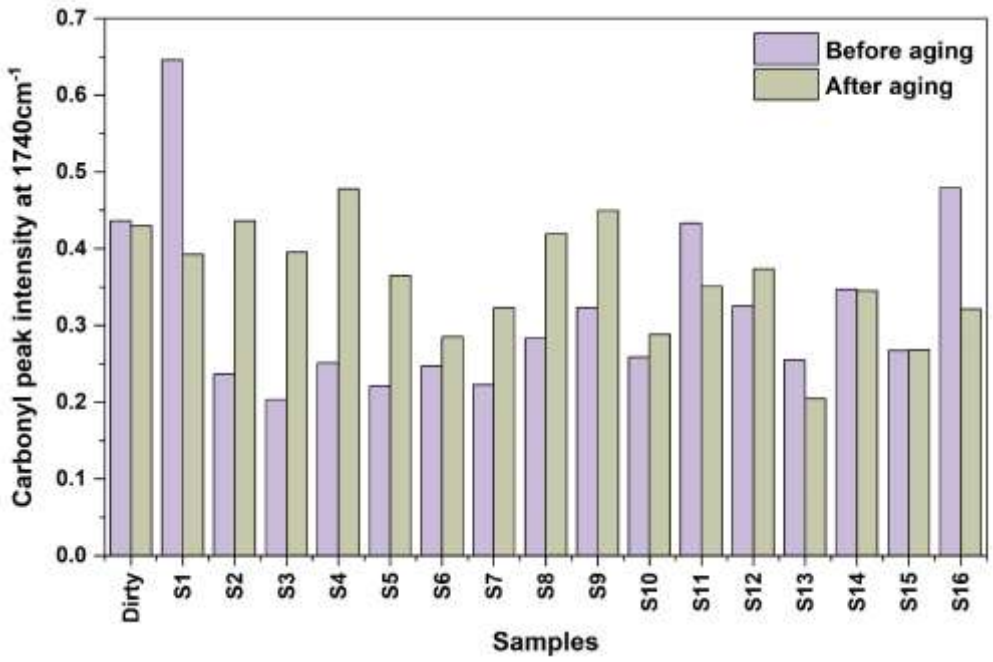


Figure 7. Intensity of the peak around 1740 cm^{-1}, corresponding to carbonyl group vibrations, in the normalized FTIR spectra obtained from the analysis of areas cleaned with hydrogels, alongside the soiled reference paper sample, before and after secondary accelerated aging.

To assess whether the observed changes extended beyond the removal of lipidic contamination to affect the cellulose structure itself, the ratio of the glycosidic-linkage bands I(1160)/I(898) was examined before and after aging for all samples, as an indicator of the cellulose backbone rather than the carbonyl-based indices used above. As shown in Table 2, most cleaned samples had I(1160)/I(898) values in the range of 2.1-4.9, higher than that of the untreated soiled reference (1.79), and this ratio remained essentially stable after accelerated aging, changes generally below 5%, suggesting no substantial additional degradation of the glycosidic linkages under the aging conditions tested. One formulation, S13 (Gellan + ZnO + NSF), showed a markedly higher and stable ratio (~6.3 before and after aging), which may reflect a more ordered cellulose surface following

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cleaning, though this was not further investigated. Overall, these results indicate that, within the sensitivity of this index, the cleaning and aging treatments examined here did not produce a marked deterioration of the cellulose backbone as reflected in the FTIR glycosidic-region bands.

Table 2. Glycosidic-linkage band ratio [I(1160)/I(898)] for each sample before and after accelerated aging.

Sample Code	Before Aging	After Aging
Ref	1.79	1.82
S1	2.35	2.35
S2	3.27	3.27
S3	4.89	4.92
S4	3.34	3.29
S5	3.57	3.74
S6	2.30	2.27
S7	2.22	2.32
S8	2.11	2.07
S9	2.12	2.08
S10	2.46	2.39
S11	2.47	2.49
S12	2.30	2.32
S13	6.30	6.26
S14	2.72	2.70
S15	2.28	2.23
S16	2.25	2.16

The role of ZnO nanoparticles in the observed cleaning behavior warrants further discussion. In the literature, ZnO nanoparticles have been reported to contribute to cleaning and surface interactions through two main pathways: photocatalytic degradation of organic compounds under UV/visible irradiation [41, 42], and surface adsorption/complexation with metal ions, as reported for heavy metal ions [43]. In the present study, hydrogel applications were carried out under ambient laboratory lighting, without a dedicated UV/visible irradiation source; a substantial photocatalytic

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contribution is therefore unlikely. The comparatively favorable performance of ZnO/NSF formulations (e.g., S13) and the intensification of the ~1620 cm^{-1} band in ZnO-containing samples after aging are more consistent with a non-photocatalytic pathway — namely, surface complexation of metal ions and/or carbonyl/hydroxyl groups, and a mild physical abrasive contribution — than with light-driven catalytic degradation. Though a mechanical abrasive action, if present, could in principle also risk fiber wear, a possibility not directly assessed in this study. Confirming the precise mechanistic role of ZnO in this system — and distinguishing the relative contributions of surface complexation, adsorption, and any residual photocatalytic activity — will require further dedicated studies, for example comparing formulation performance under controlled dark and irradiated conditions.

A number of limitations should be kept in mind when interpreting these results. The use of artificially soiled filter paper, while common in this field, is a simplified stand-in for historical paper with its inks, sizing, fillers, and decades of natural aging. Each condition was tested on a single specimen — the repeated colorimetric readings capture local uniformity rather than true replication, and a single, non-internally-referenced FTIR pellet per condition means peak intensities are only comparable in a relative sense. This precluded any inferential statistics, so differences between formulations are discussed descriptively rather than as statistically confirmed effects. Contact time was likewise tested at only two points, so the trends reported here describe that specific range rather than an optimum, and the accelerated aging used mainly captures hydrolytic and thermo-oxidative degradation rather than the slower pathways of natural aging.

Several of our mechanistic interpretations — the abrasive/adsorptive role attributed to

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ZnO, the apparent antagonism between enzyme and NSF, and the tentative FTIR assignments at 1780 and 1620 cm^{-1} — rest on overall trends rather than dedicated verification; reference spectra of the individual components would have helped confirm these assignments, but additional analyses were not feasible within the scope of this project. Before these formulations could be considered for use on actual historical artifacts, further work is needed on residue removal, ZnO retention or migration within the paper, the mechanical properties of treated paper, and testing on substrates more representative of real historical materials.

4. Conclusion

The findings of this study highlight the effectiveness of gellan gum-based hydrogels, especially when enhanced with specific additives, in tackling complex soiling on paper substrates. However, the overall effectiveness and long-term stability of the cleaning results are closely linked to the formulation and careful management of contact time.

Among the various compositions tested, those containing the anionic surfactant NSF, particularly with a one-hour contact time, yielded the most noticeable visual improvements and a marked reduction in the intensity of FTIR peaks related to lipid compounds. This outcome emphasizes the crucial role of NSF in emulsifying and removing hydrophobic substances from the surface of cellulose fibers. Additionally, incorporating ZnO nanoparticles with NSF further improved the cleaning process; since the treatments were carried out under ambient lighting without controlled UV/visible irradiation, this improvement is unlikely to reflect a photocatalytic effect and may instead be related to mild abrasive action and/or surface-mediated adsorption of contaminants by

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the nanoparticles, although these mechanisms were not directly verified in this study.

However, accelerated aging tests indicated that extended contact with certain formulations containing both ZnO and surfactants could result in undesirable color changes. The enzymatic component alone showed contact-time-dependent effectiveness, while its combination with NSF did not outperform the best-performing individual component at either contact time, and this pattern was consistent across formulations with and without ZnO. This suggests a possible antagonistic rather than synergistic interaction between enzymatic hydrolysis and NSF-mediated emulsification, which merits further mechanistic investigation.

FTIR analysis confirmed that effective removal of fatty compounds is possible, but the long-term stability of this effect varies across different formulations. Some samples exhibited a partial return of carbonyl peaks or the formation of secondary products after aging. The formulations that achieved the best balance between cleaning efficiency and color stability were those combining ZnO nanoparticles, whose contribution may involve abrasive and/or adsorptive action, with the emulsifying properties of NSF, while minimizing the residue of active components to prevent excessive penetration and retention within the paper.

These results highlight the importance of a multifaceted approach in designing cleaning hydrogels, and support the potential of gellan gum-based formulations, particularly those combining NSF and ZnO, as a promising direction for further development in paper conservation. However, before these formulations can be recommended for treatment of actual historical artifacts, several aspects require further investigation: complete removal of residual surfactant, enzyme, and gel components from the treated substrate; the

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retention, migration, and long-term fate of ZnO nanoparticles within the cellulose matrix; the mechanical properties of paper after treatment and aging; and validation on paper substrates more representative of historical materials, including aged cellulose, sizing agents, fillers, and inks. Addressing these points would be an important next step toward translating the present findings into conservation practice.

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References

1. Mazzuca C, Micheli L, Lettieri R, Cervelli E, Coviello T, Cencetti C, Sotgiu S, Iannuccelli S, Palleschi G, Palleschi A. How to tune a paper cleaning process by means of modified gellan hydrogels. Microchem J. 2016; 126:359-67. https://doi.org/10.1016/j.microc.2015.12.016

2. Abdel-Nasser M, Abdel-Maksoud G, Abdel-Aziz M, Darwish S, Hamed A, Youssef A. Evaluation of the efficiency of nanoparticles for increasing α-amylase enzyme activity for removing starch stain from paper artifacts. J Cultural Heritage. 2022; 53:14-23. https://doi.org/10.1016/j.culher.2021.11.004

3. Wahba WN, Shoshah NM, Nasr HE, Abdel-Maksoud G. The use of different techniques for removal of pressure sensitive tapes from historical paper documents: a

ACCEPTED MANUSCRIPT

review. Egyptian J Chem. 2022; 65(13):1019-31. https://doi.org/10.21608/ejchem.2022.139602.6123

4. Koochakzaei A, Saidi Mehrabad M. A multi-analytical investigation of an Iranian lithography book from the Qajar period. Inter J Conservation Sci. 2024; 15(3):1465-78. https://doi.org/10.36868/IJCS.2024.03.19

5. Abdolalizadeh M, Mohammadi Achachluei M, Oudbashi O. Studying the products and factors involved in corrosion of brass alloy in four Qajar era manuscripts. J Res Archaeom. 2024; 10(1):167-88. https://doi.org/10.61186/jra.10.1.397.

6. Abdel-Maksoud G, Emam H, Ragab N. From traditional to laser cleaning techniques of parchment manuscripts: a review. Adv Res Conservation Sci. 2020; 1(1):52-76.

7. Stockman D. Treatment options for oil stains on paper. The Book and Paper Group Annual. 2007; 26:115-26.

8. Choi S. Foxing on paper: a literature review. J Am Inst Conservation. 2007; 46(2):137-52. https://doi.org/10.1179/019713607806112378

9. Koochakzaei A, Alizadeh Gharetapeh S. Paper foxing stains on a historic manuscript from the early Qajar era: abiotic or biotic foxing? Heritage. 2021; 4(3):1366-74. https://doi.org/10.3390/heritage4030074

10. Oliveira MJA, Otubo L, Pires A, Brambilla RF, Carvalho AC, Santos PS, Neto AO, Vasquez P. Silver nanoparticles-based hydrogels synthetized by ionizing radiation for cleaning of tangible cultural heritage surfaces. Radiation Phys Chem. 2022; 199:110345. https://doi.org/10.1016/j.radphyschem.2022.110345

Accepted Manuscript

ACCEPTED MANUSCRIPT

11. Salim E, Ali Hassan RR. Alkyl dimethyl benzyl ammonium chloride as a new cleaner for washing treatments for historical printed paper. Pigm Resin Technol. 2024; 53(2):164-72. https://doi.org/10.1108/PRT-04-2022-0052

12. Mazzuca C, Micheli L, Carbone M, Basoli F, Cervelli E, Iannuccelli S, Sotgiu S, Palleschi A. Gellan hydrogel as a powerful tool in paper cleaning process: a detailed study. J Colloid Inter Sci. 2014; 416:205-11. https://doi.org/10.1016/j.jcis.2013.10.062

13. Mazzuca C, Micheli L, Cervelli E, Basoli F, Cencetti C, Coviello T, Iannuccelli S, Sotgiu S, Palleschi A. Cleaning of paper artworks: development of an efficient gel-based material able to remove starch paste. ACS Appl Mater Interf. 2014; 6(19):16519-28. https://doi.org/10.1021/am504295n

14. Mazzuca C, Poggi G, Bonelli N, Micheli L, Baglioni P, Palleschi A. Innovative chemical gels meet enzymes: a smart combination for cleaning paper artworks. J Colloid Interf Sci. 2017; 502:153-64. https://doi.org/10.1016/j.jcis.2017.04.088

15. Di Napoli B, Franco S, Severini L, Tumiati M, Buratti E, Titubante M, Nigro V, Gnan N, Micheli L, Ruzicka B. Gellan gum microgels as effective agents for a rapid cleaning of paper. ACS Appl Polym Mater. 2020; 2(7):2791-801. https://doi.org/10.1021/acsapm.0c00342

16. Guild S. Caring for paper objects. Canadian Heritage; 2018.

17. Cowan J, Guild S. Dry methods for surface cleaning paper. Canadian Conservation Institute Technical Bulletin. 2005; 11:1-12.

18. Zervos S, Alexopoulou I. Paper conservation methods: a literature review. Cellulose. 2015; 22(5):2859-97. https://doi.org/10.1007/s10570-015-0699-7

ACCEPTED MANUSCRIPT

19. Parfenov V, Galushkin A, Tkachenko TA, Aseev V. Laser cleaning as novel approach to preservation of historical books and documents on a paper basis. Quantum Beam Sci. 2022; 6(3):23. https://doi.org/10.3390/qubs6030023

20. Baglioni P, Chelazzi D, Giorgi R. Nanotechnologies in the conservation of cultural heritage: a compendium of materials and techniques. Springer; 2014.

21. Cremonesi P. Rigid gels and enzyme cleaning. In: New insights into the cleaning of paintings. 2013. p. 179-83.

22. De Filpo G, Palermo AM, Tolmino R, Formoso P, Nicoletta FP. Gellan gum hybrid hydrogels for the cleaning of paper artworks contaminated with Aspergillus versicolor. Cellulose. 2016; 23(5):3265-79. https://doi.org/10.1007/s10570-016-1021-z

23. Fu J. Hydrogel properties and applications. J Mater Chem B. 2019; 7(10):1523-5. https://doi.org/10.1039/c9tb90023c

24. Khaksar-Baghan N, Koochakzaei A, Hamzavi Y. An overview of gel-based cleaning approaches for art conservation. Heritage Sci. 2024; 12(1):248. https://doi.org/10.1186/s40494-024-01369-0.

25. Ullah F, Othman MBH, Javed F, Ahmad Z, Akil HM. Classification, processing and application of hydrogels: a review. Mater Sci Eng C. 2015; 57:414-33. https://doi.org/10.1016/j.msec.2015.07.053

26. Baglioni M, Poggi G, Chelazzi D, Baglioni P. Advanced materials in cultural heritage conservation. Molecules. 2021; 26(13):3967. https://doi.org/10.3390/molecules26133967

27 .Gyles DA, Castro LD, Silva Jr JO, Ribeiro-Costa RM. A review of the designs and prominent biomedical advances of natural and synthetic hydrogel formulations. European Poly J. 2017; 88:373-92. https://doi.org/10.1016/j.eurpolymj.2017.01.027

Accepted Manuscript

ACCEPTED MANUSCRIPT

28 Du J, Nie Z, Yu H, Xu J, Xu L, Chen Q. Protective cleaning of Chinese paper artworks with strong hydrogels: an interfacial adhesion perspective. Sci China Technol Sci. 2023; 66(9):2681-95. https://doi.org/10.1007/s11431-023-2425-0

29. Richard F, Hermans JJ, Angelova L. Rigid solvent-gels in paper conservation: a new approach to sticky problems. J Paper Conserv. 2024; 25(2):86-106. https://doi.org/10.1080/18680860.2024.2343671.

30. Ranucci E, Alongi J. Advanced polyamidoamine hydrogels for the selective cleaning of artifacts in heritage conservation. Polymers. 2025; 17(19):2680. https://doi.org/10.3390/polym17192680

31. Hassan RRA, Hassan HM, Mohamed YA, Ismail ME, Farid Y, Mohamed H, Ismail SH, Salem MZ, Abdel-Hamied M. ZnO, TiO_2 and Fe_3O_4/Carbopol hybrid nanogels for the cleaner process of paper manuscripts from dust stains and soil remains. Heritage Sci. 2023; 11(1):1-26. https://doi.org/10.1186/s40494-023-01063-7

32. Abdel-Hamied M, Hassan HM, Mohamed YA, Ismail ME, Farid Y, Mohamed H, Ismail SH, Salem MZ, Hassan RRA. Hybrid nanogels and their roles in eliminating soot stains from historical paper manuscripts. Heritage Sci. 2024; 12(1):104. https://doi.org/10.1186/s40494-024-01216-2.

33. Abdel-Hamied M, Ewes A, Salim Z, El-Sayed O, Abdel-Kader K, Tamer M, Sadek AM, Kassem M, Korany MS. Efficiency of gel poultices based on Arbocel and carboxymethyl cellulose with nanoparticle additives for cleaning paper manuscripts. Pigm Resin Technol. 2026; 65:1-16. https://doi.org/10.1108/prt-10-2025-0109.

34. Franco S, Severini L, Buratti E, Tavagnacco L, Sennato S, Micheli L, Missori M, Ruzicka B, Mazzuca C, Zaccarelli E, Angelini R. Gellan-based hydrogels and microgels:

ACCEPTED MANUSCRIPT

a rheological perspective. Carbohydrate Polym. 2025; 354:123329. https://doi.org/10.1016/j.carbpol.2025.123329.

35. Tavagnacco L, Chiessi E, Severini L, Franco S, Buratti E, Capocefalo A, Brasili F, Mosca Conte A, Missori M, Angelini R. Molecular origin of the two-step mechanism of gellan aggregation. Sci Adv. 2023; 9:4392. https://doi.org/10.1126/sciadv.adg4392

36. Morris ER, Nishinari K, Rinaudo M. Gelation of gellan—a review. Food Hydrocolloids. 2012; 28(2):373-411. https://doi.org/10.1016/j.foodhyd.2012.01.004

37. García MC, Alfaro MC, Calero N, Muñoz J. Influence of gellan gum concentration on the dynamic viscoelasticity and transient flow of fluid gels. Biochem Eng J. 2011; 55(2):73-81. https://doi.org/10.1016/j.bej.2011.02.017

38. Cremonesi P, Casoli A. Enzymes as tools for conservation of works of art. J Cultural Heritage. 2021;50:73-87. https://doi.org/10.1016/j.culher.2021.06.005

39. Singer MM, Tjeerdema RS. Fate and effects of the surfactant sodium dodecyl sulfate. Reviews of Environmental Contamination and Toxicology: Continuation of Residue Reviews. 1993; 133:95-149. https://doi.org/10.1007/978-1-4613-9529-4_3

40. Koochakzaei A, Ghane Z, Mohammadi Achachluei M. Zinc oxide nanoparticles in leather conservation: exploring the potential of hydroxypropyl cellulose/zinc oxide nanocomposite as a leather consolidation agent. Heritage. 2023; 6(12):7547-58. https://doi.org/10.3390/heritage6120396

41. Baig A, Siddique M, Panchal S. A review of visible-light-active zinc oxide photocatalysts for environmental application. Catalysts. 2025; 15(2):100. https://doi.org/10.3390/catal15020100

Accepted Manuscript

ACCEPTED MANUSCRIPT

42. Jaski AC, Silva BJG, Zimmermann LM, Riegel-Vidotti IC. Photocatalytic degradation of organic molecules by poly(vinyl alcohol)/zinc oxide hydrogels. J Nanopart Res. 2026; 28(6):149. https://doi.org/10.1007/s11051-026-06666-2.

43. Yadav A, Langyan R, Dhankhar R. Sustainable phytosynthesis of reusable ZnO-NPs for efficient heavy metal ion removal with antibacterial properties. Sustain Chem One World. 2026; 9:100171. https://doi.org/10.1016/j.scowo.2025.100171.

44. Baglioni M, Poggi G, Benavides YJ, Camacho FM, Giorgi R, Baglioni P. Nanostructured fluids for the removal of graffiti—a survey on 17 commercial spray-can paints. J Cultural Heritage. 2018; 34:218-26. https://doi.org/10.1016/j.culher.2018.04.016

45. Macken A, Smith MJ. Solvent vapour use—the unintended consequences in textile conservation. Studies Conservation. 2019; 64(6):352-62. https://doi.org/10.1080/00393630.2018.1544347

46. Baglioni M, Guaragnone T, Mastrangelo R, Sekine FH, Ogura T, Baglioni P. Nonionic surfactants for the cleaning of works of art: insights on acrylic polymer films dewetting and artificial soil removal. ACS Appl Mater Interf. 2020; 12(23):26704-16. https://doi.org/10.1021/acsami.0c06425

47. Baglioni M, Rengstl D, Berti D, Bonini M, Giorgi R, Baglioni P. Removal of acrylic coatings from works of art by means of nanofluids: understanding the mechanism at the nanoscale. Nanoscale. 2010; 2(9):1723-32. https://doi.org/10.1039/c0nr00255k

48. Ardelean E, Bobu E, Niculescu G, Groza C. Effects of different consolidation additives on ageing behaviour of archived document paper. Cellulose Chem Technol. 2011; 45(1):97.

ACCEPTED MANUSCRIPT

49. Feller RL. Accelerated aging: photochemical and thermal aspects. Getty Publications; 1995.

50. Bansa H. Accelerated ageing of paper: some ideas on its practical benefit. Restaurator. 2002; 23(2):106-17. https://doi.org/10.1515/REST.2002.106.

51. Rushdy AM, Wahbab WN, Dacrory S, Kamel S. Multi-function gellan gum hydrogel for heritage paper cleaning. Egyptian Chem. 2024; 67(5):441-50. https://doi.org/10.21608/ejchem.2023.230569.8460

52. Kida K, Inaba M. Damage due to ageing of paper brushed with iron ferrocyanide pigment (Prussian blue). Restaurator. 2013; 34(3):213-26. https://doi.org/10.1515/res-2013-0012

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